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# MACMILIAN INTERNATIONAL COLLECE EDITIONS A Guide to **Medical Entomology**

**M.W. Service** 



A Guide to Medical Entomology

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## A GUIDE TO MEDICAL ENTOMOLOGY

M W Service



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## Preface

This book is written for those whose work is concerned with medicine, hygiene, public health or parasitology but who have no specialised knowledge of medical entomology. Its aim is to provide basic information regarding the recognition of arthropod vectors of disease, their biology and life-cycles, their role in the transmission of diseases to man, and to present a guide to their control. It is hoped that the book will be used selectively to suit the varied needs and requirements of its readers. For example, it is unlikely that physicians being introduced to medical entomology will need to remember either the numbers or durations of the various stages in the life-cycles of the many different vectors. This type of information is nevertheless included in the book so that it can serve as a reference source. Paediatricians, nurses and community health workers will probably find the chapters on lice, bedbugs, scabies mites, houseflies and blowflies etc. more relevant to their needs than those describing the role of tsetse flies and blackflies in the transmission of sleeping sickness and river blindness. On the other hand students specialising in human parasitology or medical entomology will require to learn more from the book than others, and in addition will probably



Fig 1 The main zoogeographical regions of the world.

need to refer to more specialised publications for greater detail on certain topics.

Attempts have been made to present clear and precise accounts of the vectors without unnecessary detail. For this reason little space has been given to detailed descriptions of arthropod mouthparts and only sufficient morphological details are given to identify the vectors.

Chapter 2 which outlines the classification and external and internal morphology of the arthropods is considerably shorter than found in most books on medical entomology for two reasons. Firstly, it is not considered essential that students interested in medical entomology need have detailed knowledge of this aspect of general entomology, and secondly it is realised that few students read such accounts!

The 'Recommended References' at the end of the book provide a starting point for those requiring more detailed information on various vectors or topics: where possible recent review articles and generalised papers and books have been cited.

When describing the distribution of vectors it is often convenient to refer to one or more of the six zoogeographical regions of the world, which are shown in figure 1. Basically the Palearctic region is the whole of the Old World north of about the Tropic of Cancer, the Nearctic region is America north of Mexico, the Neotropical region comprises Mexico and the Americas to the south, and Africa south of the Sahara is the Afrotropical region, previously called the Ethiopian region. The Oriental region is less easily defined, but comprises all tropical lands in the east, such as the Indian subcontinent and Malaysia, but excluding New Guinea, Australia, and New Zealand, which are collectively known as the Australasian region.

The drawings which are so essential in a book of this sort have been provided by Mr S. N. McDermott who has had considerable experience in illustrating medically important arthropods. To Wednesday

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Liverpool April 1979 M. W. Service

### I Introduction to medical entomology

The study of insects is termed entomology. Medical entomology is a specialisation that involves the study of those insects that are of medical importance, for example mosquitoes, flies, lice and fleas. However, the term medical entomology is usually used in a broader sense to take into account the arachnids, a group of invertebrates that includes ticks and mites, that are not insects but nevertheless may be of considerable medical importance.

#### Historical

Some sort of association between certain insects such as fleas, flies and lice, and ill health of man has been recognised from antiquity, many of man's common ectoparasites are referred to in religious texts such as the Talmud, Koran and Bible. However, it was only during the nineteenth century that any definite proof emerged concerning the role of insects and other arthropods in the transmission of disease. For example, in 1868 Fedtschenko discovered that the guinea worm (Dracunculus medinensis) underwent its early development in a small copepod (Cyclops), it later being shown that man becomes infected by drinking water containing these infected crustacea. During the same year Melnikoff found cysts in the body of the dog-louse which Leuckart showed were those of a tapeworm (Dipylidium caninum). Its more common host, the flea, was not discovered until some twenty years later.

The most influential discovery of this period, however, was that made by the physician and parasitologist Patrick Manson. In 1877 while working in China he discovered that bancroftian filariasis (*Wuchereria bancrofti*) underwent development in mosquitoes, *Culex pipiens fatigans* (= C. quinquefasciatus). This provided the first real evidence that a pathogenic organism of man underwent obligatory development in an insect, and can therefore be rightly regarded as representing the 'birth of medical entomology'.

During the next 50 years or so a variety of insects, ticks and mites were incriminated in quick succession as vectors of diseases to man and animals, such as malaria, sleeping sickness, yellow fever, typhus, plague and relapsing fever. The rapid advances in understanding how these diseases were spread allowed the development of rational methods of control.

#### Why medical entomology?

Insects, and other arthropods such as ticks and mites (see chapter 2 for definition of these terms), can directly affect man's health and well-being by transmitting diseases, and so are said to be insects or arthropods of medical importance and are studied by medical entomologists.

Some insects, and other arthropods, do not transmit any disease but can nevertheless cause considerable nuisance and annoyance to man by their bites or stings, which may be poisonous or provoke severe irritations. Others, such as house-dust mites may induce allergies, while a few such as scabies mites actually live in the superficial layers of man's skin and are thus true parasites. Although many insects, ticks and mites cause considerable annoyance to man because of their bites, by far the most important medically are those that transmit diseases such as malaria, sleeping sickness, yellow fever and a multitude of other viruses, various types of filariasis including river blindness and many other diseases.

Great efforts have been made to control the major vector-borne diseases. In nearly all instances the most efficient control measures are those aimed at killing the vectors, whether these be insects, ticks or mites, for example spraying houses with residual insecticides to kill indoor resting *Anopheles*, vectors of malaria; dosing rivers with insecticides to kill the larval stages of blackflies, adults of which transmit river blindness; aerial insecticidal spraying of vegetation which provides resting places for adult tsetse flies which spread sleeping sickness. Because of this reliance on vector control to prevent the transmission of these diseases it is essential that the ecology and behaviour of the vectors is studied to ensure that the best control methods are selected and effectively applied. Moreover, knowledge of the biology, distribution and behaviour of vectors is necessary for a better understanding of the complexities of the epidemiology and spread of diseases, for example variations in adult longevity, feeding behaviour and predilection for human blood and seasonal abundance of Anopheles vectors will greatly affect the intensity of malaria transmission in an area.

Although medical entomology is often taught as a separate subject, it should not be divorced from the parasitological and clinical aspects of diseases. The medical entomologist should work alongside the physician and parasitologist identifying the vectors and providing information on their biology, ecology, seasonal incidence and breeding places. With such knowledge the medical entomologist is able to suggest the most suitable control procedures and the methods by which their impact on the vector population can be assessed.

#### Methods of disease transmission

An insect is said to be a vector when it transmits pathogens or parasites from one animal (including man) to another, thus Anopheles mosquitoes are vectors of malaria. Some insects are more or less accidental vectors and the methods of transferring the disease organisms are simple and relatively inefficient. Houseflies, for example, carry on their hairy legs, feet and bodies numerous pathogenic viruses, bacteria, protozoa and even helminth eggs which they pick up from excreta, pus or wounds etc. and they then deposit them on man's food. The pathogens undergo no obvious morphological changes or multiplication on the fly, which is acting solely as a mechanical vector. Moreover, these pathogens are not solely dependent on the fly for their transmission, as they are spread to man by many other methods such as people with contaminated hands handling food.

Another example of mechanical transmission is the spread of *Trypanosoma evansi* amongst horses by the contaminated mouthparts of horseflies (Tabanidae) and some other biting flies. During feeding the mouthparts of these insects become infected with trypanosomes which remain viable for short periods and can infect healthy animals if the insect feeds on them shortly afterwards. There is also mechanical transmission of myxomatosis to rabbits by fleas, mosquitoes and other blood-sucking insects.

As with the housefly the disease organisms undergo no developmental changes on or in the insect, which serves only as a mechanical vector, but in this case there is a difference because the spread of myxomatosis is dependent mainly on blood-sucking insects.

A more sophisticated relationship exists between insect vectors and pathogens when transmission to man, or animal, occurs only after the pathogens have undergone multiplication and/or some form of development within the insect. This is referred to as cyclical transmission of a disease. It follows that in cyclical transmission there is an 'incubation period' (usually lasting some 7-21 days) in the insect before the pathogens have undergone multiplication, morphological changes or migration and are in the correct state and place to be transferred to the new host. A simple example is provided by the rickettsial parasites causing louse-borne (epidemic) typhus which are swallowed by the body louse when it sucks blood from a typhus victim. The ingested rickettsiae multiply prolifically in the cells of the insect's stomach, after which they pass out with the faeces of the louse and man becomes infected when these are scratched into abrasions on the skin or inhaled. A rather more complicated development of a pathogen in an insect is evidenced by the trypanosomes responsible for South American trypanosomiasis (Chagas' disease). These are ingested by triatomine bugs when they feed on man or animals and the trypanosomes then both multiply and change into different developmental forms within the gut of the triatomine vector before the infective form of the parasite is produced and passed out in the faeces of the insect. Other trypanosomes, such as those causing sleeping sickness in man in Africa, not only undergo morphological changes and multiplication in the gut of the vector, in this instance the tsetse fly, but also have a complicated passage of migration within the insect's gut, so that the infective forms eventually occur in the salivary glands of the fly. When the tsetse fly feeds, saliva is pumped down the insect's mouthparts into the wound to prevent the blood from clotting and this results in the infective trypanosomes of sleeping sickness being injected into the host.

Disease transmission involving infected saliva of blood-sucking insects represents a more efficient procedure than most others. Malarial parasites have the most sophisticated methods of vector transmission. Not only do they multiply within the *Anopheles* mosquito but they also undergo a sexually reproductive cycle in the vector before the infective forms, the sporozoites, are formed and migrate to the salivary glands of the mosquito.

Some disease organisms such as filarial parasites,

causing elephantiasis or river blindness in man, undergo morphological changes and migration in the insect vector but no multiplication. Many of the microfilariae ingested by vectors such as mosquitoes and blackflies are destroyed in the vector's gut so that only a few survive. Those that do survive penetrate the stomach wall and migrate to the thoracic muscles of the insect where they undergo morphological changes. They eventually develop into infective forms which pass down the mouthparts of the insect, and during refeeding are deposited on the surface of the skin which they penetrate. This type of transmission is clearly more complicated than mechanical transmission, but is not as complicated as in diseases such as malaria and African trypanosomiasis where there is both multiplication of the parasites and their injection into a new host with saliva.

In a few vectors, notably ticks and mites, pathogens such as viruses, spirochaetes and rickettsiae actually penetrate the ovaries of the vector. As a result the immature stages which hatch from the eggs are infected, and this infection usually persists to the adult stage. Consequently larval and nymphal stages (immature forms) and adults which have not previously fed on any host are able to transmit diseases. This unusual hereditary method of a vector acquiring an infection and transmitting it is termed transovarial transmission. When infection is acquired by one of the immature stages and passed to later immature stages or adults then it is referred to as transstadial transmission. Both transovarial and transstadial transmission may occur in the same vector.

#### Insect-man contact

The degree of association between hosts (man or animals) and arthropods (insects, mites, ticks) varies considerably. Biting flies such as mosquitoes settle on a host for only relatively short periods to take bloodmeals, whereas triatomine bugs remain longer on a host while taking blood-meals, and some species of ticks may feed on a host for several days before dropping off. Bedbugs live in cracks and crevices in man's houses during the day but during the night leave their hiding places to feed on man. After engorging with blood they return to their daytime resting places. In contrast, fleas spend much longer periods on their hosts, but nevertheless are not permanently attached to them and frequently hop from one host to another.

Head, body and pubic lice are true ectoparasites of man and remain more or less permanently attached to his hairs or clothing. They are spread only by close contact. Even closer relationships exist with scabies mites which live in the surface layer of man's skin, and also with the maggots (larvae) of some specialised myiasis-producing flies which may penetrate deeply into man's tissues and sinuses.

Certain blood-sucking insects, such as some species of mosquitoes and tsetse flies, feed commonly or even predominantly on man and consequently have potential to be efficient disease vectors. Other species feed almost indiscriminately on man and animals or may even prefer animals to man. With these latter species, even if they are capable of transmitting various diseases to man, their importance is often reduced because there is less contact with man and therefore a reduced risk of transmission. However, this is not true with zoonotic infections where vectors become infected by feeding on animal reservoirs and then pass the pathogens to man, and where man to man feeding may be unimportant in maintaining transmission.

## Specificity of vector-parasite relationships

The degree of susceptibility and specificity of vectors for pathogens and parasites varies greatly. With mechanical vectors there is often little specificity, for example any flies landing on excreta and man's food can in theory transmit a variety of enteric pathogens, similarly any insects feeding on rabbits with myxomatosis can spread the disease. In practice, however, some insects are more important than others as mechanical vectors because of their behaviour and habits. For example, the true housefly (*Musca domestica*) is a more efficient mechanical vector of cholera, typhoid and various dysenteries than other closely related flies because it is more likely to settle on both excreta and food than most other flies.

In South America the trypanosomes of Chagas' disease can undergo cyclical development in most species of triatomine bugs and consequently many species should be able to spread the disease to man by their contaminated faeces. However, only the few species of triatomines that live in close association with man and therefore frequently feed on him are important vectors. Similarly, all species of tsetse fly are capable of transmitting human sleeping sickness, but only some of the species that feed on man are efficient vectors.

With several other diseases there is greater specificity of the parasites for an insect vector, not so much because of their ecology or behaviour but because only in certain species can the parasites multiply and undergo development. For example, malaria is transmitted to man by mosquitoes belonging only to the genus *Anopheles*, because the parasites of human malaria are unable to survive or develop in other genera of mosquitoes. However, even within the genus *Anopheles* only in a few species do the malarial parasites succeed in completing their development and infecting the salivary glands. Thus in any area only a few species of *Anopheles* are malaria vectors. Similarly, in Africa yellow fever virus is transmitted from man to man almost entirely by the mosquito *Aedes aegypti*, despite the fact that many other *Aedes* species feed on man. In most other *Aedes* species there is poor survival and very little multiplication of the virus.

A further complication is that a vector species may not have uniform behaviour throughout its range, for example Aedes simpsoni commonly bites man in some areas of Africa and as such is involved in the rural cycle of yellow fever transmission, whereas in other areas it rarely feeds on man. Moreover, although a vector may feed on man throughout its distribution its vectorial capacity may differ, for example in some areas of Africa Culex pipiens fatigans is a good vector of the filarial parasite, Wuchereria bancrofti, while in other areas, notably West Africa, it appears to be a poor vector. These examples show that the epidemiology of many vector-borne diseases is more complex than might appear at first sight.

## 2 Introduction to the arthropods (insects, mites, ticks etc.)

#### **Classification of arthropods**

All insects and arachnids belong to a very large phylum of invertebrate animals termed the Arthropoda, a phylum which contains over 85 per cent of all known species of animal. All arthropods have the body composed of numerous segments, but many of these may be fused and consequently segmentation may not be clearly visible. The entire body is covered with a tough skin called the cuticle which in parts is chemically hardened to form a protective exoskeleton. The very simple heart is dorsal, whereas the ganglionated nerve cord is ventral but in the head region it connects to a dorsal large ganglion, often called the 'brain'. The body cavity (coelom), which is the space between the alimentary canal and body wall, is often called a haemocoele because it contains the arthropod's blood.

The class Insecta is the largest within the Arthropoda; other classes within this phylum include the Arachnida (spiders, scorpions, ticks, mites etc.), Crustacea (crabs, lobsters, shrimps, *Cyclops* etc.), Diplopoda (millipedes), Chilopoda (centipedes) and Pentastomida (includes tongue worms). All these classes contain animals of greater or lesser medical importance.

#### Insecta

The body is divided into three regions – head, thorax and abdomen, but in some insects there may be little external evidence of such differentiation. The head bears a pair of antennae and may also have one pair, occasionally two pairs, of palps which are modified components of the mouthparts. Three pairs of legs arise from the thorax, and usually there are one or two pairs of wings, but some insects such as fleas and lice lack wings. There are no walking legs on any abdominal segments. Respiration is by means of air entering small openings termed spiracles in the exoskeleton of the insect's body, these lead to tracheae within the body which in turn branch into small tracheoles which ramify the body and carry oxygen to the tissues. There may be a pair of spiracles on most body segments or they may be greatly reduced in number.

#### Arachnida

There are no antennae or wings but four pairs of legs. The body is either divided into two regions, one comprising a combination of the head and throax termed the cephalothorax (prosoma) and the other the abdomen (opisthosoma), or the body appears as a single unsegmented entity, such as in ticks. Respiration occurs by air entering one or more pairs of spiracles, often called stigmata in the arachnids, situated on the body.

#### Diplopoda

These are millipedes. There is a small head which has one pair of antennae and following this is a long cylindrical and segmented body which is not differentiated into a thorax and abdomen. Each apparent segment carries two pairs of legs and two pairs of spiracles.

#### Chilopoda

These are the centipedes and are superficially rather similar to the millipedes, except that the body is flattened dorsoventrally and each body segment has only one pair of legs and spiracles.

#### Crustacea

These have two pairs of antennae and at least five pairs of legs. The body may be either single and unsegmented, or segmented and divided into a combined head and thorax and a separate abdomen. Respiration is never by tracheae.





#### Pentastomida

These include the tongue worms. They are elongated and segmented. The adults lack legs and other similar appendages, but the mouth is armed with chitinous hooks. Because of their segmented worm-like appearance they were previously classified as helminths not arthropods. They are parasitic in snakes and a variety of mammals including occasionally man.

#### Insecta

#### Classification

The class Insecta is divided into two subclasses, Apterygota and Pterygota. Within these subclasses are 28 orders (some of which may be divided into suborders) which are divided into numerous families (names ending in -idae, for example Culicidae – which are the mosquitoes), and sometimes into subfamilies (names ending in -inae, for example Culicinae, a group containing many genera of mosquitoes), and finally into genera and species, and sometimes subspecies.

Table 2.1 presents a simplified outline of this type of classification with, for example mosquitoes classified from the phylum Arthropoda down to genera.

#### External morphology of adults

Adult insects are divided into three regions – head, thorax and abdomen, divisions which in many insects such as flies are well differentiated, but in others such as fleas and some lice there is less distinction between these three regions. The thorax is subdivided into three segments the pro-, meso- and metathorax; in many insects, such as flies, these segments are more or less fused together (figure 2.1), but in others such as fleas they remain clearly demarcated (figure 15.1). The abdomen is divided into a varying number (two to eleven) of visible segments, though occasionally seg-



Fig 2.1 An adult Cyclorrhaphan fly.

mentation is very weak and all abdominal segements appear more or less fused together. Both adults and the immature stages are covered with cuticle which contains a nitrogenous polysaccharide called chitin secreted by the epidermis. The cuticle provides a tough and flexible exoskeleton, but does not afford much protection. This is achieved by parts of the cuticular covering undergoing a hardening process called sclerotisation, in which a substance called sclerotin is formed by the addition of protein to the cuticle. The exterior of the cuticle is covered with a thin layer of wax, the chief function of which is to prevent water loss.

#### Head

The head bears one pair of compound eyes, and sometimes several simple dorsally situated eyes called ocelli (figure 2.1), a pair of segmented antennae, and near the mouthparts a pair of palps which are more accurately called the maxillary palps because they are part of the maxillae of the mouthparts (figure 2.2). The length and shape of the palps varies greatly according to different insects. In addition a few insects, such as



Fig 2.2 Diagram of the head and mouthparts of a female culicine mosquito.

cockroaches and fleas (figure 15.2), have retained another pair of palps associated with the labium, and called the labial palps. In most insects, however, they are absent or are an integral part of the labium and modified to form the paired labella at the end of the labium. Both maxillary and labial palps are provided with chemoreceptors which detect smell and taste.

The mouthparts consist of a number of components. Basically the arrangement is an upper 'lip' termed the labrum and a lower 'lip' termed the labium, and in

between these two structures are a pair of mandibles (upper jaw), a pair of maxillae (lower jaw), and a single hypopharynx arising at the base of the labrum through which there is a duct from the salivary glands. The shape and size of these components varies greatly according to the feeding habits of the insects. Cockroaches for example, have chewing mouths consisting of strong jaw-like mandibles used for tearing food apart, whereas in mosquitoes the mandibles and other mouthparts are elongated and most are highly specialised for piercing the skin and sucking blood (figure 2.3). In mosquitoes the labium is fleshy and does not pierce the skin, whereas in tsetse flies and stableflies it penetrates the skin during feeding. In certain insects various components may be absent, for example there are no mandibles in fleas and both mandibles and maxillae are absent in the Cyclorrhaphous flies (see classification of Diptera in table 2.1).



Fig 2.3 Diagram of a female culicine mosquito inserting the fascicle of the proboscis into a host to take a blood-meal; note that the fleshy labium is curved and does not enter the skin.

The mouthparts of insects may project forward, as in mosquitoes and tsetse flies, or downwards such as in tabanids and houseflies. In those insects in which the mouthparts project from the head in an elongated form (for example mosquitoes, biting midges, houseflies, blowflies, bedbugs, fleas, but not lice) they are referred to as the proboscis; in blood-sucking insects the components of the proboscis that actually penetrate the host's skin are sometimes called the fascicle.

#### Thorax

The thorax bears a pair of walking legs on the pro-, meso- and metathorax, each of which is divided into two, usually small, segments called the coxa and trochanter, and three larger segments, the femur, tibia and tarsus. The tarsus may be subdivided into up to five segments (figure 2.4a). The last tarsal segment usually



Fig 2.4 (a) Leg of an insect; (b) terminal tarsal segments of a fly showing pulvilli and different forms of the empodium.

terminates in a pair of claws, which may be simple or have teeth; in lice there is only a single claw but this is very large (figure 16.2). In some flies (Diptera) a pair of pulvilli are present between the claws, these may be fleshy and are often provided with fine hairs which exude a sticky substance enabling the insects to adhere to smooth surfaces, or the pulvilli may be more hairlike. A median structure in the form of a bristle or fleshy pad called the empodium may also be present, and may also assist insects to cling to objects (figure 2.4b). In other insects pulvilli are absent and only a central median structure termed the arolium is present between the claws.

Some insects have a pair of fore wings and a pair of hind wings arising from the mesothorax and metathorax, but in the Diptera the hind wings are greatly reduced and modified to form a pair of knob-like structures called the halteres (figure 2.1) which are sensory organs concerned with flight orientation and balance. In some insects, such as beetles, the fore wings are heavily sclerotised and form horny protective wing cases called elytra, in others such as reduviid bugs the fore wings are thick and hard basally, and membraneous distally and are known as hemielytra (figure 2.5). In some Diptera there are three lobes (squamae) present on the posterior margin of the wing near its insertion to the thorax. One of these is called the thoracic squama, or sometimes just squama, and is more clearly associated with the thorax than the other



Fig 2.5 An adult triatomine bug showing the fore wings which are partially sclerotised to form the hemielytra.



Fig 2.6 Wing of a fly to show the various squamae and numbering of the wing veins.

two which are termed the alar squama and alula (figure 2.6). These lobes are often difficult to see unless the wing is stretched out away from the thorax. The presence or absence of minute hairs on the thoracic squama is useful for separating various genera of the family Calliphoridae (chapter 13). Insect wings have distinct lines of thickening called veins, their arrangement is characteristic of the families. Areas in between the veins are called cells; sometimes the veins completely enclose a part of the wing, and this results in a closed cell, for example the hatchet cell in tsetse flies (figure 11.2a). Each vein is hollow and contains a nerve cord, trachea and haemolymph. The leading edge of the wing is formed by a vein called the costa; directly behind this there may be a short subcostal vein. It is convenient to give the veins below the subcosta numbers (figure 2.6). In many ectoparasitic insects such as lice, fleas and bedbugs and some Diptera such as certain Hippoboscidae (figure 3.1b) there are no wings.

Each thoracic segment has a dorsal sclerotised region called the tergum or notum and a ventral one, the sternum, and laterally on each side a pleuron, all of which are joined together with non-sclerotised membraneous cuticle (figure 2.7). These regions may be



Fig 2.7 Diagram of a transverse section through the thorax of an insect to show sclerotised plates connected by flexible cuticle.

fused or sub-divided further into sclerites, namely tergites, sternites and pleurites. For example, in the Diptera almost the entire dorsal surface of the thorax is comprised of the tergum of the mesothorax, which is called the scutum or mesonotum, in contrast the lateral mesopleuron of many Diptera is subdivided into a number of small separate sclerites.

#### Abdomen

The abdomen is usually, but not always, subcylindrical and consists of eleven segments, but in many insects only a few are visible because some are telescoped within the body, the housefly, for example, has only four visible segments. In the females of many insects the tip of the abdomen is more rounded than in the males. The last abdominal segment of both sexes may possess bristle-like or finger-like processes called cerci (figure 2.8a), these are often more conspicuous in females than males. In many male insects such as phlebotomine sandflies and mosquitoes there is a pair of conspicuous claspers of the external genitalia at the end of the abdomen (figure 2.8b). In some insects, however, such as houseflies, there is little difference between the ending of the abdomen in the two sexes. Each abdominal segment is composed of a dorsal sclerite termed the tergum and a ventral one called the sternum which are joined laterally by a membraneous area of non-sclerotised cuticle.



Fig 2.8 Diagram of last segments of the abdomen of (a) a female insect showing cerci and (b) a male insect showing the well developed claspers.

#### Hairs and scales

The head, thorax, legs, wings and abdomen of insects may be partially or almost entirely covered with appressed, semi-erect or erect fine hairs called setae. When these setae are stout and coarse and possibly frayed they are usually referred to as bristles, and when they are greatly flattened they are called scales. Hairs and scales may be white, brown, black or almost any colour, it is the coloured scales on butterflies and moths that give them their beautiful appearence. The colour of the head, thorax, abdomen, legs and wings of mosquitoes for example is due to numerous closely appressed scales of different colours. On insect wings these scales and setae may be confined to the veins as in mosquitoes, or occur on the membraneous areas, for example the membraneous areas of the wings of biting midges (Ceratopogonidae) are often covered with small setae called microsetae. Each seta or scale has a distinct circular socket from which it arises. Other surface structures such as spines do not arise from sockets and are extensions of the cuticle.

#### Internal anatomy of adults

#### **Blood system**

Insect blood is usually colourless and is called haemolymph. It contains haemocytes which are blood cells



Fig 2.9 Diagram of a mosquito showing the arrangement of the nerve cord and circulatory system.

not concerned with respiration but are mainly phagocytic. Blood circulation is maintained by the haemolymph entering small valve-like openings, called ostia, in the distal part of a dorsal pulsating tube called the 'heart' (figure 2.9). Blood from the heart is forced forwards through its so called 'aorta' to the brain. The general activities and locomotion of the insect ensures that the blood forced out of the dorsal tube is agitated and reaches all tissues and organs of the body. The main function of insect blood is to carry nutrients to the tissues and waste products to the excretory organs (Malpighian tubules). The entire body cavity (coelom) between the alimentary canal and the insect cuticle is full of haemolymph, for this reason the coelom is often termed the haemocoel.

#### **Respiratory system**

Oxygen reaches the tissues of insects by direct gaseous

diffusion; insect blood (haemolymph) is not concerned with respiration. Small circular openings called spiracles in the cuticle of the exoskeleton of the insect allow air to enter the body. Spiracles may occur on the mesoand metathorax and the first eight abdominal segments, or be greatly reduced in number and located on only a few segments. Air entering the spiracles passes into a series of small tubes called tracheae which are provided with spiral thickenings to keep them distended and prevent them from kinking. These tracheae branch to form a network of small tracheoles which ramify through most parts of the body and sometimes penetrate the cells (figure 2.10). Oxygen diffuses across the tracheole walls into the cells and carbon dioxide from the cells enters the tracheoles and is passed out via the spiracles. In some insects, especially aquatic species, there also may be direct cuticular respiration.

The tracheal system and the spiracles, which in many insects can be partially or fully closed, also regulate water loss.



Fig 2.10 Diagram showing an insect spiracle and tracheae branching into fine tracheoles.

#### Nervous system

The central nervous system consists of a relatively large supraoesophageal ganglion in the head, termed the 'brain', which is connected to a ventral nerve cord having ganglia at intervals, often one per body segment (figure 2.9). Nerves arising from these ganglia reach various parts of the body and special sensory organs such as the compound and simple eyes (ocelli), antennae, halteres and palps and also various receptors in the body which respond to temperature, humidity, food stimuli and host odours. Many of the hairs on the insect's body serve as sensory receptors. In some bloodsucking insects, such as tsetse flies and tabanids, sight is important in locating hosts, whereas in others such as mosquitoes, host orientation is mainly governed by chemoreceptors receiving host odours. Lice on the other hand are mainly guided to their hosts by increases in temperature and humidity.

#### Alimentary canal

The structure, size and formation of the mouthparts of insects is complex and varied; insects such as cockroaches which chew solid foods have very different mouthparts from those of blood-sucking insects. Because of the different types of foods ingested there is great diversity in the morphology of the alimentary canal, but a generalised description is as follows.

The alimentary canal (figure 2.11) can be conveniently divided into three regions, the fore, mid and hind gut. The fore gut starts with the mouth and leads to the buccal cavity, pharynx, oesophagus and proventriculus (gizzard). The posterior part of the oesophagus is dilated and is referred to as the crop. In some insects, especially those ingesting fluid foods such as houseflies and blowflies and blood-sucking ones, the crop forms a distinct diverticulum of the oesophagus. The crop usually serves as a temporary food store before food is passed to the mid gut for digestion. In addition there may be other diverticula arising from the oesophagus. The muscular proventriculus acts as a valve and prevents food being regurgitated, and may have teeth or spines to aid the disintegration of food particles (figure 15.5). A pair of salivary glands is situated ventrally in the thorax, but may, as in the case of tsetse flies, extend into the abdomen. Ducts from each gland unite to form a common salivary duct which passes down the hypopharynx of the mouthparts. The composition of saliva varies according to the insects, but in blood-sucking ones it often, but not always, contains anticoagulins which prevent blood which is sucked from the host from clotting and blocking the insect's mouthparts. Saliva contains allergens and it is the host's reaction to having minute quantities of saliva injected into the skin that causes localised swellings, itching and irritation. The severity of this irritation depends not only on the species of blood-sucking insect but also on the individual reaction of the host.

The mid gut or stomach stores food during the process of digestion and may become greatly distended. It secretes the enzymes necessary for the digestion of the insect's meal. The beginning of the hind gut is marked by the presence of a variable number, often four to six, whitish opaque excretory tubules, termed the Malpighian tubules. The anterior part of the hind gut is referred to as the ileum, while the more distal and usually dilated part is called the rectum, and this is usually provided with rectal papillae or glands which reabsorb water from the faeces. The rectum terminates in the anus.

In some insects, especially the Diptera, there is a peritrophic membrane which originates as a viscous secretion from special cells at the anterior end of the mid gut. It is formed as a very delicate sausage-shaped



Fig 2.11 Diagram of a mosquito showing the alimentary canal, Malpighian tubules and the salivary glands.

sheath and extends down the mid gut and some way into the hind gut. It prevents damage, such as abrasion, to the delicate secretory cells of the mid gut during digestion. In tsetse flies it is important in the cylical development and migration of trypanosomes of sleeping sickness (chapter 11 for a detailed account). The peritrophic membrane is absent in many insects including fleas, lice, tabanids and Hemiptera, while in other insects including cockroaches another type of peritrophic membrane is formed by the delamination of the whole of the mid gut surface.

#### **Excretory system**

The Malpighian tubules (figure 2.11) are situated at the end of the hind gut and extract urine, salts and other waste products from the haemolymph. The tubules act as excretory filters and discharge waste products into their lumens from where they are passed to the hind gut. After water has been reabsorbed by the rectal papillae of the rectum the waste products are passed out with the excreta through the anus.

The number of Malpighian tubules varies in different insect species from 2-200. They are usually milky-white and opaque in appearance due to the deposition within their cells of waste products.

#### **Reproductive system**

Females: The external genital apparatus in most female

insects is small and inconspicuous, but some insects have conspicuous ovipositors.

Internally the female reproductive organs consist of a pair of ovaries comprising several egg-tubes called ovarioles within which the eggs are developed, paired oviducts, and a common oviduct (sometimes called the uterus, for example in tsetse flies) of which the lower part may be called the vagina (figure 2.12a). One or two pairs of accessory glands secrete a mucous or cement-like substance which is deposited on the eggs as they pass down through the vagina and are laid. This secretion can be a water-proofing agent or an adhesive which glues eggs to various substrates, such as lice eggs to body hairs and clothing and bedbug eggs to walls. The accessory glands also provide material for the formation of the egg-pod (ootheca of cockroaches). A most important structure of the female reproductive system is the spermatheca, which is lined with cuticle, and consists of one to three receptacles which are filled with spermatozoa from the male during copulation. (Spermathecae can be of considerable taxonomic value in distinguishing between females of some species, such as fleas and phlebotomine sandflies). As each egg passes down the oviduct into the vagina it is fertilised by spermatozoa coming from the spermatheca. Insects need mate only once because the spermatozoa contained within the spermatheca remain viable throughout the female's life and are sufficient to fertilise all eggs laid in the first and subsequent egg batches. In a few species, after fertilisation, eggs are hatched within the



Fig 2.12 Reproductive system of (a) a female mosquito and (b) a male mosquito.

uterus of the female and larvae, not eggs are deposited; such insects are said to be larviparous or viviparous (see chapters 11, 13 and 14).

Males: Externally there is generally a pair of genital claspers (figure 2.8b) which are used to grip the female during mating, an act which can last for a few seconds or many hours. In between the claspers is the aedeagus and in the centre of this is the penis. The external genital structures, often called the male genitalia or terminalia, can be of great taxonomic value in identifying male insects to species.

The internal male reproductive system consists of a pair of testes and paired vas deferens which are swollen distally to form the seminal vesicles which store spermatozoa; in some Diptera there is a single common seminal vesicle. The single or paired seminal vesicles open into the ejaculatory duct which terminates in the aedeagus. One or three pairs of small accessory glands are often present, their function is to secrete a fluid into the ejaculatory duct (figure 2.12b). Accessory glands are absent in some insects including many Diptera.

#### Growth, moulting and metamorphosis

The integument or skin of insects is sclerotised in most places and forms a protective exoskeleton which being hard and rigid, prevents them increasing in size. Adult insects never grow. Growth is restricted to the immature stages of their life-cycles and can be achieved only by casting off the exoskeleton; a process known as moulting or ecdysis. For example, a minute cylindrical maggot (larva) emerges from an egg laid by a housefly and after eating for a few days its integument splits and the maggot wriggles out from its old skin which is left behind. The newly emerged maggot swallows air and assisted by blood pressure expands the body and thus grows in size while its new integument is still soft and pliable. After this short period of growth the new integument hardens and darkens and further growth is prevented. After a few more days of feeding this new integument splits and the maggot again struggles out and expands in size before the new integument has a chance to harden. Hence a maggot grows spasmodically in discrete stages.



Fig 2.13 Diagrammatic representation of the life-cycle of a housefly as an example of holometabolous metamorphosis.

Each of the larval stages is called an instar, thus an egg hatches into a first-instar larva which after moulting passes into the second-instar larva and so on until the pupa is formed. The number and duration of the larval instars varies in different insects, houseflies and other cyclorrhaphous flies have three larval instars whereas mosquitoes have four larval instars. There is a slight complication. In many insects, such as mosquito larvae the last larval instar splits its cuticle and the pupa wriggles out, but in houseflies, blowflies and other cyclorrhaphous Diptera the cuticle of the last larval instar does not split but hardens and darkens to form a protective shell called the puparium which protects the delicate pupa which is formed inside. The transformation from the egg stage through the immature stages to the final adult form is referred to as metamorphosis, a word meaning 'change in form'. There are two types of metamorphosis. Many insects have what is termed a complete metamorphosis and are called holometabolous insects, others pass through an incomplete metamorphosis and are called hemimetabolous.

Holometabolous insects are those in which a larva, which is completely dissimilar in appearance to the adult, hatches from the egg (figure 2.13). Larvae usually feed on a different type of food to the adults and may live in a different environment, for instance, mosquito larvae occur in water whereas adults are nonaquatic. The larva passes through a series of instars and



Fig 2.14 Diagrammatic representation of the life-cycle of a bedbug as an example of hemimetabolous metamorphosis.

the final ecdysis results in the pupal stage. During the pupal period the larval tissues break down and the cells are rearranged to build up the tissues and organs of the adult insect. The pupal stage does not feed and is really a transient and quiescent stage that allows the reorganisation and transformation to occur. In some insects, such as blackflies and fleas, the larva spins a protective silken cocoon to enclose and protect the pupa. The pupal stage is usually relatively short and terminates when the hard pupal or puparial integument splits open to allow the adult to emerge.

In holometabolous insects there are therefore several larval instars and a single pupal stage, none of which resembles the adult insect. A common example of holometabolous insects is the butterflies which have caterpillars (larvae) hatching from the eggs and after growth and a series of moults finally turns into chrysalids (pupae), then after some days or weeks adult butterflies emerge. Insects of medical importance that have this type of holometabolous life-cycle include all the Diptera (for example houseflies, mosquitoes, tsetse flies) and fleas.

In hemimetabolous insects there is a gradual metamorphosis or change. The eggs hatch to produce a miniature version of the adult which lives in the same environment as the adult, has more or less the same food requirements and similar habits (figure 2.14). This stage is called a nymph and after a period of days or weeks it moults and a new nymph emerges from the exoskeleton, this grows before the cuticle hardens, and eventually moults again to produce another nymph and so on. Each nymphal stage is larger and resembles more the adult than the preceding one.

Head, body and pubic lice are hemimetabolous insects which pass through three nymphal instars, while bedbugs and triatomine bugs pass through five nymphal stages. Of these insects only the triatomine bugs have wings, but these are only fully formed in the adults, the nymphs have non-functional wings known as wing pads. Sometimes the immature forms (nymphs) of hemimetabolous insects are called larvae, but it is better to restrict this term to the immature stages of holometabolous insects, or to the first developmental stage of ticks and mites.

#### Diapause, hibernation and aestivation

At some stage during their life-cycle insects may temporarily cease to develop or be active, a condition known as quiescence. This usually occurs during environmentally unfavourable periods. The term hibernation is commonly used to describe periods when insects enter quiescence to resist cold conditions such as during the winter, whereas when they become relatively inactive to allow them to resist drought or high temperatures this is called aestivation. For example, adult houseflies become inactive during the cold winters of northern temperate areas and seek shelter in buildings etc., and are said to be hibernating. In very hot periods during the dry season in the tropics some mosquitoes stop blood-feeding and ovipositing and seek shelter in protective places, this is termed aestivation.

In some insects development of the egg, larva, nymph or adult may be arrested although external conditions appear favourable, and the insects are said to be in a state of diapause. Diapause lasting a few weeks or several years is initiated by many external factors including changes in daylength and temperature and the insects must be subjected to appropriate stimuli to 'break' the diapause. For example, eggs of many *Aedes* mosquitoes enter diapause and will not hatch even when flooded with water, unless there is a reduction in oxygen content of the water, and larvae of some temperate *Aedes* species enter diapause that is broken by increasing daylength. Diapause allows various stages of insects to survive under adverse conditions.

#### Arachnida

The class Arachnida is divided into several subclasses including the Scorpionida or Scorpiones (scorpions), Araneida or Araneae (spiders) and the Acarina or Acari (ticks and mites). Although spiders and scorpions contain species that can inflict poisonous stings and bites they are not of great medical importance. In contrast ticks and mites of the subclass Acari include ectoparasites of man and species that are vectors of disease.

## External morphology of adults of the subclass Acari

In all arachnids the head and thorax are fused to form a division of the body termed the prosoma (cephalothorax). In some arachnids such as scorpions and spiders the prosoma is clearly differentiated from the abdomen or opisthosoma (figure 2.15) but in others such as in most mites and all ticks these two divisions are fused together to form a more or less ovoid or saclike entity called the idiosoma (figure 2.16). There is usually no obvious external evidence of a segmented body although there are a few exceptions such as the follicle mites of man (*Demodex folliculorum*, figure 24.1).



Fig 2.15 A spider as an example of an arachnid having the body divided into two regions, the prosoma and the opisthosoma.



Fig 2.16 A lateral view of a hard (ixodid) tick showing the more or less sac-like body termed the idiosoma and the capitulum.



Fig 2.17 The mouthparts of a soft (argasid) tick.

Mites and ticks have no wings, antennae or compound eyes but simple eyes (ocelli) may be present. The mouthparts arise from the anterior part of the idiosoma sometimes called the false head, capitulum or ignathosoma (figure 2.16). This consists of a ring-like basal part called the *basis capituli* (figure 2.17), a pair of four segmented sensory palps sometimes called the pedipalps, a pair of cutting and piercing mouthparts called the chelicerae and a prominent 'upper lip' termed the hypostome and a poorly developed 'lower lip' which is the epistome. In ticks, but not mites, the hypostome is toothed on the ventral surface and is used together with the paired chelicerae to pierce the host's skin. There are no mandibles, maxillae or hypopharynx.

There are four pairs of walking legs divided into seven segments – the coxa, trochanter, femur, patella (genu), tibia, metatarsus and tarsus. In ticks the tarsi terminate in claws whereas most mites lack claws; in the scabies mite (*Sarcoptes scabiei*) the legs end in specialised filamentous processes (erroneously described as suckers) or long hairs. Most ticks and mites have a dorsal plate called a scutum on the anterior part of the body, in the scabies mite it is very small but in male ixodid ticks it covers almost the whole of the dorsal surface of the body (figure 21.1).

#### Internal anatomy of adults of the subclass Acari

The food channel is formed by the apposition of the hypostome and paired chelicerae, and leads to the gut which consists of the mouth, pharynx, oesophagus and mid gut which often has numerous diverticula and



Fig 2.18 Diagrammatic representation of the internal anatomy of a soft (argasid) tick.

branched tubules, and finally the rectum and anus (figure 2.18). The rectum and anus may be closed and not open to the exterior. The shape and size of the salivary gland varies greatly in different types of ticks and mites; saliva from the salivary glands passes into the buccal cavity and is concerned with digestion.

In the subclass Acari the excretory system varies greatly according to family. It may consist of (1) a pair of long, slightly coiled Malpighian tubules opening into the rectum of the hind gut, or (2) an assortment of small excretory glands opening at various points into the intestine, or (3) one or more pairs of coxal organs which discharge their fluids into tubules which open to the exterior between the coxae of the legs, or (4) a mixture of two or all three systems.

The respiratory openings of the tracheae in ticks are called spiracles or stigmata. In some mites there appears to be no definite respiratory system, gaseous diffusion apparently occurring through the delicate chitinous area of the integument. The numbers and arrangement of the spiracles and form of the respiratory system is used in classifying the subclass Acari into various subdivisions and orders.

#### Metamorphosis in the subclass Acari

Nearly all medically important mites and ticks lay eggs, but mites of the genus *Pediculoides* (=*Pyemotes*) produce adults. Eggs of mites and ticks hatch to produce a developmental stage that resembles the adult but is much smaller and has only three pairs of legs, and which is known as the larva. The acarine larva moults to give rise to a nymph which is bigger than the larva and, although not as big as the adult, resembles it in having four pairs of legs (figures 20.5 and 21.3). There may be one or several nymphal stages before the adult stage is reached. The immature stages of mites and ticks, that is the larvae and nymphs, may have similar habits and food requirements as the adult or be dissimilar to a varying degree.

## Classification of the subclass Acari into orders

#### Metastigmata (Ticks)

This order contains the soft (Argasidae) and hard (Ixodidae) ticks, which are the largest acarines. They possess a pair of spiracles (stigmata) surrounded by a rounded chitinous spiracular (stigmatal) plate on each side of the body. The hypostome of the mouthparts is used in piercing the host's skin during feeding, and has recurved teeth ventrally.

#### Mesostigmata (Mites)

Comprises the tropical rat mite (Ornithonyssus bacoti) and a few other blood-sucking mites belonging to the family Dermanyssidae. The spiracles are found dorsolaterally near the base of the coxae of the second to fourth pairs of legs. Associated with the spiracles are long grooves called peritremes, the function of which remains unknown.

#### Astigmata (Mites)

This order includes the 'stored products itch mites' which cause dermatitis in man, for example species of *Tryophagus*, *Glycyphagus*, the so called house-dust mites such as *Dermatophagoides* species and the scabies mite (*Sarcoptes scabiei*). They are all weakly sclerotised mites, with two-segmented palps, pincer-like chelicerae, and the idiosoma is often divided into two parts. Neither spiracles nor tracheae are present.

#### **Prostigmata** (Mites)

This contains a large assortment of rather dissimilar mites, including the grain itch mite (*Pediculoides* (=*Pyemotes*) ventricosus), the follicle mites (*Demodex* folliculorum) and the trombiculid mites which are vectors of scrub typhus. Chelicerae may be pincer-like, hook-like, or modified for piercing, the palps are usually long and leg-like and may have an apposable claw-like appendage. Tracheae, if present, connect with paired spiracles which are either located between the chelicerae (but are very difficult to see), or on the dorsal surface of the anterior part of the body.

# 3 Introduction to the order Diptera – mosquitoes, horseflies, houseflies, tsetse flies etc.

Adult insects belonging to the order Diptera may be readily recognised by the presence of only one pair of functional wings, not two pairs as in butterflies and moths, and wasps and bees.

In all Diptera the hind wings are absent, being replaced by a pair of small knob-like structures called halteres. These are not directly concerned with flight propulsion but control the stability and balance of the insects during their flight. Thus mosquitoes which only have one pair of wings are technically flies, because they belong to the order Diptera. All insects discussed in chapters 4-14 are therefore flies.

All Diptera undergo a holometabolous life-cycle. No dipterous larvae possess legs, but false legs called pseudopods may sometimes be present such as in larval horseflies (Tabanidae). Larvae of many flies such as mosquitoes and blackflies are aquatic, other dipterous larvae live in semi-aquatic habitats such as muddy ground or at least in situations that are very humid, such as in decaying vegetable matter, soil, animal



Fig 3.1 Hippoboscid flies: (a) adult fly of the genus Ornithomyia, (b) adult fly of the genus Melophagus.

excreta or festering wounds and sores. Larvae of a few species are wholly or partially parasitic in tissues of man or animals and cause a condition known as myiasis (chapters 13 and 14).

There are more than 50 000 species of Diptera in the world, the majority of which are of no medical importance but some of the most important vectors of diseases to man and animals belong to the order Diptera. For example, mosquitoes are vectors of malaria, tsetse flies are vectors of sleeping sickness, blackflies transmit river blindness and phlebotomine sandflies transmit leishmaniasis.

The Diptera are divided into three suborders (table 2.1, page 6). The most primitive is the Nematocera which comprises mainly small flies with a simple type of many-segmented antennae which may be relatively long and filamentous as in mosquitoes or considerably shorter such as in blackflies and phlebotomine sandflies. The next suborder, the Brachycera, contains mainly large flies having the antennae generally divided into three segments, the last of which is the largest and may be annulated or subdivided. In some species which are of no medical importance the third and last antennal segment may bear a bristle called an arista, a feature more characteristic of the Cyclorrhapha. The medically important species in the Brachycera belong to the family, Tabanidae, in which the antennae are short and stout in many genera, such as *Tabanus* and *Haematopota*, but are longer, although still relatively stout, in *Chrysops* species. Flies in the most advanced state of evolution belong to the suborder Cyclorrhapha. The antennae also consist of three segments but the last one always bears a bristle called the arista. Insects in this suborder include those that are generally called flies by the nonspecialist, such as houseflies, blowflies and tsetse flies.

In order classifications there was a fourth suborder of the Diptera called the Pupipara, however, the Pupipara are now regarded as a 'section' within the Cyclorrhapha, and contain flies of the family Hippoboscidae. They are unusual and abnormal flies with a leathery type of integument, are greatly flattened dorso-ventrally and are more or less permanently attached parasites on various animals and birds, especially domesticated livestock (figure 3.1). They cling firmly on to these animals and suck blood. Wings may be absent, reduced in size or fully developed, but if present they are rarely used. Although of veterinary importance they are of no direct medical importance and rarely bite man and therefore are not discussed further in this book.

## 4 Introduction to the mosquitoes (Order Diptera: Family Culicidae): classification, morphology, life-cycle and control principles

#### Species

Some authorities place the small non-biting flies belonging to the Dixidae and Chaoboridae as subfamilies (Dixinae and Chaoborinae) of the family Culicidae but in this book the classification given in the world catalogue of mosquitoes by Knight and Stone (1977) is followed, that is the family Culcidae contains only mosquitoes. There are some 3100 species of mosquitoes belonging to 34 genera arranged in three subfamilies, – Toxorhynchitinae, Anophelinae (anophelines) and Culicinae (culicines).

The most important man-biting mosquitoes belong to the genera Anopheles, Culex, Aedes, Mansonia, Haemagogus, Sabethes and Psorophora.

#### Distribution

Mosquitoes have a world-wide distribution, they occur throughout the tropical and temperate regions and extend their range northwards into the arctic circle; the only area from which they are absent is Antarctica. They are found at elevations of 5500 m and in mines at depths of 1250 m below sea level. Some genera have a restricted distribution and may be confined to certain areas of the world, the genera *Haemagogus* and *Sabethes* for example are found in only Central and South America. Some mosquitoes may occur in only a few countries or localities, whereas others, such as *Culex pipiens fatigans*\* (= *C. quinquefasciatus*) and *Aedes aegypti* are widespread in the tropical regions of the world.

#### Medical importance

Anopheles species are primarily of medical importance as vectors of human malaria (*Plasmodia* spp.) but they are also vectors of filariasis (Wuchereria bancrofti, Brugia malayi and B. timori) and certain arboviruses. The genus Aedes contains important vectors of yellow fever, dengue, encephalitis viruses and many other arboviruses, and also vectors of W. bancrofti and B. malayi. Certain Culex species transmit W. bancrofti and a variety of arboviruses and Mansonia species transmit Brugia malayi, and sometimes Wuchereria bancrofti and a few arboviruses. Haemagogus and Sabethes species are vectors of yellow fever and a few other arboviruses. Psorophora species are important mainly as nuisance mosquitoes, but they also transmit arboviruses including occasionally yellow fever.

Several mosquitoes in other genera have also been incriminated as vectors of various arboviruses, and many other species although not carriers of any disease can nevertheless be troublesome because of the serious biting nuisances they cause.

#### External morphology of mosquitoes

#### Adults (figure 4.1)

Mosquitoes possess only one pair of functional wings – the fore wings, the hind wings are represented by a pair of small knob-like halteres. They belong to the suborder Nematocera of the order Diptera (table 2.1, p. 6). They can be distinguished from other flies of a somewhat similar shape and size (for example Dixidae, Chaoboridae and Chironomidae) by (1) the possession of a conspicuous forwardly projecting proboscis, (2) the presence of numerous appressed scales on the thorax, legs, abdomen and wing veins,

\* Culex pipiens is a species complex: the tropical form is often called C. pipiens fatigans and this name has been used in this book to avoid confusion and introducing an unfamiliar name, although it has recently been established that more correctly the name C. quinquefasciatus has priority.



Fig 4.1 Diagrammatic representation of an adult female anopheline mosquito.

and (3) a fringe of scales along the posterior margin of the wings.

Mosquitoes are slender and relatively small insects, measuring about 4-6 mm in length, although some species can be as small as 2-3 mm while others may be as long as 10 mm. The body is distinctly divided into a head, thorax and abdomen.

The head is more or less globular in shape and has a conspicuous pair of kidney-shaped compound eyes. Between the eyes arises a pair of filamentous and segmented antennae, which in females have whorls of short hairs between the segments (that is pilose antennae) but in males, with a few exceptions in genera of no medical importance, the antennae have many long hairs giving them a feathery or plumose appearance. All species of mosquitoes can be conveniently sexed by examination of the antennae, individuals with feathery antennae are males (figure 4.2b) while those with only short and rather inconspicuous antennal hairs are females (figure 4.2a). Just below the antennae are a pair of palps, which may be long or short, dilated or pointed at their tips, depending on the sex of the adults and whether adults are anophelines or culicines (figure 4.21). Arising between the palps is the single elongated proboscis, which contains the piercing mouthparts (sometimes termed the biting fascicle) of the mosquito. In mosquitoes the proboscis characteristically projects forwards (figure 4.1).



Fig 4.2 Heads of adult mosquitoes showing central proboscis and antennae, only bases of palps shown, (a) female mosquito showing pilose (non-plumose) antennae and (b) male mosquito showing plumose antennae.

The thorax is slightly humped and is covered dorsally and laterally with scales, which may be dull or shiny and metallic, white, black or almost any colour, small bristles are also present. The numbers, distribution and colour of the scales are important characters used in the identification of different mosquito species. It is this arrangement of black and white, or coloured,



Fig 4.3 Thorax and scutellum of (a) a culicine mosquito showing trilobed scutellum and (b) an anopheline mosquito with rounded posterior margin to the scutellum.

scales on the dorsal surface of the thorax (that is the scutum or mesonotum) that gives many species (especially those of the genus *Aedes*) distinctive patterns (figure 6.5). Behind the scutum of the thorax is the small scutellum, which posteriorly has a rounded margin (figure 4.3b) in anopheline mosquitoes (except genus *Chagasia*), but is more or less trilobed in culicine mosquitoes and *Chagasia* (figure 4.3a).

The wings are long and relatively narrow. The number and arrangement of the wing veins is virtually the same for all mosquito species (figure 4.4). The veins are covered with scales which are usually brown, black, white or creamy yellow, but more brightly coloured scales may occasionally be present. The shape of the scales and the pattern they form differs considerably both in different genera and species of mosquitoes. Scales also project as a fringe along the posterior border of the wings (figure 4.4). In life the wings of resting mosquitoes are placed across each other over the abdomen in the fashion of a closed pair of scissors. The legs of the mosquito are long and slender, consisting of a short coxa and trochanter, a long femur and tibia and a long five-segmented tarsus, which terminates in, usually a pair, of toothed or simple claws. A delicate seta (empodium) is present at the end of the last tarsal segment of most species and in addition some genera (for example Culex) have a pair of small fleshy pulvilli (figure 4.5). The legs are covered with scales which are usually brown, black or white and may be arranged in patterns, often in the form of rings (figure 6.6a).

The abdomen is composed of ten segments but only the first eight are visible and well differentiated. They are covered dorsally and ventrally with mostly brown, blackish or whitish scales in mosquitoes of the subfamily Culicinae but brightly coloured ones may be present. Their arrangement, particularly dorsally on the tergites, is used as an aid in species identification. In the Anophelinae, however, the abdomen is almost or entirely devoid of these scales. The last abdominal



Fig 4.4 Diagram of a wing of a mosquito showing numbering of wing veins and fringe scales.



Fig 4.5 Last segment of the tarsus of a Culex mosquito showing empodium and fleshy pulvilli.

segment of female mosquitoes terminates in a small pair of finger-like cerci, whereas in the males a prominent pair of claspers are present (figure 2.8b). These are part of the external male genitalia (terminalia) the morphology of which is important in species identification. Within a few hours of emergence the external male genitalia starts to rotate and after about 12-24 hours it has turned through  $180^\circ$ : males are unable to mate with females before this rotation is completed.

In unfed mosquitoes the abdomen is thin and slender, but after females have bitten a suitable host and taken a blood-meal (only females bite) the abdomen becomes greatly distended and resembles a red oval balloon. When the abdomen is full of developing eggs it is also dilated, but is whitish not red in appearance.

#### Mouthparts and salivary glands

The mouthparts are collectively known as the proboscis. In mosquitoes the proboscis is elongate and projects conspicuously forwards in both sexes although males do not bite. The largest component of the mouthparts is the long, flexible, gutter-shaped labium,



Fig 4.6 Diagram of a transverse section through the proboscis of a mosquito to show position of the components of the mouthparts and the food channel.

which terminates in a pair of small flap-like structures called the labella. In cross-section the labium is seen to almost encircle all the other components of the mouthparts (figure 4.6) and it serves as a protective sheath. The individual components are held close together in life and only become partially separated during blood-feeding, or when they are teased apart for examination as illustrated in figures 2.2 and 4.7.

The uppermost structure, the labrum, is slender, pointed and grooved along its ventral surface. In between this 'upper roof' (labrum ) and 'lower gutter' (labium) are five needle-like structures, namely a lower pair of toothed maxillae, an upper pair of mandibles which are more finely toothed and finally a single untoothed hollow stylet called the hypopharynx. When a female mosquito bites a host the labella at the tip of the fleshy labium are placed on the skin and the labium which cannot pierce the skin becomes curved backwards (figure 2.3). This allows the paired mandibles and paired maxillae to cut into and pierce the skin of the host, so that together with the labrum and hypopharynx they can enter the skin. Blood from the host is obtained either by the flexible biting fascicle of the proboscis actually entering and passing along a blood capillary or from a pool of blood formed by the rupture of minute capillaries. Blood is sucked up the proboscis in the space formed by the apposition of the labrum and other stylet-like mouth-



Fig 4.7 Diagram of the head of a female culicine mosquito to show the components of the mouthparts dissected from the labium.
parts (figure 4.6). A pair of trilobed salivary glands, situated ventrally in the anterior part of the thorax (figure 2.11), pumps saliva down through the salivary ducts which unite to form a single duct which passes down the entire length of the hypopharynx. Saliva contains anticoagulins and /or haemagglutinins which prevent the blood from clotting and obstructing the mouthparts as it is sucked into the stomach.

In male mosquitoes the mandibles and maxillae are reduced in size or are absent, males cannot pierce the skin and take blood-meals.

The alimentary canal of the mosquito is depicted in figure 2.11 and the reproductive systems in figure 2.12. The mid gut and salivary glands of mosquitoes are important in the life-cycles of the malarial parasites and other pathogens.

## Life-cycle of mosquitoes

## Blood-feeding and the gonotrophic cycle

Most mosquitoes mate shortly after emergence from the pupa. Sperm passed by the male into the spermatheca of the female serves to fertilise all eggs laid by her during her lifetime, thus only one mating and insemination per female is required. With a few exceptions a female mosquito must bite a host and take a blood-meal to obtain the necessary nutrients for the development and maturation of the eggs in the ovaries. This is the normal procedure and is sometimes referred to as anautogenous development. In the few species that can develop at least the first batch of eggs, and possibly subsequent batches, without a blood-meal the process is called autogenous development. The nutrients required for autogenous egg production are either derived from food reserves carried over from the last larval instar or from adults feeding on sugary secretions. The speed of digestion of the blood-meal depends on temperature, and in most tropical species takes only two to three days, but in colder, temperate countries blood digestion may take as long as 7-14 days.

After a blood-meal the mosquito's abdomen is dilated and bright red in colour, but some hours afterwards the abdomen becomes a much darker red. As the blood is digested and the white eggs in the ovaries enlarge the abdomen becomes whitish posteriorly and dark reddish anteriorly. This condition represents a mid-point in blood digestion and ovarian development and the mosquito is referred to as being half-gravid (figure 4.8). Eventually all blood is digested and the abdomen becomes dilated and whitish due to the formation of fully developed eggs (figure 4.8). The female is now said to be gravid and she searches for suitable larval habitats in which to lay her eggs. After oviposition the female mosquito takes another bloodmeal and after two to three days (in the tropics) a further batch of eggs is matured. This process of bloodfeeding and egg maturation followed by oviposition is repeated several times throughout the female's life, and is referred to as the gonotrophic cycle. Thus a female mosquito passes through the following physiological stages (i) unfed, (ii) blood-fed, (iii) half-gravid and (iv) gravid, during the completion of a single gonotrophic cycle. Some species need to take two blood-meals before the first batch of eggs can mature, thereafter only a single blood-meal is needed for egg production.

Although male mosquitoes have a conspicuous proboscis, their mandibles and maxillae are insufficiently developed for piercing the skin and bloodfeeding, instead they feed on nectar of flowers and other naturally occurring sugary secretions. Males are consequently unable to transmit any diseases to man. Sugar feeding is not, however, restricted to males,



Fig 4.8 Diagram representing the gonotrophic cycle of a female mosquito, starting with a female in an unfed condition and passing through a blood-fed, half-gravid and gravid condition, then after oviposition the female becomes unfed again ready for another blood-meal.

females may also feed on sugary substances to obtain energy for flight and dispersal, but only in a few species (the autogenous ones) is this type of food sufficient for egg development.

## Oviposition and biology of the eggs

Depending on the species, female mosquitoes lay about 30-300 eggs at any one oviposition. Eggs are brown or blackish, and in many Culicinae are elongate or approximately ovoid in shape but this varies considerably according to the species, while in the Anophelinae they are usually boat-shaped (figure 4.9a). In many mosquitoes, such as species of Anopheles, Culex and some Mansonia, the eggs are laid directly on the water surface. In Anopheles the eggs are laid singly and float on the water, whereas those of Culex and some Mansonia (subgenus Coquillettidia) are laid stuck together in 'egg rafts' which float on the water surface (figures 4.9c and 6.13). Eggs of none of these mosquitoes can survive desiccation, consequently if they become dry they die. In the tropics eggs hatch within about two to three days, but in cooler temperate countries they may not hatch until after about 7-14 days or longer.

Other mosquitoes such as those belonging to the genera, *Aedes*, *Psorophora* and *Haemagogus*, do not deposit their eggs on the water surface but lay them just beyond the water line on damp substrates, such as mud, leaf litter, on the inside walls of tree holes and clay pots (figure 4.9b). These eggs can withstand varying degrees of desiccation for periods of weeks, months, or even years, and still remain viable and capable of hatching when they are flooded with water. Because the eggs are laid above the water line of breeding places it may be many weeks or months before they are flooded with water and thus have the opportunity to hatch. However, even when flooded, the process of hatching may be extended over long periods because the eggs hatch in instalments, and sometimes *Aedes* and *Psorophora* eggs require repeated immersions in water followed by short periods of desiccation before they are induced to hatch. The eggs may also enter a state of diapause (see chapter 2, p. 16) and will not hatch until diapause is terminated (broken) by environmental stimuli such as a reduction in oxygen content of the water or changes in daylength. In temperate regions many *Aedes* and *Psorophora* species overwinter as diapausing eggs.

## Larval biology

Mosquito larvae can be distinguished from all other aquatic insects by being legless and having a bulbous thorax that is wider than both the head and abdomen. There are four active larval instars. All mosquito larvae require water in which to develop, no mosquito has larvae that can withstand desiccation although larvae may be able to survive short periods amongst wet mud etc.

All mosquito larvae have a well developed head which has a pair of antennae and a pair of compound eyes. Prominent mouth brushes are present in most species and serve to sweep water containing minute food particles into the mouth, but a few species have predacious larvae and in some of these the mouth brushes are used to seize their prey. The thorax is roundish in outline and has various simple and branched hairs which are usually long and conspicuous. The abdomen is distinctly segmented and has nine visible segments most of which have simple or branched hairs (figure 4.10). The last segment has two paired groups of long hairs forming the caudal setae (caudal brush) and in most species a larger group of hairs forming the ventral brush (figures 4.16 and 4.24). The last segment also has a sclerotised plate, called the saddle, which may



Fig 4.9 Mosquito eggs: (a) Anopheles, (b) Aedes and (c) egg raft of a Culex species.



Fig 4.10 Mosquito larvae at the water surface: (a) Anopheles larva lying parallel to the water surface taking in air through the spiracles situated on abdominal segment eight, and with the head rotated through 180° to be in a feeding position and (b) a culicine larva hanging down from the water surface at an angle, with air being taken in through the siphon.

completely encircle the segment, and two pairs of transparent sausage-shaped gills, which may be short or long, of equal or unequal lengths. Although called gills they are not concerned with respiration but with osmoregulation.

With the exception of Mansonia species (and a few other mosquitoes) mosquito larvae must come to the water surface to breathe. Atmospheric air is taken in through a pair of spiracles situated dorsally on the eighth abdominal segment. In the subfamilies Toxorhynchitinae and Culicinae these spiracles are situated at the end of a single dark-coloured and heavily sclerotised tube termed the siphon (figures 4.10b and 4.24). Mansonia larvae possess a specialised siphon that is more or less conical and pointed at the tip, it is supplied with prehensile hairs and serrated cutting structures (figure 6.15) that enable the siphon to be inserted into the roots or stems of aquatic plants. Oxygen for larval respiration is obtained from the plants. In contrast larvae of the Anophelinae do not have siphons (figures 4.10a and 4.16).

Mosquito larvae feed on yeasts, bacteria, protozoa and numerous other plant and animal microorganisms found in the water. Some, such as *Anopheles* species, are surface feeders, whereas many others browse over the bottom of habitats. A few mosquitoes are carnivorous and cannibalistic. There are four larval instars, and in tropical countries larval development, that is the time from egg hatching to pupation, can be as short as five to seven days but many species require about 7-14 days. In temperate areas the larval period may last several weeks or months, and several species overwinter as larvae.

Mosquito larval habitats vary from large and usually permanent collections of water, such as fresh water swamps, marshes, rice fields and borrow pits to smaller collections of temporary water such as small pools, puddles, water-filled car tracks, ditches, drains, gullys, a variety of 'natural container habitats' such as waterfilled rot holes in trees (that is tree holes), rock pools, hoof prints, water-filled bamboo stumps, bromeliads, pitcher plants, leaf axils in banana plants and pineapples and other plants, water-filled split coconut husks and snail shells. Larvae also occur in wells and 'man-made container habitats', such as clay pots, water-storage jars, tin cans, discarded kitchen utensils and motor vehicle tyres. Some species prefer shaded larval habitats, others like sunlit habitats, many species cannot survive in water polluted with organic debris, whereas others can breed prolifically in water contaminated with excreta or rotting vegetables and plants. A few mosquitoes breed almost exclusively in brackish or salt water, such as in salt water marshes and mangrove swamps, and are consequently restricted mostly to coastal areas. Some species are less specific in their requirements and can tolerate a wide range of different types of breeding places.

Almost any collection of permanent or temporary water can constitute a mosquito larval habitat, but larvae are usually absent from large expanses of uninterrupted water such as lakes, especially if they have large numbers of fish and other predators which are likely to eat mosquito larvae. They are also usually absent from large rivers and fast flowing waters, except that they may occur in marshy areas and isolated pools and puddles formed at the edges of flowing water.

## **Pupal biology**

All mosquito pupae are aquatic and comma-shaped. The head and thorax are combined to form the cephalothorax which has dorsally a pair of respiratory



Fig 4.11 A pupa of an Anopheles mosquito.

trumpets (figure 4.11). The abdomen consists of ten segments but only eight are visible. Each segment has numerous short hairs and the last segment terminates in a pair of oval and flattened structures termed the paddles (figures 4.11 and 4.12). In between the paddles is a small pouch-like projection containing the developing external genital processes of the adults; in female pupae this consists of the cerci and the pouch is quite small (figure 4.12b) but in male pupae the pouch is bigger because it contains the claspers of the male genitalia (figure 4.12a). Mosquito pupae can thus be



Fig 4.12 Terminal abdominal segments of mosquito pupae: (a) a male pupa showing large genital pouch and (b) female pupa showing smaller genital pouch in front of paired paddles.

readily sexed by examining the end of the abdomen. Some of the developing structures of the adult mosquito can be seen through the integument of the cephalothorax, the most conspicuous features being a pair of dark compound eyes, folded wings, legs and the proboscis (figure 4.11). Pupae do not feed but spend most of their time at the water surface taking in air through the respiratory trumpets. Unlike the pupae of most other Diptera they are capable of active movements. If disturbed they alternately flex and extend the abdomen and this movement aided by their paddles enables them to swim up and down in the water in a jerky fashion.

Pupae of *Mansonia* are different in that they have relatively long breathing trumpets which are modified to enable them to pierce aquatic vegetation and thus obtain their oxygen in a similar fashion to the larvae (figure 6.15). As a consequence they remain submerged and rarely come to the water surface.

In the tropics the pupal period in mosquitoes lasts only two to three days, but in cooler temperate regions pupal development may be extended over 9-12 days or longer. At the end of pupal life the skin on the dorsal surface of the cephalothorax splits and the adult mosquito struggles out.

## Adult biology and behaviour

After adults have emerged from the pupae they rest either on the water surface or more usually on nearby vegetation for about an hour to allow their wings to unfold and harden sufficiently for them to fly. Mating usually occurs near breeding places and relatively soon after emergence. In some, but not all species, males form swarms and females enter these swarms to mate and become inseminated. Both adult emergence and mating usually occur early in the evening, commonly around dusk.

As already mentioned (p. 26) females of most species of mosquito require a blood-meal, either before or more usually after mating, before the eggs can develop. Many species bite man to obtain their blood-meals and a few may feed on man in preference to any other animals, but others prefer feeding on non-human animals, and many never bite man. Species that usually bite man are said to be anthropophilic in their feeding habits, while those that feed mainly on other animals (mammals, amphibia, reptiles or birds) are called zoophilic. Mosquitoes that feed on birds are sometimes called ornithophilic instead of zoophilic. Females are attracted to hosts by various attractive stimuli emanating from them, such as body odours, carbon dioxide and heat. In most species vision appears to play a minor role in host orientation but some species are attracted by the silhouette or movement of their hosts. Some species feed more or less indiscriminately at any time of the day or night, but others are mainly diurnal or nocturnal in their biting habits.

A few species of mosquitoes frequently enter houses to feed on man and are said to be endophagic in their feeding habits, whereas those that bite their hosts outside houses are called exophagic. After having

bitten man or animals, either inside or outside houses, some species rest inside houses during the time required for blood digestion and maturation of the ovaries, and they are called endophilic. In contrast mosquitoes that have fed either outdoors or inside houses and rest afterwards outdoors are termed exophilic. Female adults of Aedes aegypti (vector of yellow fever) for example are usually anthropophilic, exophagic and exophilic, whereas adults of Anopheles gambiae\* (African malaria vector) are mainly anthropophilic, endophagic and endophilic. Few mosquitoes, however, are entirely anthropophilic or zoophilic, endophagic or exophagic, endophilic or exophilic, instead most show various degrees of these behavioural patterns, in other words all these terms are relative. Moreover, the feeding behaviour of a species may change. For example, in certain areas and at certain seasons a species may bite man predominantly (anthropophilic) inside houses (endophagic) and remain in houses afterwards (endophilic), whereas at other times, especially if there are few people but many animals in the area, the species may become predominantly zoophilic and also exophagic and exophilic. Some species, however, are less adaptable in their feeding behaviour and for example will never rest inside houses or enter them to feed on man, and many mosquitoes that feed on birds will rarely bite man as an alternative host.

The biting behaviour of female mosquitoes may be very important in the epidemiology of disease transmission. For example, mosquitoes that feed on man predominantly out of doors and late at night will not bite many young children because they will be indoors asleep at this time. Thus, young children will be less likely infected with any diseases these mosquitoes might be transmitting. During hot and dry periods of the year substantial numbers of people may sleep out of doors and as a consequence be bitten more frequently by exophagic mosquitoes than during cool or wet seasons when people remain outdoors only during the early night or evening. Some mosquitoes bite predominantly within forests or wooded areas, consequently man will only get bitten when he visits these places. Clearly the behaviour of both people and mosquitoes may be relevant in disease transmission.

The resting behaviour of adult female mosquitoes may be an important consideration in control measures. In many malaria control campaigns the interior surfaces of houses such as walls and ceilings are sprayed with residual insecticides such as DDT to kill adult mosquitoes resting on them. This approach will, of course, only be effective in controlling malaria if the mosquito vectors rest indoors.

Mosquitoes usually disperse less than about 2 km

and most only fly a few hundred metres from their breeding places, but a few species, principally those inhabiting temperate and cold northern climates, may disperse much further, especially just after emergence. Mosquitoes sometimes get transported long distances by wind.

In tropical countries adult mosquitoes probably live on average only about two to three weeks, but in temperate climates the average life may be four to six weeks or longer, and species which overwinter as fertilised and hibernating adults live for many months. It seems that males usually have a shorter life-span.

## Classification of mosquitoes into the subfamilies Toxorhynchitinae, Anophelinae and Culicinae

## Subfamily Toxorhynchitinae

This subfamily contains only one genus, Toxorhynchites, which in older classifications was called Megharinus and the subfamily name was Megharininae. The genus contains about 65 species which are found in the tropics of Asia, Africa, Central and South America and also eastern parts of the U.S.A. and coastal areas of Russia and Japan.

Adults are large and colourful mosquitoes, being metallic bluish or greenish with orange and red tufts of hairs on some abdominal segments. Adults are easily recognised by the possession of a proboscis that is recurved in both sexes and incapable of piercing the skin to take blood-meals (figure 4.13). Consequently, since neither sex can bite they are of no medical importance. They are day-fliers and feed on natural sugary secretions. Their larvae are large and stout and often dark reddish in colour and, like the Culicinae, have a siphon. They can best be recognised by their mouth brushes which are composed of only about ten flattened, non-pectinate, stout setae, instead of a large number of pectinate setae as possessed by most culicine larvae. Larvae are predacious on larvae of other mosquitoes and on their own kind. They have occasionally been introduced into areas in the hope that their predacious habits will help reduce the numbers of pest mosquitoes. They are mainly found in natural container habitats (for example tree holes, pitcher plants, bamboo stumps) and man-made containers (for example tin cans, pots).

The two most important subfamilies of mosquitoes are the Anophelinae (anophelines) and Culicinae (culicines), both contain man-biting species that are important disease vectors, and it is important to be able to distinguish between these two subfamilies.



Fig 4.13 Head of Toxorhynchites adults showing curved proboscis of (a) a female and (b) a male.

## Subfamily Anophelinae

Adult Anophelinae (anophelines) have the ventral surface (sternites) of the abdomen entirely or mostly devoid of scales, and these are usually also absent from the dorsal surface (tergites).

Only three genera are included in the subfamily. The subgenus *Bironella* occurs only in Australia, New Guinea, Melanesia and Moluccas, and adults can be identified by the wavy appearance of vein four and the upper branch of vein five; larvae have two pairs (not one pair as in *Anopheles*) of palmate hairs on the thorax. *Chagasia* species are found only in Central and South America. They can be identified by having the scutellum trilobed (not rounded posteriorly as in *Anopheles*); larvae can be distinguished from other anophelines by the peculiar shape of the abdominal palmate hairs (figure 4.14a). Neither of these two genera are of any medical importance.



## Anopheline eggs

Eggs are laid singly on the water surface. In most species they are typically boat-shaped and laterally have a pair of floats (figures 4.9a and 4.15) which in



Fig 4.15 Dorsal and lateral views of an egg of an Anopheles.

Fig 4.14 Abdominal palmate hairs from larva of (a) Chagasia species and (b) Anopheles species.

The third and most important genus is *Anopheles*, which contains important malaria vectors. The following characters can identify and separate this genus from any of the 30 genera of the Culicinae. some species such as *An. pseudopunctipennis* can completely surround the egg. They are unable to withstand desiccation.

## Anopheline larvae

Larvae lack a siphon, with the result that when they are at the water surface they lie parallel to it (figure 4.10a)



Fig 4.16 Dorsal view of an Anopheles larva.

and are not subtended at an angle as are the Culicinae. Larvae are surface feeders and spend most of their time at the water surface. Examination under a microscope shows that the abdomen has small, brown, sclerotised plates, called tergal plates, on the dorsal surface of abdominal tergites one to eight, in addition most or all of these segments have a pair of well developed palmate hairs, sometimes called float hairs (figure 4.14b and 4.16). These abdominal palmate hairs and the



Fig 4.17 Lateral view of the terminal segments of an Anopheles larva.



Fig 4.18 Pupa of an Anopheles, (a) showing short and broad respiratory trumpet and (b) abdominal segments showing well developed spines at the corners.

single pair on the thorax come into contact with the water surface and aid in keeping the larvae parallel to the surface. On either side of abdominal segment eight there is a group of spines which are joined together at their base to form a structure called the pecten, which is best seen in lateral view (figure 4.17). All these structures identify larvae as belonging to the genus Anopheles.

## Anopheline pupae

The respiratory trumpets are short and broad distally, thus appearing conical (figures 4.11 and 4.18a) whereas in most Culicinae the trumpets are narrower and more cylindrical. The most reliable characteristic for identifying anopheline pupae is the presence of short peglike spines situated laterally near the distal margins of abdominal segments two or three to seven (figure 4.18b). In the culicines there are no such spines.

## Anopheline adults

Adult Anopheles usually rest with the body at an angle to the surface, that is with proboscis and abdomen in a straight line (figure 4.19). In some species they rest at almost right angles to the surface, whereas in others, such as *An. culicifacies*, the angle is much smaller. This is a very useful characteristic allowing adults resting in houses and elsewhere to be readily identified as *Anopheles*. Most, but not all *Anopheles* mosquitoes, have the dark (usually blackish) and pale (usually whitish or creamy white) scales on the wing veins arranged in 'blocks' or specific areas (figure 4.20) to form a distinctive spotted pattern, which differs according to the species. A few species, however, have the veins covered more or less uniformly with dark (often brown) scales.

The most reliable way to distinguish between adult Anopheles and Culicinae is by examination of their heads. The first procedure is to determine the sex of the adults; female mosquitoes have non-plumose antennae, while males have plumose antennae. If the adults are females and also Anopheles then the palps will be about as long as the proboscis and usually lie closely alongside it (figure 4.21), and may be marked, especially the apical half, with broad or narrow rings of pale scales. In male Anopheles the palps are also about as long as the proboscis but are distinctly swollen at the ends and are said to resemble 'Indian clubs' (figure 4.21). They may also have rings of pale scales apically.



Fig 4.19 Resting positions on a wall of an anopheline and a culicine adult.



Fig 4.20 Wing of an Anopheles species showing the characteristic arrangement of dark and light scales in distinct 'blocks' on the veins.



Fig 4.21 Diagram of the heads of adult male and female anophelines and culicines to show differences in the form of the palps. The palps usually lie close to either side of the proboscis but are illustrated here as pulled away from it for a clearer presentation.

In male *Anopheles* the ends of the palps sometimes bend outwards away from the proboscis.

Other minor differences between *Anopheles* and the Culicinae are that in the *Anopheles* the scutellum in both sexes is rounded posteriorly and has setae along the entire edge (figure 4.3b), only one spermatheca is present in the females, and in both sexes the middle lobe of the salivary glands is considerably shorter than the two outer lobes (figure 4.22a).



Fig 4.22 Salivary glands from adult mosquitoes: (a) Anopheles species showing short middle lobe and (b) culicine mosquito showing all lobes more or less equal in size.

The principal characters for separating the various stages in the life-cycles of anopheline and culicine mosquitoes are given in table 4.1.

#### Subfamily Culicinae

There are 30 genera in this subfamily, the most important medically are Aedes, Culex, Mansonia, Haemagogus, Sabethes and Psorophora. The following characters serve to separate the Culicinae from the Anopheles mosquitoes. Methods for distinguishing between the more important genera within the Culicinae are given in chapter 6.

## Culicine eggs

Eggs never have floats, they are laid either singly (for example *Aedes*) or in the form of egg rafts that float on the water surface (for example *Culex* and some *Mansonia*) or are glued together as sticky masses that are stuck to the undersides of floating vegetation (some *Mansonia*) (figures 4.9, 4.23, 6.13 and 6.14).

Table 4.1 Guide to the separation of mosquitoes into subfamilies Anophelinae and Culicinae

Stage	Anophelinae	Culicinae
Eggs	Laid singly, possess floats.	Laid singly or in egg rafts or masses. Never possess floats.
Larvae	Never have a siphon. Lie para- llel to water surface. Have ab- dominal palmate hairs and ter- gal plates.	All larvae have a short or long siphon. Subtend an angle from the water surface. No palmate hairs or tergal plates.
Pupae	Breathing trumpets short and broad apically. Short peg-like abdominal spines on segments two or three to seven.	Breathing trumpet short or long, opening not broad. No spines or abdominal segments three to seven.
Adults (both sexes)	Rest at an angle to any surface. In most species dark and pale scales on wing veins arranged in distinct 'blocks'.	Rest with the bodies more or less parallel to the surface. Scales on wing veins not arranged in 'blocks', scales frequently all brown or blackish or a mixture of pale and dark scales scattered on veins.
Adult females (non-plumose antennae)	Palps about as long as proboscis.	Palps much shorter than proboscis.
Adult males (plu- mose antennae)	Palps about as long as proboscis and swollen at ends.	Palps about as long as proboscis, but never swollen at ends, but may be hairy distally.



Aedes

Fig 4.23 Mosquito eggs: Culex egg raft floating on water surface, Mansonia (Mansonioides) eggs glued to undersurface of a leaf of a floating aquatic plant and individual eggs of Aedes species which are deposited on damp surfaces.



Fig 4.24 Dorsal view of a culicine larva with segments seven to nine turned in a lateral position to show siphon, comb and other structures.

## Culicine larvae

All culicine larvae possess a siphon (figure 4.24), which may be long or short. They hang upside down and at an angle from the water surface when they are getting air (figure 4.10b) except, however, *Mansonia* larvae which insert their specialised siphons into aquatic plants and remain submerged (figure 6.15).

There are no abdominal palmate hairs or tergal plates on culicine larvae. The pecten teeth present on the culicine siphon, should not be confused with the pecten or pecten plate found in *Anopheles* larvae (figure 4.17), they are different structures, which unfortunately have the same name.

## Culicine pupae

The length of the respiratory trumpets is variable, but the trumpets are generally longer, more cylindrical and their openings narrower (figure 4.25) than in *Anopheles*. Abdominal segments two to seven lack peglike spines, although they have numerous setae (figure 4.26).



Fig 4.25 Pupa of a culicine, (a) showing an elongated and relatively narrow respiratory trumpet and (b) position at the water surface.



Fig 4.26 Abdominal segments of a culicine pupa showing hairlike setae at corners of the segments.

## Culicine adults

In life adults rest on surfaces with the thorax and abdomen more or less parallel to the surface, only the proboscis may form a slight angle with the surface (figure 4.19). The wing veins are often covered with scales of a uniform brown or black colour, sometimes, however, there are contrasting dark and pale scales but they are not usually arranged in distinctive areas or 'blocks' as found in many *Anopheles* adults.

The most reliable method for identifying the Culicinae is to examine their heads. In females (which have non-plumose antennae) the palps are shorter than the proboscis; in males (which have plumose antennae) the palps are about as long as the proboscis but are not swollen distally and hence do not appear clubbed (figure 4.21). They may, however, be turned upwards distally, and in many species they are covered with long hairs, so that superficially they can appear to be somewhat swollen distally, but more careful inspection shows that the palps in male culicines are not clubbed.

Other minor differences that separate the Culicinae from *Anopheles* are that in culicines the scutellum is trilobed and the scutellar setae are restricted to these lobes (figure 4.3), there are two to three spermathecae in the females, and the middle lobe of the salivary glands is about as long as the outer two lobes (figure 4.22).

## Medical importance of mosquitoes

Although in many temperate countries mosquitoes may be of little or no importance as disease vectors they can nevertheless cause considerable annoyance due to their bites. The greatest numbers of mosquitoes are found in the northern areas of the temperate regions especially near or within the arctic circle, where the numbers biting can be so great at certain times of the year as to make almost any outdoor activities impossible. Because of their elongated mouthparts female mosquitoes have little difficulty in biting through clothing such as socks, shirts, trousers and woollen garments, but clothing with a much closer weave of material may prevent this.

The diseases mosquitoes transmit to man are described in chapters 5 and 6.

## Mosquito control

Greater efforts have been made to control mosquitoes than any other biting insects and a vast literature has accumulated on control operations.

Control measures which are directed against specific vectors, such as *Aedes aegypti*, *Culex pipiens fatigans* and the malaria vectors, are described in more detail in chapters 5 and 6, only the broader principles of control are outlined here.

Control measures can be directed at either the immature aquatic stages or the adults, or at both stages simultaneously.

## Control directed at the immature stages

## **Biological (naturalist) control methods**

Although often termed naturalist control there is little if anything natural about the process, since either the incidence of predators, parasites or pathogens in any habitat must be greatly increased to obtain worthwhile control, or they have to be introduced into habitats from which they were originally absent. Such environmental manipulation is not natural. Because biological control methods (biocontrol) do not cause any chemical pollution they are sometimes advocated as a better approach than insecticidal methods, but they are usually more difficult to implement and maintain. Moreover, if predators are used it is unlikely that they will prey exclusively on mosquito larvae and pupae but also eat harmless or beneficial insects, and contrary to earlier beliefs it seems that resistance may be developed to some mosquito pathogens.

Predators Several attempts have been made to control mosquitoes by using predators. The most commonly used predators are fish, and species in several genera such as Lebistes, Poecilia, Sarotherodon ( = Tilapia), Panchax, have been used, but two subspecies of Gambusia, namely G. affinis affinis and G. affinis holbrooki, commonly referred to as 'mosquito fish' have been employed more extensively than any others. Originating in the U.S.A. Gambusia have been introduced into the Pacific Islands, Europe, the Middle East, south-east Asia and Africa in attempts to control mosquito larvae. Some predatory fish can breed in saline waters and can therefore be introduced into salt water habitats. Fish are unsuitable for the control of mosquitoes which breed in small containers or small pools and puddles which rapidly dry out. However, some fish such as species of Nothobranchius and Cynolebias which are the so called 'instant' or 'annual' fish have drought resistant eggs and these are more suitable for introducing into small temporary habitats that repeatedly dry out. Polluted waters are also usually unsuitable habitats for most fish.

Although fish have sometimes greatly reduced the numbers of larvae in certain habitats, such as borrow pits, ponds and rice fields, they have not proved effective in reducing the size of mosquito populations over large areas, nor have they significantly decreased the incidence of mosquito-borne diseases.

Other predators of mosquito larvae and pupae include tadpoles (larvae) of frogs and toads and various aquatic insect larvae, but these have rarely proved effective control agents. A few mosquitoes have predacious larvae, for example *Toxorhynchites* species, and these have been introduced into container habitats in certain areas (for example Fiji, Samoa, Hawaii) to control larvae of other container-breeding mosquitoes. Results have not been very encouraging.

Pathogens There are numerous pathogens, such as viruses (for example iridescent and cytoplasmic polyhedrosis viruses), bacteria (for example Bacillus thuringiensis and B. sphaericus), protozoa (for example Nosema, Thelohania) and fungi (for example Coelomomyces, Lagenidium, Culicinomyces) that cause larval mortality. There are also several parasitic nematodes that kill mosquito larvae, and the most promising for control purposes are Reesimermis nielseni and more importantly Romanomermis culicivorax, which has in the past been misidentified as R. nielseni which appears to be more specific in its hosts.

These parasites and pathogens appear harmless to man. Although there is interest in the possibilities of mass rearing or cultivating parasitic nematodes and various pathogens, especially *Bacillus thuringiensis*, to control mosquitoes, investigations and trials are still mainly at the experimental stage. A useful compilation of pathogens of mosquitoes and other medically important arthropods has been published by Roberts and Strand (1977) and is given in the references at the end of the book.

## Genetic methods

One technique is to colonise in the laboratory large numbers of mosquitoes whose genetic make-up has been altered. For example, genetic manipulation and selective rearing can produce mosquitoes that are refractory to infection with human diseases, such as malaria or filaria, and then these are released into the environment in the hope that they may successfully compete with the natural population of susceptible ones and eventually replace them. It may be possible to combine refractiveness with insecticide resistance so that if populations are sprayed after their release the refractory individuals will survive whereas the natural non-refractory population will be reduced in size. Another possibility is the replacement of a vector mosquito with a non-vector species which in the field will compete with the vector and eventually replace it. This is known as species replacement. Other methods aim to select genes that cause large distortions in the sex ratio, so that excessive numbers of male mosquitoes are produced. Not only are males non-vectors, but introducing a distorted sex ratio gene into populations should lead to reductions in population size.

Sterile-male release techniques involve the production and release into the field of male mosquitoes that are sterile. Sterilisation can be achieved in the laboratory by several methods, such as radiochemical irradiation, use of chemosterilants (for example apholate, hempa, tepa, metepa) or production of infertile hybrid males by crossing closely related species. The aim is to introduce these sterile males into wild populations to compete with natural fertile males for female mates and so result in an increased proportion of infertile inseminations. The eggs produced will be sterile and fail to hatch, thus causing a reduction, and hopefully the elimination, of the species.

Genetical methods of control can be aimed at either substantially reducing the size of the vector population, or replacing it with a non-vector species or strain.

None of these genetic methods is simple and they may prove more difficult to implement than more conventional insecticidal measures. There have been a few limited field trials, some of which have shown promise, but genetic control of mosquitoes is still only in the experimental stage. The main advantage of this approach is that it overcomes the increasing problems of insecticide resistance which is fast developing in many mosquito species, and there is no contamination of the environment with insecticides, – but there are also difficulties. Advanced technology and expertise is required, also the ability to rear vast numbers of mosquitoes to be released into the field to compete with or replace natural populations. Not all the important vector mosquitoes can be reared in the laboratory, let alone on an enormous scale. Moreover, it is essential that reared mosquitoes live as long and are as healthy and vigorous as wild populations, otherwise they will be unable to compete with them and result in their elimination. It is also possible that females may evolve a mechanism to avoid mating with sterile males. Another problem is that there may evolve changes in mosquito-parasite relationships, so that the parasites (malaria, filaria) are able to develop in strains of mosquitoes that were originally selected for their refractiveness to infection.

# Physical (mechanical or environmental) control methods

Filling in, source reduction and drainage A simple form of control consists of filling in and thus completely eradicating breeding places. For example, larval habitats ranging in size from water-filled tree holes, ponds to small marshes can be filled in with rubble, earth, sand etc. Other container habitats such as abandoned cans, metal drums, canoes, pots and tyres can be removed and the breeding sources greatly reduced or eliminated. Mosquito breeding in containers which are used to collect and maintain drinking water, such as water-storage jars and village pots, could be minimised by covering up their openings, but this is seldom very popular with the owners and the practice becomes neglected. In some areas the introduction of a reliable piped water supply should help reduce the dependence on water-storage containers, and thereby reduce breeding of species such as Aedes aegypti. Some species breed in septic tanks, faulty soak-away pits, large water-storage tanks and cisterns, but this type of breeding can be prevented if existing covers are repaired or new ones fitted.

Larval breeding places such as ponds, borrow pits, fresh and salt water marshes can be drained. An important advantage of filling in, draining or removing larval habitats is that such measures can lead to permanent control, but this approach is not always feasible. It is impossible, for example, to fill in all scattered, small and temporary collections of water such as pools, vehicle tracks and puddles which may appear during the rainy season. Larger and more permanent habitats, such as swamps, may prove too costly to drain. Moreover, the local people may understandably not want certain breeding places such as rice fields, ponds which may be used as fish farms, and borrow pits providing drinking water for man and his livestock, to be either drained or filled in; such habitats are an essential part of life. In some countries large collections of water may be used for recreation such as boating or fishing, while wet and marshy areas may be important nature conservation sites, consequently there will be objections to eliminating these habitats even if they constitute mosquito breeding places. In all instances the feasibility of eradicating breeding places must be assessed individually.

Habitat changes If it is impossible to eliminate aquatic habitats it may be possible to alter them to make them unsuitable for mosquito breeding. For example, many mosquitoes breed in small and more or less isolated pools or marshy areas that commonly form at the edges of streams, especially when these have a twisting and winding course. By increasing the water flow rate, straightening and steepening the banks of streams and possibly slightly altering their course to prevent the formation of small pools, breeding can be greatly reduced.

Shallow ponds that have sloping edges and a fluctuating water level that results in exposed muddy areas may provide ideal breeding sites for many *Aedes* and *Psorophora* mosquitoes, because they lay their eggs not on the water surface but on exposed muddy and waterlogged soil. Mosquito breeding can be prevented in these habitats by changing the sloping shore line to one that has well demarcated and steep, almost vertical, banks. Although the water level may still fluctuate it will no longer expose large marshy and muddy areas, consequently breeding by *Aedes* and *Psorophora* mosquitoes will be eliminated or greatly reduced.

Ecological changes can also prevent mosquito breeding. For example, cutting down overhanging vegetation to increase sunlight on the water may prevent breeding by species that like shaded habitats, whereas planting vegetation near water may stop breeding by sun-loving species. Similarly, the removal of rooted or floating vegetation may result in reducing mosquito breeding, especially by species of *Mansonia* which require plants to get their oxygen requirements, on the other hand the presence of a dense coverage of vegetation can prevent breeding by species that prefer exposed water surfaces.

Impoundments An alternative to draining marshy areas is to excavate them to form areas of deep water with well defined vertical banks. This process is called impoundment. It completely alters the habitat and makes it unsuitable for mosquitoes which were previously breeding in either numerous small pools scattered over extensive marshy areas or larger expanses of shallow water having dense growths of vegetation. Both small and large, fresh and salt water marshy areas can be converted to impounded waters.

Whenever an aquatic habitat is not eliminated but

modified to prevent breeding of certain mosquitoes there is the danger that such environmental changes will favour the breeding of other species that were previously either absent or present in only small numbers.

## Chemical control methods

Paris green One of the earlier chemicals used to prevent mosquito breeding was Paris green, a copper acetoarsenite, which was applied to the water surface as a very fine dust at the rate of about 1 kg/ha. After ingestion by mosquito larvae during filter feeding it acted mainly as a stomach poison. It was particularly effective against surface feeding mosquitoes such as Anopheles. More recently Paris green has been incorporated into small granules of vermiculite or bentonite which float on the water surface, and sand granules formulated as small pellets which sink to the bottom. This latter formulation is particularly effective against mosquito larvae that feed on the bottom, and also in shallow water against surface-feeders. Granular and pellet formulations are better at penetrating dense covers of aquatic vegetation than dusts and are therefore more suited to habitats with vegetation.

Oils Another method of controlling mosquito larvae is by the application of mineral oils to the water. Originally diesel oils, fuel oils or kerosene (paraffin), sometimes mixed with filtered oils from motor vehicles, were sprayed on to water. Larvae are killed not so much by the oils physically blocking their tracheae and suffocating them, but due to a combination of the fumigant effects of the oil's toxic aromatic hydrocarbons and by the more stable fraction of the oils interfering with air intake at the water surface. About 300-500 litres of oil per hectare are needed because of the poor spreading power of these oils, although the addition of castor oil helps increase this. The spreading power of oils is referred to as the spreading pressure, kerosene for example has a value of about 8-13dynes/cm, but a good larvicidal oil should have a spreading pressure of at least 23 dynes /cm, and if used on areas with much emergent vegetation which must be penetrated a spreading pressure of about 40 dynes/cm may be needed. Specially prepared commercial oils (for example Flit MLO, ARCO larvicide, Malariol HS) containing spreading agents have been introduced which have much better spreading powers, so that only about 50-100 or even only 10-20 litres /ha are needed.

Larval habitats have to be treated with chemicals such as Paris green or oils about every seven to ten days in most tropical areas to ensure that larvae hatching from eggs are killed before they pupate and give rise to adults. Less frequent applications can be made in cooler temperate areas because the speed of development of the immature stages of the mosquito is much slower.

Residual insecticides The advent in the 1940s of residual insecticides such as DDT and other organochlorines such as HCH and dieldrin and later the organophosphate and carbamate insecticides, ushered in a new phase of mosquito control. The use of Paris green and oils was more or less abandoned because much better control was achieved by spraying breeding places with these insecticides, either in oil solutions or more frequently as emulsions. If emulsions are used the insecticide is dissolved in organic solvents such as xylene, diesel or fuel oils or kerosene, containing small quantities of special emulsifying additives such as Triton. Only relatively small quantities of the concentrated oil solutions are carried to the field, because before spraying a small quantity of the insecticidal solution is vigorously mixed with water in the sprayer. The resultant emulsion which is formed is sprayed on to the water at rates varying from 15-45 litres/ha. The small droplets which fall on to the water surface separate out into water and globules of oil containing the insecticide which coalesce to form a very thin film on the water surface.

A set-back in the use of organochlorine larvicides is the development by some mosquitoes of insecticide resistance. Another difficulty is their extreme persistence in the soil and in animal and plant tissues which makes them biological pollutants. The World Health Organization no longer recommends their use as mosquito larvicides and some countries have banned their use on aquatic habitats. In their place, the less persistent and biodegradable organophosphate and carbamate insecticides are now recommended to kill mosquito larvae. The organochlorines, however, can still be used for residual spraying of houses (pp. 42-43, 51-52 and 201).

The insecticides commonly used for controlling mosquito larvae include organophosphates such as temephos (Abate), fenthion (Baytex), malathion and chlorpyrifos (Dursban). The latter is more toxic to most larvae than Abate but causes higher mortalities amongst fish and other aquatic life, and is consequently not so widely used.

Application procedures and dosage rates Liquid formulations do not easily penetrate dense growths of aquatic vegetation, better penetration is achieved if insecticides are applied as granules or pellets. Some granules are formulated to sink to the bottom of breeding places, whereas others float on the water surface, and some are made for rapid release of toxicants, others for a slower release. Gelatin capsules containing oil solutions of insecticides are also available. They are dispersed by hand or machine and float on the surface of breeding places where the gelatin envelope dissolves to release the insecticidal contents.

Insecticides for larval control are often sprayed from knapsack-type sprayers carried on the back of operators, but they can also be dispersed from various insecticidal spraying machines mounted on the back of landrovers or other suitable vehicles. Sometimes, especially if large and/or inaccessible areas require spraying, aerial applications are made from helicopters or light aircraft.

The dosage rate per hectare varies according to the insecticide, the mosquito species, and the method of application. For example, ground application rates are usually two to four times greater than for aerial applications because of less efficient application which does not always result in adequate coverage. Dosage rates usually have to be increased if the water is highly polluted or contains substantial amounts of vegetation.

Although organochlorine, organophosphate and carbamate insecticides are known as residual insecticides they have very little residual effect when used as mosquito larvicides, consequently they must be sprayed on larval habitats about as frequently as oils, that is every seven to ten days in most tropical areas.

Alternative insecticides and chemicals Other insecticides and compounds used in mosquito control include natural and synthetic pyrethrum compounds, other organophosphates such as dichlorvos (DDVP), organic thiocyanates such as butyl carbitol (Lethane 384) and carbamates such as carbaryl (Sevin).

Less orthodox chemicals include the synthetic insect juvenile and moulting hormones and insect growth regulators, such as Altosid, Dimilin and farnesol which interfere at some stages with the growth and metamorphosis of the immature stages of mosquitoes. Other compounds include long-chain aliphatic amines, various wetting agents, surfactants and lecithin-type substances which interfere with larval and pupal respiration at the water surface. These compounds are at mainly the experimental stages of development and evaluation.

*Eggs and pupae* Most insecticides will not kill mosquito eggs, nor will their presence in the water deter gravid females from ovipositing. Chemical control measures against the immature stages of mosquitoes are mainly directed at killing larvae; relatively few insecticides are very efficient at killing mosquito pupae. However, because of the short duration of the pupal life this is not usually a serious disadvantage.

## Integrated control

It has become fashionable to advocate integrated control, which usually means combining biological

and insecticidal methods. For example, the introduction of predacious fish to breeding places which are also sprayed with insecticides that have minimum effect on the fish. However, it is better to regard integrated control as any approach that takes into consideration more than one method, whether these are directed at only the larvae or adults, or both.

## Control directed at adults

## **Personal** protection

Much can be done to reduce the likelihood of being bitten by mosquitoes. Houses, hospitals and other buildings can have windows, doors and ventilators covered with mosquito screening made either of strong plastic or non-corrosive metal, but it is essential that it is kept in good repair. Screens with 18-20 meshes per inch will exclude most mosquitoes, closer mesh screening will keep out smaller biting flies some of which may be vectors, but it will appreciably reduce ventilation and light. Screening in most cases need only be extended to about the second or third floors of buildings because few mosquitoes will fly higher than this to enter buildings. If houses are unscreened, or if screening is defective, mosquito nets with 23-26 meshes per inch can be used. These should be tucked in under the mattress or bedding, never allowed to drape loosely over the bed. Torn nets are useless. Nets should be placed over beds before sunset. The main disadvantage of nets is that they reduce ventilation.

Small spray guns (for example Flit-guns) filled either with pyrethrum dissolved in kerosene or some other knock-down insecticides can be used early each evening in rooms to kill resting mosquitoes that may later try to bite the occupants. Small canisters of aerosols containing a fast-acting knock-down insecticide such as natural or synthetic pyrethrum or dichlorvos are widely sold for killing houseflies and other obnoxious insects including mosquitoes. When used properly they can be an effective, but costly, method of killing mosquitoes. Residual insecticides, such as DDT, HCH and malathion, are sometimes added to aerosols to prevent insects knocked down by pyrethrum from recovering, but their presence does not impart any appreciable residual effects and aerosols have to be used repeatedly.

Suitable insect repellents, in the forms of oils, lotions, creams or aerosols, can provide temporary protection. The best commercially available repellents contain one or more of the following chemicals, butyl mesityloxide oxalate (Indalone), diethytoluamide (DET), dimethyl phthalate (DMP), ethyl hexanediol and chloro-diethylbenzamide. Oil formulations are often better than creams or lotions although the latter may be cosmetically more acceptable. Repellents are normally applied to all exposed areas of skin, such as wrists, arms, hands, neck and face taking care to avoid the eyes otherwise severe irritation and inflammation may occur. They should also be applied to the ankles even if socks are worn. Mosquitoes may not, however, be deterred from biting through clothing. To prevent this, clothing may be lightly sprayed with suitable repellents such as diethyltoluamide and butyl ethylpropanediol, but the latter should not be applied directly to the skin. Normally, repellents remain effective for only about two hours, but clothing specially impregnated with repellents may remain effective for several weeks or months.

## Aerosols, mists and fogs

Oil solutions or emulsions of insecticides can be introduced into a cold high velocity air stream generated by spraying machines to produce mists, fogs or aerosols of insecticide. Alternatively, oil formulations of insecticides can be introduced into special heated chambers or into the hot exhaust fumes of spraying machines for vaporisation and the production of thermal fogs or aerosols. Usually the term mist is applied to formulations that are composed of droplets with a volume median diameter (vmd) of about 50-100  $\mu$ m, and aerosols and fogs consist of droplets with a vmd of less than  $50 \mu m$ . The term vmd refers to the median droplet size of a spray, in other words one half of the volume of the spray is composed of droplets larger than this while the other half consists of smaller droplets.

Fogging and aerosol producing machines can be mounted on vehicles or on aeroplanes and helicopters. Insecticidal mists or fogs are usually used to kill adult mosquitoes, especially those resting in vegetation or other areas that cannot be reached by more conventional methods, but indoor resting adults may also be killed.

Many different insecticides can be used, but cold aerosol generators are usually employed with natural pyrethrums and synthetic pyrethroids such as bioresmethrin because they may not be stable in the high temperatures produced by thermal foggers. Because of this, and also other reasons, cold aerosol generators and cold foggers are increasingly replacing thermal foggers. Whatever insecticide is used there is very little residual effect. Consequently although resting mosquitoes are killed, the sprayed area may become rapidly reinvaded by mosquitoes flying in from adjacent areas or by newly emerged individuals originating from breeding sites within the area. Repetitive applications may therefore be needed before the mosquitoes in an area are controlled. Aerosol applications are often combined with other control measures, such as larviciding and the spraying of houses with residual insecticides.

Ultra-Low-Volume (ULV) applications The main bulk of any liquid insecticidal formulation consists of the diluent or solvent, the actual amount of the active insecticide is small. Greater efficiency can be achieved if concentrated insecticidal solutions are sprayed sparsely over an area, than if much larger applications containing the same quantity of insecticide but dispersed in a large volume of solvent are used. An insecticidal tank on a vehicle or aeroplane filled with concentrated insecticide solution can spray much larger areas before refilling than if it contained more conventional formulations. This technique is known as ultra-lowvolume (ULV) spraying. Very small droplets forming a fog or aerosol of concentrated insecticide are produced. Whereas a standard rate of application of an insecticide solution might be 5-28 litres/ha a ULV application will be only about 74 ml-1.1 litre/ha.

With aerial applications the droplet size of the insecticide leaving the aeroplane should be bigger  $(150-200 \,\mu\text{m})$  than for ground-based applications  $(50-100 \,\mu\text{m})$  because the droplets decrease in size as they fall to the ground due to evaporation. The height at which spraying is undertaken will also determine optimum droplet size. Small droplets are generally required to kill adult mosquitoes because it is desirable to maintain a dense cloud of droplets in the air near the ground to kill resting and flying individuals. For larviciding larger droplets  $(150-250 \,\mu\text{m})$  are required so that they fall as rapidly as possible on to the water.

ULV techniques are more applicable to insecticides with low volatility and high specific gravity, because these characters will tend to reduce losses due to drift and evaporation. Malathion is a suitable insecticide that is often used in ULV applications, others include fenthion (Baytex), chlorpyrifos (Dursban) and naled (Dibrom), and also synthetic and natural pyrethrums, but these need small quantities of diluent or solvent to be added because they are too volatile to be used alone.

Applications of aerosols and fogs are best made in calm weather and usually in the late evenings or early mornings when there are usually fewer thermals rising from the ground and less turbulence. Local conditions of terrain and climate as well as application techniques and type of insecticides will dictate the timing and exact operational procedures.

Usually there is little residual effect from ULV applications, but concentrates of Abate have given good control of *Aedes aegypti* for several weeks.

Residual house-spraying Some mosquitoes, such as many but not all malaria vectors, Culex pipiens fatigans and some Mansonia species, rest inside houses or manmade animal shelters before and/or after bloodfeeding. This knowledge prompted the widespread adoption in malaria campaigns of spraying the interior surfaces of walls and roofs or ceilings of houses, and sometimes animal shelters, with water dispersable powders of residual insecticides such as DDT. The whitish powder of insecticide that is deposited on these surfaces kills mosquitoes that rest on them. For greater details of this method see the section on control in chapter 5 and chapter 28 on insecticides.

Dichlorvos strips Strips of polyvinyl chloride plastic impregnated with the fumigant organophosphate insecticide dichlorvos (DDVP) are sold in many countries for killing household insects such as houseflies and mosquitoes. Their effectiveness depends on the buildup of a minimum concentration of fumigant (about 0.  $I\mu g$  /litre air), consequently the number of strips per room depends on its size. A single  $5 \times 25$ -cm strip is required per  $28 \text{ m}^3$  of space and will probably give a high kill of mosquitoes for eight to ten weeks. They are not very effective in houses or rooms with a lot of ventilation, and should not be used in rooms in which infants, old people or those with bronchial complaints are confined. Although under certain conditions they can be useful in killing household insects, they have not proved effective on a large scale in controlling housefrequenting (endophilic) mosquitoes.

## 5 Anopheles mosquitoes (Order Diptera: Family Culicidae, Subfamily Anophelinae)

## Species

The subfamily Anophelinae contains the genera Bironella, Chagasia and Anopheles, but as explained in chapter 4, the former two have a restricted distribution and are of no medical importance. The genus Anopheles contains about 380 species.

## Distribution

Anopheles mosquitoes have a world-wide distribution, occurring not only in tropical areas but also in temperate regions; they are, however, absent from Polynesia and Micronesia.

### Medical importance

The most important disease carried by Anopheles mosquitoes is human malaria (Plasmodium spp.), but only comparatively few species are efficient malaria vectors. Some Anopheles species are also vectors of filariasis, especially that caused by Wuchereria bancrofti, but a few also transmit Brugia malayi and B. timori. Certain species are also vectors of arboviruses.

# External morphology of Anopheles mosquitoes

## Adults

The main features distinguishing adults of the Anophelinae, and in particular the genus *Anopheles*, from other mosquitoes have been given in the previous chapter, but a brief summary is presented here.

Most, but not all, *Anopheles* have spotted wings, that is the dark and pale scales are arranged in small blocks or areas on the veins (figure 4.20). The number, length and arrangement of these dark and pale areas differs considerably in different species and provide useful characters for species identification. Unlike the Culicinae the dorsal and ventral surfaces of the abdomen are entirely or almost entirely devoid of appressed scales, but in some species there may be small but conspicuous lateral tufts of scales projecting from the margins of the abdomen. In all *Anopheles* the scutellum is rounded in outline (figure 4.3b). In both sexes the palps are about as long as the proboscis and in males, but not females, they are enlarged (that is clubbed) at their ends (figure 4.21).

## Life-cycle of Anopheles

After mating and blood-feeding, gravid Anopheles lay some 50–200 small (1 mm long) brown or blackish boat-shaped eggs (figure 4.15) on the water surface. In a few species the eggs are not boat-shaped, but more oval in outline. In most Anopheles there is a pair of conspicuous lateral air-filled chambers called the floats on the eggs, which in a few species completely extend round the egg. These floats help maintain the eggs floating on the water surface. Anopheles eggs cannot withstand desiccation and in tropical countries they hatch within two to three days, but in colder temperate climates hatching may not occur until about two to three weeks, the duration depending on temperature. Two Anopheles species overwinter in the egg state, but this is a most unusual phenomenon.

Anopheles larvae have a dark brown or blackish sclerotised head and a roundish thorax with numerous simple and branched hairs and a single pair of thoracic palmate hairs. There are nine visible abdominal segments of which segments one to six or seven usually have dorsally a pair of palmate hairs (figures 4.14b and 4.16), which help maintain the larvae in a horizontal position at the water surface (figure 4.10a). Segments one to eight have a median sclerotised light or dark brown structure called the tergal plate, which varies in size and shape in different species; in addition each segment may possess one to three very small accessory tergal plates posterior to the main one (figure 4.16). Paired spiracles are present dorsally on the posterior end of the eighth visible segment; there is no siphon. On each side, just below and lateral to the spiracles is a sclerotised structure bearing teeth, somewhat resembling a comb and called the pecten. The ninth visible segment has dorsally the sclerotised saddle and two pairs of tufts of hairs called the caudal (dorsal) setae, and ventrally a larger collection of tufted hairs comprising the ventral brush (figure 4.17). At the end of the last abdominal segment are four sausage-shaped transparent gills which have an osmoregulatory function and are not used for respiration.

As in all mosquitoes there are four larval instars. Anopheles larvae are filter feeders and unless disturbed remain at the water surface feeding on bacteria, yeasts, protozoa and other microorganisms and also breathing in air through their spiracles. When feeding, larvae rotate their heads through  $180^{\circ}$  so that the ventrally positioned mouth brushes can sweep the underside of the water surface. Larvae are easily disturbed by shadows or vibrations and respond by quickly swimming to the bottom of the water to resurface some seconds or minutes afterwards.

Anopheles larvae occur in many different types of both small and temporary and large and more permanent habitats, ranging from fresh and salt water marshes, mangrove swamps, grassy ditches, rice fields, edges of streams and rivers, ponds, borrow pits, puddles, hoof prints, wells, discarded tins and a few species sometimes occur in water-storage pots. Other larvae occur in water-filled tree holes and in the Neotropical region (Central and South America and the West Indies) a few breed in water that collects in the leaf axils of epiphytic plants growing on tree branches, such as bromeliads which somewhat resemble pineapple plants. Some Anopheles prefer habitats with aquatic vegetation, others habitats without vegetation, some species like exposed sunlit waters while others prefer more shaded breeding places. In general Anopheles prefer clean and unpolluted waters and are usually absent from habitats that contain rotting plants or are contaminated with faeces.

In tropical countries the larval period frequently lasts only about seven days, but in cooler climates the larval period may be about two to four weeks, and in temperate areas some *Anopheles* overwinter as larvae and consequently may live many months.

In the comma-shaped pupa the head and thorax are combined to form the cephalothorax, which dorsally has a pair of short trumpet-shaped breathing tubes which have broad openings (figure 4.18a). There are eight visible abdominal segments each having numerous short setae and segments two or three to seven have distinct short peg-like spines. The last segment terminates in a pair of oval paddles (figure 4.18b). Pupae normally remain floating at the water surface with the aid of the pair of palmate hairs on the cephalothorax, but when disturbed they vigorously swim down to the bottom with characteristic jerky movements. The pupal period lasts two to three days in tropical countries but sometimes as long as one to two weeks in cooler climates.

## Adult biology and behaviour

Most Anopheles are crepuscular or nocturnal in their activities, thus emergence from the pupae, mating, blood-feeding and oviposition normally occur in the evenings, at night or in the early morning around sunrise. Some species such as Anopheles albimanus, a malaria vector in Central and South America, bite man mainly outdoors (exophagic) from about sunset to 2100 hours, whereas in Africa species of the Anopheles gambiae complex, which contains probably the world's most efficient malaria vectors, bite mainly after 2300 hours and mostly indoors (endophagic). As already discussed in chapter 4 the times of biting and whether adult mosquitoes are exophagic or endophagic may be important in the epidemiology of diseases.

Both before and after blood-feeding some species will rest in houses (endophilic) whereas others will rest outdoors (exophilic) in a variety of natural shelters, such as amongst vegetation, in rodent burrows, in cracks and crevices in trees, under bridges, in culverts, in termite mounds, in caves and amongst rock fissures and cracks in the ground. Most Anopheles species are not exclusively exophagic or endophagic, exophilic or endophilic, but exhibit a mixture of these extremes of behaviour. Similarly, few Anopheles feed exclusively on either man or non-humans, most feed on both man and animals but the degree of anthropophilism and zoophilism varies according to species. For example, An. culicifacies, an important Indian malaria vector, frequently feeds on cattle in addition to man, whereas in Africa An. gambiae sensu stricto (of the gambiae complex) feeds more rarely on cattle and thus maintains a stronger mosquito-man contact. This is one of the reasons why An. gambiae is a more efficient malaria vector than An. culicifacies.

## Medical importance

### **Biting nuisance**

In some areas *Anopheles* mosquitoes constitute a biting problem although they may not be vectors of any disease. However, non-vector mosquitoes that cause a biting nuisance are usually various culicines not anophelines.

## Malaria

Anopheles are the most notorious of all vectors of disease to man. Only mosquitoes of the genus Anopheles can transmit Plasmodium species causing malaria in man, namely P. falciparum (malignant tertian), P. vivax (benign tertian), P. malariae (quartan malaria) and P. ovale (ovale benign tertian). Because the sexual cycle of the malaria parasites occurs in the Anopheles vector, it is conventional to call the mosquito the definitive host and man the intermediate one.

Male and female malaria gametocytes are ingested by female mosquitoes during blood-feeding on man. They pass to the mosquito's stomach where they undergo cyclical development that includes a sexual cycle termed sporogony. Only gametocytes survive in the mosquito's stomach, all other blood forms of the malaria parasites (the asexual forms) are destroyed. Male gametocytes (microgametocytes) extrude flagella which are the male gametes (microgametes), and the process is called exflagellation. The microgametes break free and fertilise the female gametes (macrogametes) which have formed from the macrogametocytes. As a result of fertilisation a zygote is formed which elongates to become an ookinete. This penetrates the wall of the mosquito's stomach and reaches its outer membrane where it becomes spherical and develops into an oocyst. The nucleus of the oocyst divides repeatedly to produce numerous spindleshaped sporozoites. When the oocyst is fully grown (about  $60-80\,\mu\text{m}$ ) it ruptures and thousands of sporozoites are released into the haemocoel of the mosquito. The sporozoites are carried in the insect's haemolymph to all parts of the body, but most penetrate the salivary glands. The mosquito is now infective and sporozoites are inoculated into man the next time the mosquito bites. It has been estimated that there may be some 70 000 sporozoites in the salivary glands of vectors.

Occysts can be seen on the stomach walls of vectors about four days following an infective blood-meal, and after about eight days they are fully grown and rupture. Sporozoites are usually found in the salivary gland after 9-12 days, but the time required for this cyclical development (exogenous or extrinsic cycle) depends on both temperature and *Plasmodium* species. For example, at  $24^{\circ}$ C sporogony in *P. vivax* takes only nine days, in *P. falciparum* 11 days, in *P. malariae* 21 days, and at  $26^{\circ}$ C sporogony of *P. ovale* is completed in 15 days.

Sporozoite rates, that is the percentage of female vectors with sporozoites in the salivary glands, vary considerably not only from species to species of mosquitoes, but also according to locality and season. Sporozoite rates are often about 1-5 per cent in species such as An. gambiae and An. arabiensis of the An. gambiae complex, but less than 1 per cent in many other species such as An. albimanus and An. culicifacies. For practical purposes it can be said that once a vector becomes infective it remains so throughout its life, although it has not been established that this is so for all vector— Plasmodium combinations.

Malaria formerly occurred in parts of the southern U.S.A. and in many areas of Europe, but due to changes in agricultural practices, life-style and also control measures, malaria was eradicated from these areas, all of which it should be remembered were at the periphery of its distribution. During the early 1960s the incidence of malaria was greatly reduced in some tropical countries such as India and Sri Lanka, but for various reasons, including insecticide resistance, bad surveillance, poorly trained and supervised personnel, lack of money and unusual weather, malaria outbreaks recurred in both countries during the late 1960s, and the situation in the 1970s has deteriorated even further. In other areas such as Africa there has as yet been little or no impact on malaria transmission.

## Simian malaria

There are several species of monkey malaria (*Plas-modium cynomolgi* complex, *P. simium*, *P. knowlesi*) and there have been very rare cases where man has become infected, both in the laboratory and in the field, with these parasites. Only *P. malariae* of man has been found in monkeys, – the chimpanzee. It is generally agreed that there is very little chance of man becoming infected with monkey malaria and that monkeys are not reservoirs of any of the four human malarias.

## Important malaria vectors

Notes are given below on the principal larval habitats and biting behaviour of adults of some of the more important malaria vectors. In such brief notes it is impossible to list all the countries in which the vectors occur, and moreover, their importance as vectors and their behaviour may differ in different areas of their distribution. These notes are no more than a guide, consequently for greater details of the behaviour and importance of *Anopheles* as malaria vectors in different countries other reference works and books should be consulted.

#### Malaria vectors of Mexico and Central America An. albimanus

Texas and Florida in the U.S.A. through Central America to Colombia, Ecuador, Venezuela and the Antilles. Larvae in fresh or brackish waters such as pools, puddles, marshes, ponds, lagoons, especially those containing floating or grassy vegetation. Prefers sunlight. Feeds on man and domestic animals, indoors and outdoors, after feeding adults rest mainly outdoors.

#### An. albitarsis

Central America to South America and Trinidad. Larvae nearly always in sunlit ponds, large pools and marshes with filamentous algae. Bites almost indiscriminately man and domestic animals, outdoors and also indoors, usually rests outdoors after feeding.

#### An. aquasalis

Lesser Antilles, Trinidad, Tobago and other nearby islands, Central America to northern parts of Brazil. Tidal salt water marshes, lagoons, salt water regions of rivers, estuaries, rarely fresh water. Sunlit or shaded habitats. Bites man and domestic animals, indoor or outdoors, rests mainly outdoors.

#### An. azetecus

Mexico at heights of 1500-2200 metres. Pools, ponds, lakes, especially with vegetation, canals, even polluted waters. Bites man indoors and outdoors, rests mainly outdoors after feeding.

#### An. darlingi

Mexico through Central America to Argentina and Chile. Fresh water marshes, lagoons, rice fields, swamps, lakes and ponds, pools, edges of streams, especially with vegetation. Mainly shaded habitats. Feeds mainly on man indoors, remains indoors after feeding.

#### An. pseudopunctipennis

Antilles, southern U.S.A. to Argentina. Pools, puddles, seepage waters, edges of streams, especially habitats with algae. Prefers sunlight. Feeds almost indiscriminately on man and domestic animals, indoors or outdoors, rests outdoors after feeding.

#### An. punctimacula

Mexico through Central America to Peru, Brazil, Argentina and Trinidad. Small pools, swamps, grassy pools at edges of streams, prefers shade. Bites man and domestic animals, indoors and outdoors, rests indoors or outdoors after feeding.

#### Malaria vectors of South America An. albimanus

See Central America.

An. albitarsis See Central America.

An. aquasalis See Central America.

#### An. bellator

Trinidad, Venezuela, Surinam, Guyana and Brazil. Larvae occur only in water collected in leaf axils of bromeliads, which are epiphytes on trees, prefers partially shaded habitats. Bites man during daytime in shaded forests, also at night and may enter houses. Rests indoors after feeding. Although man is favoured host also bites domestic animals.

#### An. cruzii

Costa Rica, Panama, Ecuador, Bolivia, Colombia, Peru, Brazil and Venezuela. Larvae occur in water collected in leaf axils of bromeliads, partial shade preferred. Bites man indoors and outdoors, after feeding rests indoors or outdoors, main importance as a malaria vector in coastal areas of Brazil.

#### An. darlingi See Central America.

#### An. nuneztovari

Guyana, Venezuela, Colombia, Brazil and Bolivia. Larvae occur in muddy waters of pools, vehicle tracks, hoof prints, small ponds, especially in and around towns, prefers sunlight. Mainly feeds on animals, but also bites man outdoors, rests outdoors after feeding. Can be an important vector in Colombia and Venezuela.

#### Malaria vectors of Africa south of the Sahara An. gambiae complex

Consists of six very similar species, separated by banding patterns of their polytene chromosomes. They differ in certain aspects of their biology and behaviour.

An. gambiae (formerly called species A of the gambiae complex) is widespread in nearly all African countries south of the Sahara and is probably the world's most efficient malaria vector. Larvae occur mainly in temporary habitats such as pools, puddles, hoof prints, borrow pits, but also rice fields. Bites man both indoors and outdoors, in some areas also feeds on domestic animals, rests predominantly indoors after feeding but may rest outdoors.

An. arabiensis (formerly species B of the gambiae complex) is also widespread in most African countries, but possibly prefers rather drier savanna areas. Larval habitats the same as those of An. gambiae. Adults bite man and animals, indoors and outdoors, and afterwards rest indoors or outdoors. This species may have a greater tendency than An. gambiae to bite animals and rest outdoors. An important malaria vector, but generally not so efficient as An. gambiae.

An. quadriannulatus (formerly species C of the gambiae complex) occurs in Ethiopia, Zanzibar, Rhodesia, Mozambique and southern Africa, not considered an important malaria vector as it mainly feeds on cattle.

Species D is known only from mineral springs in the Semliki forest of Uganda; it is a rare species and is not considered a malaria vector.

An. melas is a salt water breeding species of the gambiae complex, it occurs along the coast of West Africa to Congo. Very common in lagoons and mangrove swamps, does not breed in fresh water. Adults behave similarly to An. gambiae. Malaria vector in many coastal areas.

An. merus is the East African equivalent of An. melas, it breeds in salt water lagoons and swamps along the coast of East Africa. Biting behaviour of adults similar to that of An. gambiae, can be a vector in certain coastal areas.

#### An. funestus

Widespread in Africa south of the Sahara. The most important vector after An. gambiae and An. arabiensis (see gambiae complex above). More or less permanent waters, especially with vegetation, such as swamps, marshes, edges of streams, rivers, ditches. Prefers shaded habitats. Bites man predominantly but also domestic animals, feeds indoors and also outdoors, after feeding rests mainly indoors, but also outdoors.

#### Other Anopheles

An. nili, An. moucheti, An. hargreavesi and An. hancocki may also be malaria vectors of minor importance in certain localities.

#### Malaria vectors of North Africa and the Middle East

(Morocco, Libya, Egypt, Turkey, Syria, Iran, Iraq and Saudi Arabia etc.)

#### An. claviger

England through Europe to North Africa, Palestine, Iran, Iraq and Asia Minor, to Afganistan, but vector only in Middle East countries. Ponds, marshes, wells, cisterns, rock pools. Sunlight or shade. Bites man and domestic animals indoors and outdoors, and after feeding rests indoors or outdoors.

#### An. labranchiae

England through Europe to Morocco, Algeria and Tunisia, now a

vector only in North Africa. Brackish waters in coastal marshes, fresh waters of rice fields, marshes and edges of grassy streams and ditches, prefers sunlight. Bites man and also domestic animals indoors and outdoors, rests mainly in houses or animal shelters after feeding.

#### An. pharoensis

Palestine, Israel, Saudi Arabia, Syria, Egypt and most of Africa south of the Sahara, only a vector of importance in Egypt. Marshes, swamps, rice fields, ponds especially those with abundant grassy or floating vegetation. Bites man and animals indoors or outdoors, rests outdoors after feeding.

#### An. sacharovi

Italy, Greece, Eastern Europe, Palestine, Turkey, Syria, Jordan, Lebanon, Iran, Iraq and to Central Russia; malaria vector in Palestine area and Middle East countries. Fresh or brackish waters of coastal or inland marshes, pools, ponds, especially those with vegetation. Prefers sunlit habitats. Bites man and animals indoors or outdoors, usually rests in houses or animal shelters after feeding.

#### An. sergentii

Canary Islands, Algeria, Tunisia, Egypt, Syria, Palestine, Turkey, Afghanistan and Pakistan to north-west India; vector in Egypt, Palestine and Saudi Arabia. Rice fields, borrow pits, ditches, seepages, slow flowing streams, sunlit or partially shaded habitats. Bites man or animals indoors or outdoors, rests in houses and caves after feeding.

#### An. stephensi (includes mysorensis)

Egypt, Iraq, Iran, Saudi Arabia, Oman, Bahrain, Afghanistan, Pakistan, Sri Lanka, India, Burma, Thailand and China. An important vector over much of its range, especially in and around towns. Breeds in man-made habitats associated with towns, such as cisterns, wells, gutters, water-storage jars and containers, drains, fresh water or brackish waters, and even polluted waters, and in rural situations in grassy pools and alongside rivers. Adults bite man indoors and outdoors and rest mainly indoors after feeding.

#### An. superpictus

Greece, Mediterranean area, Iran, Iraq, Saudi Arabia, Palestine, Jordan, Turkey, Afghanistan and Pakistan. Flowing waters such as torrents of shallow water over rocky streams, pools in rivers, muddy hill streams, vegetation may be present, prefers sunlight. Bites man and animals indoors and outdoors, after feeding rests mainly in houses and animal shelters, but also in caves.

#### Malaria vectors of the Indian Subcontinent

(Afghanistan, Pakistan, India, Nepal, Bengal, Sri Lanka, Bangladesh etc.)

#### An. culicifacies

Oman, Bahrain, Afghanistan, Pakistan through India, Sri Lanka, Bangladesh, Burma, Thailand, Indochina and southern China; probably most important vector in much of Pakistan, India, Bangladesh and Sri Lanka. Great variety of clean and polluted habitats, irrigation ditches, rice fields, swamps, pools, wells, borrow pits, edges of streams, occasionally in brackish waters. Sunlit or partially shaded habitats. Prefers domestic animals but commonly bites man indoors or outdoors, rests mainly indoors after feeding.

#### An. fluviatilis

Oman, Bahrain, Iran, Iraq, eastern Saudi Arabia, Pakistan, Afghanistan, India, Sri Lanka, Bangladesh, Burma, Thailand, Indonesia, Indochina, China and Taiwan. Important vector in Pakistan, India and Bangladesh. Most flowing waters, such as hill streams, pools in river beds, irrigation ditches, prefers sunlight. Bites man and domestic animals indoors and outdoors, rests both indoors and outdoors after feeding.

#### An. minimus (includes flavirostris)

India, Sri Lanka, Burma, Malaysia, Thailand, Indochina, Taiwan, Formosa, Sumatra, Java, and the Philippines to southern China. Flowing waters, such as foothill streams, springs, irrigation ditches, seepages, also rice fields and borrow pits. Prefers shaded areas of sunlit habitats. Feeds mainly on man, but also domestic animals, mainly feeds and rests indoors. In the Philippines subspecies *flavirostris* bites indoors but rests outdoors after feeding. Some authorities regard *flavirostris* as a distinct species, not a subspecies of *An. minimus*.

#### An. stephensi

See North Africa and Middle East.

#### An. sundaicus

India, Burma, Malaysia, Thailand, Indonesia, Java, Sumatra, Borneo, Taiwan, China and Indochina. Salt or brackish waters, lagoons, marshes, pools, seepages, especially with putrifying algae and aquatic weeds, mainly a coastal species, but found in freshwater inland pools in Java and Sumatra. Prefers sunlight. Bites man and domestic animals indoors and outdoors, and rests mainly indoors after feeding.

#### An. superpictus

See North Africa and Middle East.

#### An. varuna

India, Sri Lanka, Nepal, Burma and possibly Thailand. This species is similar to *An. minimus* and has often been confused with it, appears to be a malaria vector in central and northern India. Larvae occur in stagnant ditches, pools, slow-running streams, wells, in sunlit or shaded conditions. Bites man and animals indoors and outdoors, commonly rests indoors after feeding.

#### Malaria vectors of south-east Asia

(Assam, Burma, Thailand, Indochina, Indonesia, Malaysia, Taiwan, China, Philippines etc.)

#### An. aconitus

Eastern India, Sri Lanka, Malaysia, Indochina, Indonesia and southern China. Rice fields, swamps, irrigation ditches, pools, streams with vegetation. Prefers sunlit habitats. Adults feed indoors or outdoors on man but also commonly on animals, adults rest indoors or outdoors after feeding.

#### An. balabacensis and An. leucosphyrus

Two closely related and similar species. An. leucosphyrus occurs in Malaya, Sumatra, Borneo and Sarawak. A forest species which bites and rests outdoors, a malaria vector in Borneo, Sarawak and Sumatra. An. balabacensis is more widely distributed – India, Sri Lanka, Burma, Malaysia, Thailand, Philippines, Indochina, Borneo, Java, Taiwan and China. Forest species, larvae found in hoof prints, small muddy pools, vehicle ruts. Prefers shaded habitats. Adults bite animals and man mainly outdoors, after feeding adults rest outdoors.

#### An. campestris

There has been considerable confusion over the identity of this species, it has sometimes been misidentified as An. donaldi but more frequently as An. barbirostris. Many references in the literature refer to An. barbirostris as an important vector of both malaria and filariasis, but An. barbirostris is predominantly zoophilic, and the real vector is An. campestris. Consequently most references giving An. barbirostris as a vector refer in fact to An. campestris, a species found along the coasts and deltas of Malaya and Thailand, possibly in other mainland areas of south–east Asia. Larvae in deep waters usually

having some vegetation. Partial shade preferred, larvae often accumulate in shaded corners of rice fields, also ditches, earthern wells and sometimes in brackish waters. Bites man and animals indoors and outdoors, substantial numbers rest indoors after feeding.

An. culicifacies

See Indian subcontinent.

#### An. hyrcanus

This mosquito has been reported from Europe, the northern Mediterranean, North Africa, across central and northern Asia to Japan, but what was formerly considered a single species is now known to consist of a species group. Many records incriminating An. *hyrcanus* in the transmission of malaria refer to closely related species such as An. *lesteri*, An. *sinensis* and An. *nigerrimus*, and in these brief notes these species have been identified as malaria vectors and not An. *hyrcanus* sensu stricto (see below), although this may be a vector in some areas.

#### An. lesteri and An. sinensis

There has been much taxonomic confusion over the different mosquitoes in this group, some forms occur in northern Mediterranean areas, others in India, and several in the Far East. An. sinensis was considered an important vector in China, but this was probably based on misidentifications, and the most important vector in the group is probably An. lesteri. This species occurs in Thailand, Malaysia, Borneo, Philippines, Korea and southern China to Japan. It is likely an important vector in certain areas of China. Larvae in cool, clean waters, some forms can occur in brackish water, prefers shaded habitats. Bites man and animals indoors and outdoors, rests indoors and outdoors after feeding.

#### An. letifer and An. umbrosus

India, Thailand, Indonesia, Malaysia, Philippines, Sumatra and Borneo. This species is very similar to An. umbrosus, which has a similar distribution and has in the past been confused with it. An. umbrosus, however, is probably not such an important malaria vector as formerly supposed, many sporozoites found in the salivary glands are probably of rodent malarias. An. letifer bites man more often than An. umbrosus, but also commonly feeds on animals, it seems to be a malaria vector in certain areas. Larvae often in stagnant waters, especially on coastal plains, such as pools, swamps and ponds. Prefers shade. Bites animals and man mainly outdoors, rests outdoors after feeding.

#### An. maculatus

India, Sri Lanka, Malaysia, Indonesia, Indochina, Borneo, Taiwan, Philippines and southern China. Found in or near hilly areas, in seepage waters, pools formed in streams, edges of ponds, ditches, rice fields. Prefers sunlight. Bites domestic animals and also man indoors and outdoors, rests mainly outdoors after feeding.

An. minimus See Indian subcontinent.

#### An. nigerrimus

Formerly regarded as a subspecies of *An. hyrcanus*. India, Sri Lanka, Burma, Thailand, Malaysia, Indochina, Borneo and China. Larvae in deep ponds, rice fields, irrigation ditches and swamps with much vegetation, prefers sunlight. Bites man and animals mainly outdoors, rests mainly outdoors after feeding.

#### An. pattoni

China, north of about 30th parallel. Larvae in pools in or near streams, rock pools, especially habitats with algae, prefers sunlight. Bites man indoors and outdoors.

#### An. subpictus

Iran, Pakistan, India, Sri Lanka, Burma, Malaysia, Thailand, Indonesia, Indochina, China, New Guinea, Java and Celebes. May be important malaria vector in Celebes, Java and Indochina. Larvae in muddy pools near houses, gutters, borrow pits, also in brackish waters. Bites animals mainly but also man indoors and outdoors, rests indoors or outdoors after feeding.

#### An. sundaicus

See Indian subcontinent.

#### Malaria vectors of the Australasian area An. punctulatus complex

Moluccas, New Guinea, Solomon Islands, New Hebrides to northern Australia. This is a species complex consisting of at least four species – An. punctulatus, species 'No. 1' and species 'No. 2' of An. farauti, and An. koliensis. All four species are malaria vectors. In general larval habitats are swamps, edges of slow flowing streams, springs, puddles, hoof prints, pools, wells, water-storage containers and other man-made receptacles. Water may be either fresh or slightly brackish or even polluted. Sunlight or shade. Adults bite man indoors or outdoors, rest indoors or outdoors after feeding, but in New Guinea rest mainly outdoors. An. koliensis seems to prefer to breed in marshy pools or pools at edges of forest streams. Adults usually both bite man and rest after feeding outdoors.

## Medical importance continued

## Filariasis (table 6.1)

Certain Anopheles species transmit filarial worms of Wuchereria bancrofti, Brugia malayi, and B. timori, which cause filariasis in man. W. bancrofti causes filariasis in people living in most tropical regions of the world (Central America and South America, Africa and Asia, including the Pacific area), and also in some subtropical areas such as the Mediterranean region and Australia. In many of these areas it is mainly an urban disease. In contrast B. malayi is more of a rural disease and has a more restricted distribution occurring only in Asia, in countries such as southern India, Malaysia, Indonesia, Thailand, Indochina, China, New Guinea, Philippines and Polynesia. Both diseases occur in two basic forms – nocturnal periodic and nocturnal subperiodic (sometimes called aperiodic).

In the nocturnal periodic forms of these two parasites, most of the microfilariae in man are in the blood vessels supplying the internal organs such as the lungs, during the day, but at night, especially during the middle part, they migrate to the peripheral blood system and lymph vessels. Because of this marked diel (24 hours) periodicity of the microfilariae they are mainly ingested by mosquitoes feeding on man at night. *Anopheles* mosquitoes which mainly bite at night are therefore among the vectors of the nocturnal periodic form of *W. bancrofti* which is found throughout most of the tropics except in the Pacific (where it is replaced by the diurnal subperiodic form). *Anopheles* are also vectors of the periodic form of *B. malayi* which is found more or less throughout the range of the parasite. The mosquitoes involved in transmission differ according to the area, but many of the principal malaria vectors are also important filarial vectors, for example An. campestris (formerly identified as An. barbirostris), An. darlingi, An. punctulatus complex, An. letifer, An. funestus, An. gambiae complex, An. sundaicus, An. sinensis, An. minimus, An. maculatus etc. are vectors of nocturnal periodic W. bancrofti while An. campestris, An. lesteri, An. sinensis and others are vectors of the nocturnal periodic form of B. malayi. Female Anopheles can be infected with both malarial and filarial parasites and thus transmit both diseases. (Other night-biting mosquitoes such as Culex and Mansonia species are also vectors of nocturnal periodic and subperiodic filariasis (chapter 6)).

In nocturnal subperiodic W. bancrofti and B. malayi the microfilariae exhibit a reduced periodicity and are present in the peripheral blood during the day as well as at night but there remains some degree of periodicity. For example, subperiodic W. bancrofti (which is found in Polynesia) has a small peak in microfilarial density during the daytime and can therefore be called diurnally subperiodic, whereas subperiodic B. malayi in West Malaysia, South Vietnam, Thailand, Sabah, Palewan Islands exhibits a slight peak of microfilariae at night and can be called nocturnally subperiodic. Culicine mosquitoes which bite during the daytime, such as certain Mansonia and Aedes species, are important vectors of subperiodic filariasis (table 6.1).

Filarial development of W. bancrofti and B. malayi within the mosquito vector and the basic mode of transmission from mosquito to man is the same for all vectors. Basically the life-cycle in the mosquito is as follows. Microfilariae ingested with the blood-meal pass to the stomach of the mosquito (in some vectors such as Anopheles many may be destroyed during their passage through the oesophagus), and within a few minutes they exsheath and penetrate the stomach wall and pass into the haemocoel from where they migrate to the thoracic muscles of the mosquito. In the thorax the small larvae become more or less inactive, grow shorter but considerably fatter and develop after two days into the 'sausage-shaped' forms. They undergo two moults and the resultant third-stage larvae become active, leave the muscles, and migrate through the head and down the fleshy labium of the proboscis. This is the infective stage and is formed some ten days or more after the microfilariae have been ingested with a bloodmeal.

When the mosquito takes a blood-meal infective (third-stage) larvae (about 1.2-1.6 mm long) rupture the skin of the labella of the labium and crawl on to the surface of the host's skin. Several infective larvae may be liberated on the skin when a vector is biting, but many of these die so that only a few manage to find a

skin abrasion, sometimes the small lesion caused by the mosquito's bite, and thus enter the skin and pass to the lymphatic system. It should be noted that the salivary glands are not involved in the transmission of filariasis, and also that there is no multiplication or sexual cycle of the parasites in the mosquito.

There are no known animal reservoirs of W. bancrofti, it is a disease solely of man.

In contrast, Brugia malayi is a zoonosis. The nocturnal subperiodic form is essentially a parasite of swamp inhabiting monkeys, especially the so-called 'leaf monkeys' (Presbytis spp.); man becomes infected when he lives at the edges of these areas. Other reservoirs include wild and domestic cats, dogs and pangolins. The nocturnal periodic form is more adapted to man and there do not appear to be any important animal reservoirs, but in some areas monkeys may act as reservoirs.

Infection rates of infective larvae in Anopheles vectors vary according to the mosquito species and local conditions, but they often vary from about 0.1-5 per cent for W. bancrofti and from about 0.1-3 per cent for B. malayi.

Brugia timori is known only from Timor islands in Indonesia, its microfilariae are nocturnally periodic and are transmitted to man by Anopheles mosquitoes, probably by An. campestris of the An. barbirostris group.

The presence of filarial worms in the thoracic muscles of mosquitoes, or infective worms in the proboscis, does not necessarily implicate mosquitoes as vectors of bancroftian or brugian filariasis. There are several other mosquito-transmitted filariae, for example various Setaria species infecting cattle, Dirofilaria repens and D. immitis infecting dogs, and various other species of Brugia, such as B. patei in Africa, and B. pahangi in Asia which infect animals but not man. Careful examination is therefore essential to identify the filarial parasites found in mosquitoes as those of W. bancrofti or B. malayi.

Table 6.1 (p. 68) summarises the distribution and vectors of filariasis.

## Arboviruses

The word arboviruses is derived from the term 'arthropod-borne-virus'. An arbovirus infection in either man or non-human hosts produces viraemia and the virus is ingested by blood-sucking insects such as mosquitoes when they take blood-meals. Within the vector the virus undergoes multiplication and/or cyclical development before it is transmitted by the infected arthropod during refeeding. An arbovirus therefore undergoes obligatory development in an arthropod host; yellow fever and dengue are typical arboviruses transmitted by Aedes mosquitoes. In contrast the virus causing poliomyelitis is not an arbovirus

because it does not undergo multiplication and/or development in an arthropod and, although it can be transmitted by certain flies such as houseflies, this is purely mechanical transmission. The time taken for the infected mosquito to become infective, that is the extrinsic incubation period of the arbovirus, varies according to temperature, the species of arbovirus and mosquito. There are known to be at least 100 different arboviruses, but taking into consideration about another 60 viruses probably transmitted by arthropods and about 190 that are also possibly arboviruses the total is about 350. Many of the known arboviruses are transmitted by culicine mosquitoes, in particular *Aedes* and *Culex* species, others are spread by ticks and other arthropods.

In 1959-1960 a major epidemic of a painful but non-fatal disease, called O'nyong nyong (an African word meaning 'joint-breaker') was identified in Uganda and Kenya and later in other countries of East and Central Africa. It was discovered that it was spread by the An. gambiae complex and An. funestus. This was the first time an Anopheles mosquito was incriminated with the spread of any arbovirus. About 20 other arboviruses of man have since been found to be transmitted by Anopheles, some are also transmitted by culicine mosquitoes but others are known only or principally from Anopheles, such as Guaroa (An. neivai -man, -Colombia), Nyando and Tanga (An. funestus -man, -East Africa), Tataguine (An. gambiae complex -man, -West Africa) and Kowanyama and Trubanaman (An. annulipes - man, fowl, horses, kangaroos – Australia).

## Control

The principal methods of mosquito control are outlined in the preceding chapter, here methods only specifically directed at *Anopheles* are considered.

In certain areas predatory fish such as Gambusia have reduced larval populations but the impact has never been sufficient to interrupt disease transmission. Paris green dusts and larvicidal oils were extensively used prior to the introduction of the residual insecticides and helped reduce malaria transmission in localised areas of economic or social importance, such as principal towns, coffee and tea estates, rubber plantations and mining encampments.

Draining swamps and marshes has in some areas reduced the numbers of *Anopheles* vectors, but draining or filling-in habitats is impracticable against some important vectors. For example, larvae of the *An.* gambiae complex, principal malaria vectors in Africa, occur in temporary small pools and puddles while those of *An. balabacensis*, an important vector in south-east Asia, occur in forest swamps. In both these situations water management control procedures are not feasible.

## **Residual house-spraying**

In most malaria campaigns control is now focused on the adults and the most widely practised method is the application of water dispersable powders of residual insecticides to the interior surfaces of walls, ceilings and roofs of houses. In the absence of resistance DDT is the best insecticide, and this is usually sprayed as a water dispersable (wettable) powder at the rate of  $2g/m^2$ . Even in highly endemic areas houses need be sprayed only at six monthly intervals, and where malaria transmission is very seasonal a single spraying a year may be sufficient to give good control. In some areas a better procedure may be to spray four times a year but with only  $1g/m^2$  of DDT; this, however, increases labour costs. Local conditions dictate which procedures are adopted.

The spraying technique consists of making a five per cent suspension from 50 or 75 per cent stocks of DDT wettable powder and placing this in a Hudson-type cylindrical pressurised sprayer which is pumped up to a pressure of 40 p.s.i.  $(275 \text{ kN/m}^2)$ . The T-shaped nozzle at the end of the spraying lance is held 45-50 cm from the surface to the sprayed and moved at a speed of 0.5 m/sec, – this procedure results in a deposit of  $2g/m^2$ .

Alternative organochlorine insecticides are HCH which is normally applied at a recommended dose of  $4g/m^2$  three to four times a year and dieldrin at the rate of  $6g/m^2$  once a year. Dieldrin and HCH are more toxic to mosquitoes and man than DDT, and HCH is more volatile than either DDT or dieldrin. The safest of these three insecticides is DDT, dieldrin is the most toxic and should only be used for indoor spraying under strict safety measures. One of the few disadvantages of DDT is that it may be rather irritant to mosquitoes causing them to leave sprayed surfaces before they have picked up a lethal dose of insecticide.

If mosquitoes are resistant to DDT then organophosphate insecticides such as malathion, fenthion (Baytex), fenitrothion (Sumithion), or carbamates such as carbaryl (Sevin) and propoxur (Arprocarb) can be used at rates of about  $1.5-2.0g/m^2$ , but they are less persistent and spraying may have to be repeated at three to four monthly intervals. They are also many times more expensive than DDT and the increased number of spraying cycles needed will again increase costs. Furthermore, they are more toxic to man and greater safety measures have to be introduced during control campaigns.

The effectiveness of residual spraying of houses depends on the indoor resting habits of the mosquitoes.

It is not essential, however, that malaria vectors are killed on their first contact with sprayed surfaces because Anopheles mosquitoes must live at least 10-14 days before they can transmit malaria. Therefore, even if they need several periods of contact with insecticides before they are killed, malaria transmission will still be prevented, so long as their longevity is reduced to less than 10-14 days. It is possible, therefore, to have large biting populations of vectors too young to transmit malaria. In practice, however, anophelines are usually killed by residual insecticides at an early age which results in a decrease in numbers of ovipositions and eventually large reductions in population size of vectors. But, if spraying ceases, any remaining small populations of vectors can build up again, and if malarial parasites are still present in the human population then there is likely to follow a recrudescence of malaria transmission. Even if malaria vectors have been completely eradicated by insecticidal spraying, there remains the possibility that the area will be reinvaded by mosquitoes flying in from outside the control area and breeding become re-established.

## Alternative control measures

It has become increasingly obvious that many malaria vectors, especially some of those in Central and South America and south-east Asia, are unfortunately exophilic, hence spraying houses with residual insecticides will be of little use in reducing malaria. Another problem with house spraying is that it may alter the behaviour of vector population. For example, it may kill the endophilic proportion of the vector population but by so doing reduce competition between the larvae of endophilic and exophilic adults and thus allow an increase in the production of exophilic adults. Consequently, after some years of house spraying there may be a substantial increase in the exophilic, and possibly exophagic, proportion of the vector population with the result that malaria again becomes a problem because of large outdoor resting and biting populations. Alternative control measures are needed to combat these difficulties. One method is to use outdoor applications of aerosols of malathion, bioresmethrin or some other suitable insecticide which may not only kill the exophilic populations but also indoor resting adults. Another approach is to refocus attention on larvicides. Mosquito nets and mosquito screening can reduce the risk of acquiring malaria and other mosquito-borne diseases, but such measures will not be effective against vectors that bite during the day or early evening before people have gone to bed.

It seems unlikely that efficient malaria control will be achieved or maintained by relying on a single method of attack. Malaria is most likely to be conquered by integrated control, combined with improvement of housing, general hygiene and education, even so it seems that it will be a long time before the complex problem of malaria is solved.

## Malaria control and malaria eradication

In 1955 the eighth World Health Assembly stated that world-wide malaria eradication, except in Africa south of the Sahara, was technically feasible, although it recognised that there was a sense of urgency in achieving this because insecticide resistance in Anopheles had been reported in 1950. In 1968 the twenty-second World Health Assembly realised it had been over optimistic and declared that global malaria eradication was not at present possible, although it remained the ultimate goal, and that for the time being malaria control should be the aim. The difference between malaria eradication and malaria control is that malaria eradication means the total cessation of transmission and elimination of the reservoir of infection in man so that at the end of the anti-malaria campaign there is no resumption of transmission. Malaria control means reducing malaria transmission to an acceptable rate, that is to a level that no longer constitutes a major public health problem. This has the implication that control measures have to be maintained indefinitely, because if they are relaxed malaria prevalence will rise. The feasibility of control will depend not only on scientific considerations but the financial and public health resources of the community or country.

## 6 Culicine mosquitoes (Order Diptera: Family Culicidae, Subfamily Culicinae)

## Species

The subfamily Culicinae contains 30 genera of mosquitoes, of which the medically important ones are Culex, Aedes, Mansonia, Sabethes, Haemagogus and Psorophora. The most important vector species include Aedes aegypti, Ae. africanus, Ae. simpsoni, Ae. albopictus, the Ae. scutellaris group, Culex pipiens fatigans\* (= C. quinquefaciatus), C. tritaeniorhynchus, Mansonia uniformis, M. dives, M. bonneae and Haemagogus spegazzinii falco (= H. janthinomys).

## Distribution

The Culicinae have a world-wide distribution. The genera *Culex*, *Aedes*, and *Mansonia* are found in both northern temperate regions and throughout all tropical areas. The genus *Psorophora* is American, occurring from southern Canada down to Argentina and in the Caribbean islands, while *Haemagogus* and *Sabethes* are found in only Central and South America.

## Medical importance

Certain Aedes mosquitoes are vectors of yellow fever in Africa and Aedes, Haemagogus and Sabethes are yellow fever vectors in Central and South America; Aedes species are also vectors of the classical and haemorrhagic forms of dengue. Species in all six of the more important culicine genera (see above) can transmit a variety of other arboviruses. Certain Culex, Aedes and Mansonia species are also important vectors of filariasis (Wuchereria bancrofti or Brugia malayi).

## External morphology of the Culicinae

Characters separating the subfamily Culicinae from the Anophelinae have been given in chapter 4 and ar

\* See footnote on p. 22.

summarised in table 4.1 on page 35.

It is not easy to give concise and reliable but nontechnical methods for distinguishing the eggs, larvae and adults of the six more important culicine genera. Most text books on medical entomology present methods for identifying only Culex, Aedes and Mansonia, which admittedly are the most important genera. In the following sections, however, taxonomic characters are given for identifying Psorophora, Haemagogus and Sabethes in addition to the three more common genera, but it has not been possible to avoid using some technical terminology. In practice people working outside the Americas can ignore the descriptions of these latter three genera. Brief notes are given below on the biology and habits of the adults and the principal larval habitats of some of the more important species in these six genera.

## Notes on Culex mosquitoes

## Distribution

More or less world-wide, but they are absent from the extreme northern parts of the temperate zones.

## Eggs

Usually brown, long and cylindrical, laid upright on the water surface and placed together to form an egg raft which can comprise up to about 300 eggs (figure 4.9c), no glue or cement-like substance binds the eggs to each other, adhesion is due to surface forces holding the eggs together. Eggs of a few other mosquitoes such as some *Culiseta* species and those of the subgenus *Coquillettidia* of *Mansonia* also deposit their eggs in rafts.

## Larvae

The siphon is often long and narrow, but it may be

short and fat, there is always more than one pair of subventral tufts of hairs, none of which is near the base of the siphon (figure 6.1). These hair tufts may be small, consisting of very few short and simple hairs



Fig 6.1 Terminal segments of a larva of a Culex species showing a relatively long siphon with several subventral tufts of hairs and an enlarged comb scale.

which may be missed unless larvae are carefully examined under a microscope. These characters will separate *Culex* larvae from the other five important genera.

## Adults

Frequently, but not always, the thorax, legs and wing veins are covered with sombre, often brown scales. The abdomen is often covered with brown or blackish scales but some whitish scales may occur on most segments. Adults are recognised more by their lack of ornamentation than any striking diagnostic characters. The tip of the abdomen of females is blunt and the cerci are retracted. The claws of all tarsi are simple and those of the hind tarsi are very small, and all tarsi have well developed fleshy pulvilli (figure 2.4b). Additional characters are the presence of narrow fringe scales on the alula of the wings (figure 6.2), absence of postspiracular bristles and the absence of scales and bristles from the spiracular area (figure 6.3).



Fig 6.2 Base of the wing of a Culex species showing fringe of narrow scales on alula.



Fig 6.3 Diagram of the side of the thorax of an adult mosquito showing the names of the various sclerites: - apn, anterior pronotum; lme, lower mesipimeron; p, paratergite; pa, prealar area; pp, prosternum; ppn, posterior pronotum; psp, postspiracular area; sp, spiracular area, stp, sternopleuron; ume, upper mesipimeron.

## Biology

Eggs are laid in a great variety of aquatic habitats. Most *Culex* species breed in ground collections of water such as pools, puddles, ditches, borrow pits, and rice fields, a few including *C. p. fatigans* occur in man-made container habitats such as tin cans, water receptacles, bottles and storage tanks. Only a few species occur in tree holes and even fewer in leaf axils. The most important species, *C. p. fatigans* which is a filariasis vector, breeds in water polluted with organic debris, such as rotting vegetation, household refuse and excreta, and larvae are commonly found in partially

blocked drains and ditches, soak-away pits, septic tanks, in village pots, especially the abandoned ones in which water is polluted and unfit for drinking. It is a mosquito that is associated with urbanisation and towns with poor and inadequate drainage and sanitation. Under these conditions its population increases rapidly.

Culex tritaeniorhynchus, an important vector of Japanese encephalitis, breeds prolifically in cleaner habitats, especially in rice fields; in southern Asia larvae are not uncommon in slightly polluted waters such as fish ponds which have had manure added.

C. p. fatigans and many other Culex species bite man and other hosts at night, and some like C. p. fatigans commonly rest indoors both before and after feeding, but they also often shelter in outdoor resting places.

### Notes on Aedes mosquitoes

## Distribution

World-wide, their range extends well into the northern and arctic areas where they can be vicious biters and serious pests of man and livestock.

## Eggs

Eggs are usually black, more or less ovoid in shape and are always laid singly (figure 4.9b). Careful examination shows that the egg shell has a distinctive mosaic pattern (figure 4.23). Eggs are laid on damp substrates just beyond the water line, such as on damp mud and leaf litter of pools, on the damp walls of clay pots, rock pools and tree holes.

Aedes eggs can withstand desiccation, the intensity and duration of which varies, but in many species they can remain dry but viable for many months. When flooded some eggs may hatch within a few minutes but others of the same batch may require prolonged immersion in water before they hatch, thus hatching may be spread over several days or weeks. Even when eggs are soaked for long periods some may fail to hatch because they require several soakings followed by short periods of desiccation before they can be induced to hatch. Even if environmental conditions are favourable, eggs may be in a state of diapause and will not hatch until this resting period is terminated. Various stimuli including reduction in the oxygen content of the water, changes in day length and illumination, and temperature etc. may be required to break diapause in Aedes eggs.

Many Aedes species breed in small container habitats (tree holes, plant axils etc.) which are susceptible to drying out, thus the ability of eggs to withstand desiccation is clearly advantageous, but this together with the property of Aedes eggs to hatch in instalments can create problems with controlling the immature stages (p. 69).

### Larvae

Aedes species have a short barrel-shaped siphon and only one pair of subventral tufts (figure 6.4) which



Fig 6.4 Terminal segments of a larva of an Aedes species showing short siphon with a single subventral tuft of hairs and enlarged comb and pecten scales.

never arise less than quarter distance from base of siphon. There are at least three pairs of setae in the ventral brush, and the antennae are not greatly flattened and there are no enormous setae on the thorax. These characters should separate *Aedes* larvae from most culicine genera, but not unfortunately from larvae of *Psorophora* and *Haemagogus*. Thus, larvae collected in North America fitting the above description could be either *Aedes* or *Psorophora*, while in Central and South America they could belong to any of these three genera.

Most American Aedes can be separated from *Psorophora* species by not having the mouth brushes modified for predation, and no Aedes has the comb teeth arising from a sclerotised plate. Also, most Aedes larvae have the comb teeth arranged in two or more rows, in Aedes species that do not have this arrangement the saddle does not encircle its abdominal segment (figure 6.4).

In Central and South America Aedes larvae can usually be distinguished from those of Haemagogus by *Aedes* species having either larger or more strongly spiculate antennae.

## Adults

Many, but not all, *Aedes* adults have conspicuous patterns on the thorax formed by black, white or silvery scales (figure 6.5), in some species yellow scales

cans and tyres. For example, Ae. aegypti breeds in village pots and water-storage jars placed either inside or outside houses, larvae occur mainly in those with clean water intended for drinking. In some areas Ae. aegypti also breeds in rock pools and tree holes. Aedes africanus, an African species involved in the sylvatic transmission of yellow fever, breeds mainly in tree holes and bamboo, whereas Aedes simpsoni, another



Fig 6.5 Dorsal surface of the thorax of adult Aedes mosquitoes showing pattern of black and white scales on the scutum and scutellum: (a) Aedes aegypti with typical 'lyre'-shaped marks, (b) Ae. albopictus and (c) Ae. vittatus.

are present. The legs often have black and white rings (figure 6.6a). Aedes aegypti, often called the yellow fever mosquito, is readily recognised by the 'lyre'shaped silver markings on the lateral edges of the scutum (figure 6.5a). Scales on the wing veins of Aedes mosquitoes are narrow, and are usually more or less all black except may be at the base of the wing. In Aedes the abdomen is often covered with black and white scales forming distinctive patterns, and in the female it is pointed at the tip and the cerci protrude (figure 6.6a). Additional characters are, spiracular area without scales or bristles (figure 6.3) (which separates adults from Psorophora species which have scales and bristles) postspiracular bristles present, pulvilli absent or hairlike (figure 6.7).

## **Biology**

Although some *Aedes* species breed in ground pools, many, especially tropical species, are found in natural or man-made container habitats such as tree holes, bamboo stumps, leaf axils, rock pools, village pots, tin African yellow fever vector, breeds almost exclusively in leaf axils, especially those of banana plants, yams and pineapples. Aedes albopictus, vector of dengue in south-east Asia, resembles Aedes aegypti in breeding principally in domestic containers. Larvae of Aedes polynesiensis occur in man-made and natural containers, especially split coconut shells, and larvae of Aedes pseudoscutellaris are found mainly in tree holes and bamboo stumps; both of these mosquitoes are important vectors of diurnal subperiodic bancroftian filariasis. Aedes togoi, a vector of bancroftian and brugian filariasis, breeds principally in rock pools containing fresh or brackish water.

The life-cycle from egg to adult can take as little as six to seven days but it is often 10-12 days, and much longer in temperate climates.

Adults of most *Aedes* species, including the yellow fever and filarial vectors, bite mainly during the day or early evening. Most biting occurs outdoors and adults usually rest outdoors although larval habitats may be water-storage jars placed inside houses. In some areas during the dry season adults may rest indoors as well as outdoors.



Fig 6.6 Aedes adult, (a) abdomen showing pattern of white scales and pointed tip (b) hind leg showing pattern of black and white bands (rings) of scales.



Fig 6.7 Terminal tarsal segments of adult mosquitoes showing central empodium and (a) toothed claws and pair of hairlike pulvilli and (b) simple claws and no pulvilli.

## Notes on Psorophora mosquitoes

## Distribution

This genus occurs only in North, Central and South America, where the species may be confused with *Aedes* mosquitoes.

## Eggs

Eggs are very similar to those of *Aedes* both in appearance and hatching behaviour. They are laid mainly on damp mud, leaf litter and soil which is destined to become flooded, for example at the edge of pools and marshy areas in meadows, in forests or woods, *Psorophora* are not container breeders. There is no simple way of distinguishing the eggs from those of *Aedes*.

## Larvae

Larvae have a single subventral tuft on the siphon arising, as in *Aedes* species, not less than quarter distance from base of the siphon. Unlike *Aedes* species many *Psorophora* have the mouth brushes composed of thick coarsely serrated setae adapted for predation (figure 6.8), and the comb usually arises from a sclerotised



Fig 6.8 Psorophora larva, (a) head of a larva showing pectinate mouth brushes and (b) three of the pectinate hairs from a mouth brush.

plate (figure 6.9). They also differ by having the comb teeth in a single and regular row, abdominal segment nine completely encircled by the sclerotised saddle and the ventral brush composed of many setal tufts which extend almost to the base of segment nine (figure 6.9).



Fig 6.9 Terminal segments of a larva of a *Psorophora* species, showing comb scales arranged on a plate, extensive ventral brush, and saddle encircling the segment.

## Adults

The thorax and legs are usually covered with black and white scales, and in the female the abdomen is pointed, and in general the adults often appear very similar to *Aedes* species. *Psorophora* adults, however, can be distinguished from *Aedes* species by the spiracular area having scales and one or more bristles (figure 6.3).

## Biology

*Psorophora* species breed mainly in ground collections of water such as rice fields, small pools formed in woods and forests and extensive marshy areas in meadows. The eggs can, like those of *Aedes*, withstand desiccation and hatch in instalments. Adults bite outdoors and mainly during the day. They are frequently very numerous in North America and may constitute a serious biting nuisance. Certain species, but mainly *Psorophora ferox*, are vectors of various viruses including certain of the encephalitis viruses.

*Psorophora* mosquitoes are less important medically than the other five culicine genera described in this chapter.

## Notes on Haemagogus mosquitoes

## Distribution

Found only in Central and South America.

## Eggs

Usually black and ovoid laid singly in tree holes and other natural container habitats and occasionally in man-made ones. There is no simple method of distinguishing *Haemagogus* eggs from those of *Aedes* and *Psorophora*, except that *Psorophora* eggs are rarely deposited in container habitats.

## Larvae

Larvae have a single subventral tuft arising, as in *Aedes* larvae, not less than quarter distance from the base of the siphon. They resemble *Aedes* larvae but can usually be separated by the following combination of characters. Antennae short and either without or with only very few spicules, ventral brush arising from a sclerotised boss (figure 6.10), if the saddle does not com-



Fig 6.10 Terminal segments of a larva of a Haemagogus species showing comb scales arranged on a plate and ventral brush arising from a sclerotised boss.

pletely encircle abdominal segment nine then the comb teeth are on a small sclerotised plate. From *Psorophora* they can usually be distinguished by their much shorter antennae.

## Adults

Adults are very colourful mosquitoes that can be easily recognised by the presence of broad, flat and bright metallic blue, red, green or golden coloured scales, covering the dorsal part of the thorax, and by the exceptionally large anterior pronotal thoracic lobes (figure 6.11a). They can be separated from *Sabethes*, which are also colourful mosquitoes with large anterior pronotal lobes, by the absence of scales or bristles on the spiracular area (figure 6.3). *Haemagogus* species do not have paddles on the legs, a conspicuous feature of many, but not all, species of *Sabethes*.

## Biology

Eggs can withstand desiccation. Larvae occur mostly in tree holes and bamboo stumps, but also in rock pools, split coconut shells and sometimes in assorted domestic containers. They are basically forest mosquitoes. Adults bite during the day, but mostly in the tree tops where they feed on monkeys, but under certain environmental conditions, such as at edges of forests, during tree felling operations or during the dry season, they may descend to the forest floor to bite man and other hosts. The most important species are Haemagogus spegazzinii falco (= H. janthinomys) and H. capricornii, both are sylvan vectors of yellow fever.

## Culicine mosquitoes

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## Notes on Sabethes mosquitoes

## Distribution

Found only in Central and South America.

## Eggs

Very little is known about the eggs of Sabethes species, but it appears that they are laid singly, have no prominent surface features such as bosses or sculpturing, and are incapable of withstanding desiccation. The eggs of Sabethes chloropterus, a species sometimes involved in the sylvatic cycle of yellow fever, are rhomboid in shape and can thus be readily identified from most other culicine eggs (figure 6.11b).

#### Larvae

The siphon has many hairs placed ventrally, laterally or dorsally, it is relatively slender and is at least three and a half times as long as the saddle on abdominal segment nine (figure 6.12). Sabethes larvae can usually be



Fig 6.12 Terminal segments of a Sabethes larva showing a single pair of hairs in ventral brush, numerous simple hairs on the siphon and absence of a pecten on the siphon.

separated from other mosquito larvae by having only one pair of setae in the ventral brush which are as long or nearly as long as the lower caudal setae, comb teeth arranged in a single row or at most with three or four detached teeth and the absence of a pecten.



Fig 6.11 Sabethes mosquito, (a) diagram of head and thorax to show the large pronotal lobes forming a 'collar' behind the head, (b) an egg and (c) hind leg showing hairs forming a 'paddle'.

## Adults

The dorsal surface of the thorax is covered with appresssed iridescent blue, green and red scales, the anterior pronotal lobes are very large (figure 6.11a) and the prealar bristles are absent (figure 6.3). Adults of many species have one or more pairs of tarsi with conspicuous paddles composed of hairs, their presence immediately distinguishes *Sabethes* from all other mosquitoes (figure 6.11c). Species which lack these paddles may be confused with *Haemagogus*, but *Sabethes* have scales on the spiracular area (figure 6.3) whereas in *Haemagogus* there are no spiracular scales or bristles.

## Biology

Larvae occur in tree holes and bamboo stumps, and a few species are found in leaf axils of bromeliads and other plants. They are forest mosquitoes. They bite during the day, mainly in the tree canopy, but like *Haemagogus* adults may descend to ground level at certain times to bite man and other hosts. *Sabethes chloropterus* has been incriminated as a sylvan vector of yellow fever.

## Notes on Mansonia mosquitoes

## Distribution

Principally a genus of wet tropical areas, but certain species are found as far north as Sweden and others as far as south as Tasmania. Older classifications referred to this genus under the name of Taeniorhynchus. Some modern classifications recognise Mansonia and Coquillettidia as distinct genera, but in the present book only one genus Mansonia is recognised, which contains four subgenera – Coquillettidia, Mansonioides, Rhynchotaenia and Mansonia, the medically most important species belong to the former two subgenera.

## Eggs

Some species, such as those belonging to the subgenus *Coquillettidia*, lay their eggs on the water surface in the form of egg rafts. These often resemble *Culex* rafts, but in certain species the eggs are arranged in fewer rows so that elongated rafts are produced (figure 6.13) which can be distinguished from the less elongated *Culex* egg rafts (figures 4.9c and 4.23). The individual eggs are cylindrical, brown or brownish-black and usually with some sculpturing or small bosses. Egg rafts of the subgenus *Coquillettidia* are found only in more or less permanent collections of water having rooted or free



Fig 6.13 Egg raft of a Mansonia species of the subgenus Coquillettidia.

floating vegetation. Eggs cannot withstand desiccation.



Fig 6.14 Eggs of a Mansonia species of the subgenus Mansonioides, (a) egg mass stuck to undersurface of a leaf of an aquatic plant and (b) a single egg.

Other species, such as those of the subgenus *Mansonioides*, lay their eggs in sticky compact masses, often arranged as circular rosettes, which are glued to the undersurfaces of floating vegetation (figures 4.23 and 6.14). Individual eggs are dark brown-black, cylindrical but with tube-like extensions apically which are usually darker than the rest of the eggs (figure 6.14b). Eggs cannot withstand desiccation and hatch within a few days.

## Larvae

Larvae of the genus *Mansonia* are very easily recognised because they have specialised siphons adapted for piercing aquatic plants to obtain air (figure 6.15b, c). The siphon is conical with the apical part dark and heavily sclerotised, it has prehensile hairs and serrated processes for inserting into plants. A few larvae of the genus *Mimomyia* (formerly regarded as a subgenus of *Ficalbia*) also have siphons modified for obtaining air from plants, but they are not so highly modified and serrated processes are absent.

## Adults

Adults frequently have the legs (figure 6.16c), palps, wings and body covered with a mixture of dark (usually brown) and pale (white or creamy) scales giving the insect a rather dusty appearance. The speckled pattern of dark and pale scales on the wing veins, gives the wings the appearance of having been sprinkled with salt and pepper (figure 6.16a) and provides a useful character for identifying the adults. Closer examination shows that the scales on the wing veins are very broad and often asymmetrical giving them an almost heart-shaped appearance (figure 6.16b), in other mosquitoes these scales are longer and narrower. Additional features are that all tarsal claws are simple and pulvilli are absent.

## Biology

Eggs are laid either in rafts floating on the water surface or in egg masses glued to the undersurface of vegetation and hatch within a few days. All larval habitats have aquatic vegetation either rooted such as grasses,



Fig 6.15 Immature stages of Mansonia species, (a) pupa with respiratory trumpets inserted into an aquatic plant to obtain air, (b) larva with siphon inserted into a plant, (c) siphon of a larva showing serrated structures and (d) respiratory trumpet of a pupa.


Fig 6.16 Mansonia adult, (a) wing showing distribution of dark and pale scales on the wing veins which gives the wing a 'pepper and salt' appearance, (b) a few wing scales to show their broad shape, and (c) hind leg showing arrangement of dark and pale scales.

rushes and reeds or floating such as *Pistia stratiotes*, *Salvinia*, and *Eichhornia*. Larvae occur in permanent collections of water such as swamps, marshes, ponds, borrow pits, grassy ditches, canals, backwaters of rivers and even in the middle of rivers if they have floating vegetation. Wherever the water lettuce, *Pistia stratiotes*, is present breeding by *Mansonia* should be suspected.

Pupae have elongated and pointed respiratory trumpets which are modified for inserting into aquatic plants for respiration (figure 6.15a, d).

Larvae and pupae only detach themselves from plants and rise to the surface of the water if they are disturbed. Because they are more or less permanently attached to plants the immature stages are frequently missed in larval surveys, and it may be difficult to identify breeding places with certainty, unless special collecting procedures are undertaken, such as the collection of plants to which the immature stages are thought to be attached. It is often difficult to control breeding of *Mansonia* species by conventional insecticidal applications, because of difficulties of getting the insecticides to the larvae which may be some distance below the water surface, (see pp. 69-70).

Adults of most species bite during the night, but some species are day-biters. After feeding most *Mansonia* species rest outdoors but a few rest indoors. There are no species that are cosmotropical in distribution or of medical importance. The most important vector species belong to the subgenus Mansonioides, such as M. uniformis a vector of nocturnal periodic Brugia malayi in the Indian subcontinent and southeast Asia, and also of Wuchereria bancrofti in New Guinea, and M. annulifera, M. annulata and M. indiana, vectors of nocturnal subperiodic B. malayi in southeast Asia and sometimes also in the Indian subcontinent. In Africa filariasis (W. bancrofti) is not transmitted by Mansonia species, but several species, especially M. africana and M. uniformis, are vectors of arboviruses.

# Medical importance

#### **Biting nuisance**

In several areas of the world much money is spent on mosquito control, not so much because mosquitoes are vectors of disease but because they are such troublesome biters. For example, some of the best organised mosquito control operations are in North America where more money is spent on killing culicine mosquitoes than is expended in most tropical countries where they are transmitting filariasis and arboviruses. In northern temperate and subarctic parts of the Nearctic and Palearctic regions much greater numbers



Fig 6.17 Diagrammatic representation of the sylvatic, rural and urban cycles of transmission of yellow fever in Africa.

of *Aedes* mosquitoes can be encountered biting man than in tropical countries. Although they are not transmitting diseases to man in these areas they can make life outdoors almost intolerable.

# Yellow fever

The arbovirus causing yellow fever occurs in Africa and tropical areas of the Americas, it does not occur in Asia or elsewhere, although mosquitoes capable of transmitting the disease occur in many countries. Yellow fever is a zoonosis, being essentially a disease of forest monkeys which under certain conditions can be transmitted to man.

In Africa the yellow fever virus occurs in certain cercopithecid monkeys inhabiting the forests and it is transmitted amongst them mainly by Aedes africanus. This is a forest dwelling mosquito that breeds in tree holes and bites mainly in the forest canopy soon after sunset, that is just in the right place and at the right time to bite monkeys going to sleep in the tree tops. This sylvatic, forest or monkey cycle as it is sometimes called maintains a reservoir of infection in the monkey population (figure 6.17). In Africa monkeys are little affected by yellow fever, but occasionally they die. Some species of monkeys involved in the forest cycle, such as the red-tailed guenon, descend from the trees to steal bananas from farms at the edge of the forest. In this habitat the monkeys get bitten by different mosquitoes including Aedes simpsoni, a species which bites during the day at the edge of forests and which breeds in leaf axils of bananas, plantains and other plants such as Colocasia and pineapples. If the monkeys have viraemia, that is yellow fever virus circulating in their peripheral blood, Ae. simpsoni becomes infected, and if the mosquito lives long enough it can transmit yellow fever to other monkeys or more importantly to man. This transmission cycle occurring in clearings at the edge of the forest involving monkeys, Ae. simpsoni and man is sometimes referred to as the rural cycle (figure 6.17). When man returns to his village or travels to towns he is bitten by different mosquitoes, including Ae. aegypti, a domestic species breeding mainly in man-made containers such as water-storage pots, abandoned tin cans and vehicle tyres. If man is showing viraemia Ae. aegypti becomes infected and yellow fever is then transmitted amongst the human population by this species, this is the urban cycle of yellow fever transmission (figure 6.17).

It is possible for man to become infected in the forest by bites of *Ae. africanus*, but the likelihood of man acquiring yellow fever by a canopy feeding mosquito is remote. The epidemiology of yellow fever is complicated by differences in the feeding behaviour of different populations of *Ae. aegypti*, but more especially Ae. simpsoni. In some areas for example, yellow fever may be circulating amongst the monkey population yet rarely get transmitted to man because local populations of Ae. simpsoni are predominantly zoophilic. Other primates in Africa such as Galago species may also be reservoirs of yellow fever.

In Central and South America the yellow fever cycle, although similar, differs in certain aspects (figure 6.18). As in Africa it is a disease of forest monkeys mainly Cebid ones, and it is transmitted amongst them by forest dwelling mosquitoes, the most important being Haemagogus spegazzinii falco (=H. janthinomys) other Haemagogus species, Ae. leucocelaenus and Sabethes chloropterus, although this last species appears to be an inefficient vector. These are arboreal mosquitoes that bite in the forest canopy and breed in tree holes. New World monkeys are more susceptible to yellow fever than African monkeys and they frequently become sick and die. When people enter the jungle to cut down trees for timber mosquitoes which are normally biting monkeys at tree canopy height may descend and bite man, and if they are infected man develops yellow fever. The disease is then spread from man to man in villages and towns, as in Africa, by Aedes aegypti (figure 6. 18).

The intrinsic incubation period of yellow fever in man is about four to five days, usually a little less in monkeys. Thus, after four or five days the virus appears in the peripheral blood, that is viraemia is produced, and this occurs irrespectively of whether monkeys or man are showing overt symptoms of the disease. Viraemia lasts only two days, after which the virus disappears from the peripheral blood never to return and the individual is immune. Monkeys and man are therefore infective to mosquitoes for only about two days in their entire lives. A relatively high titre of yellow fever (and also any other arbovirus) is needed before it can pass across the gut cells into the haemolymph from where it invades many tissues and organs of the mosquito's body including the salivary glands, where virus multiplication occurs. This is the extrinsic cycle of development and takes from 5-30 days depending on temperature, species of arbovirus and mosquito host. A mosquito must therefore live sufficiently long before it becomes infective and capable of transmitting yellow fever or any other virus.

Mosquito dissections cannot show whether mosquitoes are infected with yellow fever, or any other virus. To determine whether mosquitoes are infected with arboviruses several females are ground up and inoculated into young mice or other susceptible hosts, and then after an incubation period any viruses developing in these hosts are passaged to others. Arbovirus isolations and identifications are usually carried out in specially equipped virology laboratories.



#### Dengue

A number of different viruses (dengue types 1, 2, 3, 4, 5, and ?6) are responsible for true dengue, a disease (or diseases) that was first reported in epidemic form in India and Java, but subsequently from many areas of the world including southern U.S.A., West Indies, Central and South America, Greece, West and East Africa, south-east Asia, Hawaii and Australia. More recently a more severe form which tends to cause infant mortality termed haemorrhagic dengue has appeared in many areas of south-east Asia, such as Thailand, Malaysia, Vietnam, Laos, the Philippines and also India. Both the classical and haemorrhagic forms are transmitted principally by Ae. aegypti, but in south-east Asia Ae. albopictus, and in Uganda Ae. simpsoni, are less important vectors. Mosquitoes of the Ae. scutellaris group may also transmit dengue in the Pacific islands and New Guinea. There are no known animal reservoirs, but it is possible that monkeys and other primates are involved.

#### The encephalitis viruses

Several viruses transmitted by mosquitoes predominantly in the Americas but also elsewhere, are included in this category, for example California encephalitis (CAL) group viruses, Eastern Equine encephalitis virus (EEE), Western Equine encephalitis virus (WEE), Venezuelan Equine encephalitis virus (VEE), St Louis encephalitis (SLE) and Japanese encephalitis virus (JE). With the exception of California encephalitis (CAL) all these viruses involve a zoonosis with birds, especially herons and passerines feeding and /or nesting near marshes. The three equine encephalitis viruses are also very virulent in horses. These diseases are sometimes referred to as encephalomyelites viruses, and sometimes the word equine is omitted from their names, for example VEE may be called Venezuelan encephalitis.

There are about 12 viruses in the California group of arboviruses, most of which occur in North America but a few are found in South America (Bocas, Melao), Africa (Tahyna) and Europe (Tahyna, Inkoo). They are mainly transmitted by *Aedes* and *Culex* mosquitoes. In the U.S.A. California encephalitis virus (CAL) is spread by *Aedes* in particular by *Ae. melanimon*. In addition to man vertebrate reservoirs of the California group viruses include rabbits, squirrels and other rodents, and occasionally bats.

Western Equine encephalitis (sometimes called 'sleeping sickness', but this term is to be avoided as it may be confused with African sleeping sickness caused by trypanosomes parasites transmitted by tsetse flies (chapter 11)) and St Louis encephalitis viruses are widely distributed in the U.S.A. and extend into northern parts of South America. They are basically arboviruses infecting birds and are transmitted principally by *Culex tarsalis*, a rice field breeding mosquito, other *Culex* mosquitoes and *Culiseta* (= *Theobaldia*) *inornata* (the genus *Culiseta* is not discussed in this book). These mosquitoes transmit the disease to birds and also to man or horses. In towns SLE infects not only wild birds but also poultry and is spread by several *Culex* species including *C. nigripalpus* and *C. pipiens pipiens*. Bats may also be involved in the zoonotic cycle.

Eastern Equine encephalitis occurs mainly in the eastern area of the U.S.A., but extends down into South America, and is also found in parts of Asia, Europe and Australasia. It is principally a disease of birds and in North America is spread mainly by Culiseta melanura and Mansonia perturbans; it is transmitted to man and horses, and sometimes also birds, by various Aedes species. Venezuelan Equine encephalitis virus is probably the most important of these encephalitis viruses. It is found in southern U.S.A. through Central to South America and is transmitted amongst birds, and occasionally bats by several Culex and Aedes mosquitoes, but in Central and South America the virus occurs in forest rodents, marsupials and monkeys. Man and horses become infected. Other mosquito genera, especially Mansonia species, have been incriminated in spreading VEE, and also Simulium flies (chapter 7).

Japanese encephalitis occurs not only in Japan but in India, China, Malaysia, Korea and other areas of south-east Asia. The basic transmission cycle involves birds, mainly herons, egrets and ibises, but pigs are also important reservoir hosts particularly in warm areas, especially as they develop a high viraemia. Transmission to birds, man and pigs is mainly by *Culex tritaeniorhynchus* which is a very common rice field breeding mosquito. *Culex gelidus* probably maintains the virus in the pig to pig. transmission. Bats also become infected.

All these encephalitic viruses have a complex epidemiology involving several different transmission cycles having different animal reservoirs and mosquito vectors.

The viraemia produced in man by VEE, SLE, JE and EEE and sometimes also by WEE is so low that the disease cannot be transmitted from man to man or from man to any other susceptible hosts by mosquitoes, thus man is a 'dead-end' host in the cycle. Horses are similarly dead-end hosts in the encephalitis viruses infecting them.

#### Other arboviruses

There are many other arboviruses transmitted to man by mosquitoes, such as Chikungunya (CHIK) in East Africa and India; West Nile (WN) in Africa, Europe, Israel and Asia; Bunyamwere (BUN) in Africa; Ilheus (ILH) in Brazil, Trinidad and Panama, and Murray Valley encephalitis (MVE) in Australia. A few mosquito-borne arboviruses are found in Europe as far west as France, but there is no evidence that any arboviruses are transmitted by mosquitoes in Britain. Some arboviruses are transmitted by *Psorophora* species and by genera of mosquitoes not referred to in this book.

Transovarial transmission is sometimes an important phenomenon with rickettsiae and arboviruses infecting ticks and mites (chapters 21 and 23), but there is little evidence that it is epidemiologically important in mosquitoes. Nevertheless, it has been demonstrated that certain arboviruses (especially California group viruses) invade the ovaries of mosquitoes and may persist in the eggs after they have been laid and get carried over to the resultant larvae and adults.

# Filariasis (table 6.1)

The development of the filarial worms in the mosquito vectors is briefly described in the preceding chapter (p. 50).

i) Wuchereria bancrofti occurs throughout the tropics and also in certain subtropical areas. It is essentially an urban disease, there are no animal reservoirs, the parasites can develop only in man and mosquitoes. The nocturnal periodic form of Wuchereria bancrofti is transmitted by various Anopheles species (chapter 5) and throughout most of its distribution by Culex pipiens fatigans. This mosquito occurs throughout the tropics and breeds mainly in man-made containers and polluted waters such as septic tanks, cess pits, polluted drains and ditches, pots and water-storage jars especially those having organic pollution. It is a mosquito that has increased in many towns due to increasing urbanisation and the resultant proliferation of insanitary collections of water. It is principally a night biter. After feeding adults may rest in houses. In New Guinea Mansonia uniformis and night biting Culex species are also vectors of W. bancrofti, but in contrast Mansonia species do not transmit filariasis in Africa. In Japan and China, Aedes togoi which breeds in rock pools containing brackish water and also in rainfilled receptacles such as pots and cisterns, is also a vector of nocturnal periodic bancroftian filariasis although it is more usually thought of as being a vector of brugian filariasis. It bites man early in the evenings around sunset. In the Philippines Aedes peocilus is the most important vector of nocturnal periodic W. bancrofti. Larvae occur in leaf axils of banana, plantain and Colocasia plants; adults bite man in the early part of the night mainly indoors, but also sometimes outdoors. Adults rest outdoors after feeding.

The diurnal subperiodic form of W. bancrofti occurs in Polynesia, where the nocturnal periodic form is absent. The most important vector is Aedes polynesiensis, a day-biting mosquito that feeds mostly outdoors but may enter houses to bite; adults rest almost exclusively outdoors. Larvae occur in natural containers such as split coconut shells, leaf bracts and also in crab holes and in man-made containers such as discarded tins, pots, vehicle tyres, canoes and drums. Aedes pseudoscutellaris is another outdoor day-biting mosquito that is a vector of diurnal subperiodic bancrofti in Fiji. It mainly breeds in tree holes and bamboo stumps but larvae are also found in crab holes. In New Caledonia Ae. polynesiensis is absent and Aedes vigilax which breeds in brackish or fresh water in rock pools and ground pools is the most important vector. Adults rest and feed outdoors mainly during the dav.

In Thailand a nocturnal subperiodic form of W. bancrofti occurs that is transmitted by the Aedes niveus group of mosquitoes.

It should be noted that although several Aedes mosquitoes are vectors of filariasis, especially the bancroftian form, Ae. aegypti is not considered to be a vector of filariasis to man.

Natural infection rates of mosquitoes with infective larvae of W. bancrofti vary from about 0.1-5 per cent, depending greatly on the vector species and local conditions.

ii) The nocturnal periodic form of Brugia malayi occurs throughout most of Asia, from India, Sri Lanka, Malaysia, Indonesia, Borneo, China to parts of Japan. There do not appear to be any important animal reservoirs, although cats may possibly be infected. It is transmitted mainly by night-biting Mansonia mosquitoes, such as M. indiana, M. annulifera, M. uniformis and also various Anopheles species (chapter 5). These Mansonia species breed in more or less permanent collections of water having floating or rooted vegetation, such as swamps and ponds. Adults bite man mainly outdoors and rest outdoors afterwards, but they will also bite indoors and rest indoors in some areas. In China, Korea and Japan Ae. togoi is a vector of nocturnal periodic B. malayi.

The nocturnal subperiodic form of *B. malayi* occurs in Malaysia, Brunei, Thailand, Vietnam and the Philippines and is transmitted by *Mansonia* mosquitoes mainly by *M. annulata*, *M. dives*, *M. bonneae* and *M. uniformis*. Larvae occur in habitats with much vegetation, such as swampy forests. Adults bite mainly at night but also during the daytime, especially species such as *M. dives* and *M. bonneae*. The subperiodic form of *B. malayi* is essentially a disease of swamp monkeys, especially the leaf monkeys (*Presbytis* spp.), but domestic and wild cats such as civet cats, and pangolins appear to be minor reservoirs of infection.

Species and forms of filariasis	Geographical distribution	Vectors	Zoonotic reservoir
Wuchereria bancrofti (a) Nocturnal periodic	Throughout tropics (but not Polynesia)	Anopheles species, including many of the malaria vectors of different areas	None
	,, ,, ,, ,, New Guinea China and Japan Philippines	Culex pipiens fatigans Mansonia uniformis Aedes togoi Aedes peocilus	
<ul><li>(b) Diurnal subperiodic</li><li>(c) Nocturnal subperiodic</li></ul>	Polynesia Fiji New Caledonia Thailand	Aedes polynesiensis Aedes pseudoscutellaris Aedes vigilax Aedes niveus group	None
<ul> <li>Brugia malayi</li> <li>(a) Nocturnal periodic (principally open swamps)</li> </ul>	Asia, from India to Japan	Anopheles species including many malaria vectors of dif- ferent areas	Not import ant, poss- ibly some exist
	,, ,, ,, ,, ,, ,, China, Korea and Japan	Mansonia annulifera, M. uniformis, M. indiana etc. Aedes togoi	
(b) Nocturnal subperiodic (mainly in swampy forests)	Malaysia, Brunei, Philippines, Thailand, Vietnam	Mansonia dives, M. bonneae, M. annulata, M. uniformis etc.	Monkey, es- pecially leaf- monkeys ( <i>Presbytis</i> spp.), civet cats and pangolins
Brugia timori (a) Nocturnal periodic	Timor island in Indonesia	Anopheles species of the barbirostris grp, probably An. campestris	None

Table 6.1 Summary of mosquito-borne filariasis vectors

Table 6.1 summarises the vectors of mosquito-borne filariasis.

Natural infection rates of mosquitoes with infective larvae of *B. malayi* range from about 0.1-2 or 3 per cent, which are slightly lower rates than with *W. bancrofti*, but these vary according to mosquito species and local conditions.

As mentioned in chapter 5 the discovery of filarial worms in mosquitoes does not necessarily imply that they are vectors of either *W. bancrofti* or *B. malayi* because mosquitoes are also vectors of several other filarial parasites of animals.

# Control

The use of suitable repellents, mosquito nets and

mosquito screening of houses and other personal protection measures discussed in chapters 4 and 5 can give some relief from culicine mosquitoes. It is, however, often more difficult to obtain protection from culicines than anophelines because many of them bite outdoors and during the daytime. Spraying the interior surfaces of houses with residual insecticides as practised for *Anopheles* control, is not usually applicable to culicines, because most species do not rest predominantly in houses. The main method of attack involves larval control, although sometimes aerial ULV applications are used to kill adult culicine mosquitoes.

Control measures against Ae. aegypti, Ae. polynesiensis and Ae. albopictus, species which breed mainly in man-made containers in both rural and urban areas, are often aimed at reducing the numbers of larval habitats, that is control by source reduction. Thus, people are encouraged not to store water in pots inside or outside houses or allow water to accumulate in discarded tin cans, bottles and vehicle tyres etc. However, persuading people to cooperate in reducing peridomestic breeding of these vectors is often unsuccessful unless local legislation is strictly enforced. Piped water supply to houses can do much to reduce *Ae. aegypti* breeding.

In situations where source reduction is not feasible, insecticidal applications to breeding sites can be attempted. Although insecticidal solutions and emulsions will kill Aedes larvae they will usually have no effect on their dry but viable eggs which have been deposited at the edges of larval habitats and which will hatch when the water level rises and floods them. Aedes, and also Psorophora, mosquitoes breeding in pools, ponds and marshy areas can be controlled by ground-based or aerial applications of granular organochlorine or organophosphate insecticides, either before or after habitats have become flooded, that is pre- or post-flood treatments. Insecticidal granules landing on dry or muddy ground remain more or less inactive until the habitats become flooded. When this occurs previously dry aedine eggs hatch but at the same time flooding results in the release of insecticide from the granules which kills the newly hatched larvae. This technique helps to overcome the problem of controlling aedine mosquitoes, such as Aedes and Psorophora species, whose eggs may hatch in instalments over extended periods after flooding. The more persistent the insecticides incorporated in these granules or pellets the longer effective control can be achieved. Organochlorines, especially dieldrin, are particularly suitable, but their use may be banned or discouraged because of their extreme persistence in the soil, plant and animal tissues. For this reason most granular preparations used to control Aedes larvae are formulated from organophosphates, such as chlorpyrifos (Dursban) and temephos (Abate).

When insecticides are applied to kill mosquitoes breeding in water used for drinking it is essential that they have extremely low mammalian toxicity and impart no taste or odour to the water. The only insecticide that can safely be recommended for treating such water is temephos. Granular formulations of this insecticide can remain effective in killing *Ae*. *aegypti* larvae for above five weeks after their application.

In many countries Ae. aegypti has developed insecticide resistance to DDT, dieldrin and HCH, and sometimes also to organophosphate insecticides such as malathion; in Thailand Ae. aegypti is resistant to the synthetic pyrethroid bioresmethrin. Insecticides that are commonly used for the control of Ae. aegypti larvae include temephos (Abate), chlorpyrifos (Dursban), fenthion (Baytex), malathion and carbaryl (Sevin).

Ground-based or aerial ULV applications of insecticides such as malathion, naled (Dibrom), chlorpyrifos (Dursban), propoxur (Arprocarb), fenitrothion (Sumithion) and synthetic pyrethroids such as bioresmethrin or resmethrin have been used to kill adult *Ae. aegypti*. Aeroplane ULV spraying has on several occasions helped to control *Ae. aegypti* during dengue outbreaks. In emergencies such as dengue or yellow fever epidemics several control methods are usually employed simultaneously to kill the vectors.

Culex pipiens fatigans, an important filariasis vector, can often best be controlled by improving sanitation and installing modern sewage systems, but often this is not feasible and insecticidal measures have to be employed. In many areas C.p. fatigans has developed resistance to the organochlorine insecticides, and resistance to organophosphate compounds is rapidly increasing, and this consequently limits the insecticides that can be effectively used. Larval habitats should be sprayed at weekly intervals. Relatively large dosage rates usually have to be applied because most insecticides are less effective in the presence of organic pollution, which is characteristic of most C. p. fatigans breeding places. In the U.S.A. aerial ULV spraying has frequently been used against C. pipiens and various vectors of the encephalitis viruses. Some important encephalitis vectors, such as C. tarsalis in the U.S.A. and C. tritaeniorhynchus in many parts of Asia, have become resistant to DDT, dieldrin and HCH and in addition several organophosphate compounds.

Mansonia mosquitoes are usually controlled by removing or killing the aquatic weeds upon which the larvae and pupae depend for their oxygen requirements. Weeds such as Pistia stratiotes can sometimes be manually removed from small areas such as borrow pits, alternatively they can be sprayed with herbicides, such as paraquat, diquat, 2,4-dichlorophenoxyacetic acid (2,4-D), dichlobenil and 4-chloro-2-methylphenoxyacetic acid (MCPA) or pentachlorophenol (PCP). The last herbicide has proved exceptionally good in killing Salvinia, an aquatic weed frequently associated with Mansonia breeding. Altering aquatic habitats by the removal or destruction of weeds may result in ecological changes that allow the habitat to be colonised by mosquito species that were previously excluded by the dense covering of weeds

If insecticides are used to control Mansonia larvae, then granules or pellets are more suitable than liquid formulations because they can penetrate vegetation and sink to the bottom of breeding places and release their chemicals through the water. However, species such as *M. dives* and *M. bonneae*, which are important vectors of brugian filariasis breed in extensive swampy forests and are almost impossible to control because of the large and often inaccessible areas involved.

The spread of insecticide resistance amongst many

culicine vectors has caused renewed interest in some of the older control methods, such as the use of high spreading oils and granular preparations of Paris green (chapters 4 and 28 for greater details).

# 7 Blackflies (Order Diptera: Family Simuliidae)

#### Species

There are some 12 genera of Simuliidae (some authorities recognise more), but only three, Simulium, Prosimulium and Austrosimulium, contain regular manbiting species. Medically, by far the most important genus is Simulium as it contains important vector species such as Simulium damnosum\*, S. neavei, S. ochraceum, S. metallicum and S. callidum.

# Distribution

Blackflies have a world-wide distribution. Of the three anthropophilic genera, *Austrosimulium* is restricted to the Australasian region, *Prosimulium* is basically a genus of northern temperate regions but a few species occur in Africa, Central and South America and Australia. Species of *Simulium* occur in all parts of the world, except New Zealand, Hawaii and some minor island groups.

#### **Medical** importance

Certain species in Africa (S. damnosum and S. neavei) and Central and South America (S. ochraceum and S. metallicum) transmit to man the parasitic nematode Onchocerca volvulus, which causes onchocerciasis (river blindness). In Brazil S. amazonicum has been found infected with filariae indistinguishable in their vector stages and microfilariae from Mansonella ozzardi, a filarial parasite that is usually regarded as nonpathogenic, and which is normally transmitted to man elsewhere in Central and South America by Culicoides species (chapter 9).

#### Simulium species

## External morphology

#### Adults (figure 7.1)

The Simuliidae are commonly known as blackflies, but in some areas, in particular Australia, they may be called sandflies. As explained in chapter 8 this latter terminology is confusing and best avoided, because biting flies of the family Ceratopogonidae and flies of the subfamily Phlebotominae are also called sandflies.

Adult blackflies are quite small, about 1.5-4 mm long, relatively stout-bodied, and when viewed from the side have a rather humped thorax. As their vernacular name indicates they are usually black in colour, but many have contrasting patterns of white, silvery or yellowish hairs on their bodies and legs, while others may be predominantly or largely orange or bright yellow.

The head has a pair of large compound eyes which are separated on top of the head in females (a condition known as dichoptic) (figure 7.2a), whereas in the males the eyes occupy almost all of the head and touch on top of it and in front above the bases of the antennae (a condition known as holoptic) (figure 7.2b). The antennae are short and stout, distinctly segmented but without long hairs. The mouthparts are short and relatively inconspicuous but the five-segmented maxillary palps, which arise at their base, hang downwards and are easily seen (figure 7.1). Only females bite. The biting fascicle consists of a labrum with apical teeth, a pair of mandibles and a pair of maxillae both having fine teeth at their tips, and the hypopharynx. Behind these biting and piercing mouthparts is the relatively

<sup>\*</sup> Formerly regarded as a single species called *Simulium damnosum* but now known to consist of at least 25, possibly more, distinct species or cytotypes, the majority of which are more or less indistinguishable on external morphology. These species, however, can be separated by cytogenetical, and some by biochemical, methods. Some of these species differ in their behaviour and ecology and also in their efficiency as onchocerciasis vectors. For convenience and simplicity they are all referred to in this book as *S. damnosum*.



Fig 7.1 Lateral view of an adult simuliid.



Fig 7.2 Front view of a head of (a) a simuliid female showing dichoptic eyes and (b) a male showing holoptic eyes.

large labium which terminates in a pair of fleshy labella. The arrangement and morphology of the mouthparts is similar to those of the biting midges (Ceratopogonidae, chapter 9). The mouthparts are short and broad and do not penetrate very deeply in the host's tissues. The teeth on the labrum stretch the skin, and the rasp-like action of the maxillae and mandibles cuts through the skin and ruptures the fine blood capillaries. The small pool of blood produced is then sucked up by the flies. This method of feeding is ideally suited for picking up the microfilariae of Onchocerca volvulus which occur in man's skin not blood.

The thorax is covered dorsally with very fine and appressed hairs, which can be black, white, silvery, yellow or orange and may be arranged in various patterns. The relatively short legs are also covered with very fine and closely appressed hairs, and may be unicolorous or have contrasting bands of pale and dark colour. The wings are characteristically short and broad, and lack scales or prominent hairs. Only the veins near the anterior margin are well developed; the rest of the wing is membraneous and has an indistinct venation (figure 7.1). The wings are colourless or almost so. When at rest the wings are closed flat over the body like the blades of a closed pair of scissors.

The abdomen is short and squat and covered with inconspicuous closely appressed fine hairs. In neither sex are the genitalia very conspicuous. Blackflies are most easily sexed by looking at the eyes.

# Life-cycle

When first laid eggs are pale, often whitish, but darken to a brown or black colour. They are about 0.1-0.4mm long, more or less triangular in shape but with rounded corners, and have smooth unsculptured shells (figure 7.3a) which are covered with a sticky substance. They are always laid in flowing water, but the type of breeding place differs greatly according to species. Habitats can vary from small trickles of water, slow flowing streams, lake outlets, water flowing from dams to fast flowing large rivers and rapids. Some species prefer lowland streams and rivers while others are found in mountain rivers. In some species such as S. ochraceum, one of the South American vectors of onchocerciasis, eggs are scattered over the surface of flowing waters while females are in flight. In most species, however, ovipositing females alight on partially immersed objects such as rocks, stones and vegetation, to lay their eggs. Usually some 150-800 eggs are laid in sticky masses or strings on a level with or just below the water line on submerged objects. Females may crawl underneath the water and become completely submerged during oviposition. There may be a few favoured oviposition sites in a stream or river, resulting in many thousands of eggs from many females being found together. S. damnosum, for example, frequently has such communal oviposition sites.

Eggs of S. damnosum hatch within about one day, but in many other tropical species the egg stage lasts two to four days. Eggs of species inhabiting temperate and cold northern areas may not hatch for many weeks, and some species pass the winter as diapausing eggs.

There are six to eight larval instars. The mature larva is about 5-13 mm long, according to species, and easily distinguished from all other aquatic larvae (figure 7.3b). The head is usually black or almost so and has a prominent pair of feeding brushes (cephalic fans) while the weakly segmented, cylindrical body is often whitish, but may be darker or sometimes even green-



Fig 7.3 (a) Egg of a simuliid, (b) last instar larva of a simuliid species of the Simulium damnosum complex showing body covered with minute dark setae.

ish. The body is slightly swollen beyond the head and in most, but not all, species distinctly swollen towards the end of the body. The rectum has finger-like gills which on larval preservation may be extruded and visible as a proturberance from the dorsal surface towards the end of the abdomen. Ventrally, just below the head is a small pseudopod called the proleg which is armed with small circles of hooklets.

Larvae do not swim but remain sedentary for long periods on submerged vegetation, rocks, stones and other debris. Attachment is achieved by the posterior hook-circlet (anal sucker of many previous authors) tightly gripping a small silken pad which is produced by the larva's very large salivary glands and is firmly glued to the substrate. Larvae can nevertheless move about and change their position. This is achieved by alternately attaching themselves to the substrate by the proleg and the posterior hook-circlet, thus they move in a looping manner (figure 7.4). When larvae are disturbed they can deposit sticky saliva on a submerged object, release their hold and be swept downstream for some distance at the end of a silken thread. They can then either swallow the thread of saliva and regain their original position, or reattach at sites further down-



Fig 7.4 Diagram showing the method of progression of a simuliid larva.

stream. Larvae normally orientate themselves to lie parallel to the flow of water, with their heads downstream. They are mainly filter-feeders, ingesting with the aid of the large mouth brushes suspended particles of food, but a few species have predacious larvae and others are occasionally cannibalistic. Larval development may be as short as one to two weeks, depending on species and temperature, but in some species may be extended to several months and in some species larvae overwinter.

Mature larvae, which can be recognised by a blackish mark termed the gill spot (respiratory organ of the future pupa) on each side of the thorax (figure 7.3b), spin with the silk produced by the salivary glands, a protective slipper-shaped brownish cocoon: this is firmly stuck to submerged vegetation, rocks or other objects (figure 7.5). Its shape and structure varies greatly according to species. After weaving the cocoon the enclosed larva pupates. The pupa has a pair of usually prominent filamentous or broad thin-walled respiratory gills; their length and shape and number of filaments or branches provide useful taxonomic characters for species separation. These gills and the anterior part of the pupa often project from the entrance of the cocoon (figure 7.5). In both tropical and non-tropical countries the pupal period lasts only two to six days and is unusual in not appearing to be dependent on temperature. On emergence adults either rise rapidly to the water surface in a protective bubble of gas, which prevents them from being wetted, or they escape by crawling up partially submerged objects such as vegetation or rocks. A characteristic of many species is the more or less simultaneous mass emergence of thousands of adults. On reaching the water surface the adults immediately take flight.

The empty pupal cases with gill filaments still attached, remain enclosed in their cocoons after adults have emerged and retain their taxonomic value. Consequently, they provide useful information on the species of simuliids that have recently bred and



Fig 7.5 Dorsal view of a cocoon of a simuliid species which has broad thin-walled respiratory filaments on the pupa, and lateral view of a simuliid species which has filamentous pupal respiratory filaments.

successfully emerged from various habitats.

A few African blackfly species, such as Simulium neavei, have a very unusual aquatic existence. Their larvae (except the first instar) and pupae do not occur on submerged rocks or vegetation but on other aquatic arthropods such as the bodies of the immature aquatic stages (nymphs) of mayflies (Ephemeroptera) and various crustacea including fresh water crabs. Such an association is termed a phoretic relationship. Eggs, however, are never found on these animals or anywhere else, but it is likely that they are laid on submerged vegetation or stones (see also p. 76).

#### Adult behaviour

Both male and female blackflies feed on plant juices and naturally occurring sugary substances, but only females take blood-meals. Biting occurs out of doors at almost any time of the daylight hours, but each species may have its preferred times of biting. For example, in Africa S. damnosum has a biting peak in the morning and another in the afternoon, whereas in South America S. ochraceum bites predominantly early in the morning, between 0800-1000 hours. Many species seem particularly active on cloudy and overcast days and in thundery weather. Species may exhibit marked preferences for feeding on different parts of the body, for example S. damnosum feeds mainly on the legs whereas S. ochraceum prefers to bite the upper part of the body. When feeding on animals, adults crawl down the fur of mammals or feathers of birds to bite the host's skin and they may also enter the ears to feed.

Many species of blackflies feed almost exclusively on birds (ornithophilic) and others on non-human mammalian hosts (zoophilic), but several species also bite man. Some man-biting species seem to prefer various large animals such as donkeys or cattle and bite man only as a poor second choice, while others appear to find man an almost or equally attractive host. No species bites man alone. In many species sight seems important in host location, but host odours may also be important. After feeding, blood-engorged females shelter and rest in vegetation, on trees and in other natural outdoor situations until the blood-meal is digested, which in the tropics takes two to three days but in non-tropical areas may take three to eight days or longer, the speed of digestion depending mainly on temperature. Relatively little is known about blackfly longevity, but it seems that adults of most species live two to three weeks, but some individuals may live as long as about three months.

Female blackflies may fly considerable distances from their emergence sites to obtain blood-meals and may also be dispersed large distances by winds. For example, it is not exceptional for adults of *S. damnosum* to be found biting 60-100 km from their breeding sites. The long distances involved in dispersal have great relevance in control programmes, because areas freed from blackflies can be reinvaded from distant breeding sites.

In temperate and northern regions of the Palaearctic and Nearctic regions biting nuisance from simuliids is seasonal because adults die in the autumn and the new generations of adults do not appear until the following spring or early summer. Although in many tropical areas there is continuous breeding throughout the year, there may nevertheless be dramatic increases in population size during the rainy season.

#### Medical importance

#### Annoyance

In both tropical and non-tropical areas of the world blackflies can cause a very serious biting problem. Their bites can be painful. Although the severity of the reaction to bites differs in different individuals, localised swelling and inflammation frequently occurs accompanied by intense irritation lasting for several days or even weeks. A form of dermatitis has been reported in Central Europe following the bites of Simulium erythrocephalum, and in the U.S.A. biting by S. jenningsi is said to have caused asthma. The classical example of the nuisance that can result from blackflies was the seasonal exodus during the eighteenth century of people from the Danube valley areas in Central Europe largely to save their domestic animals from attacks by the enormous numbers of S. colombaschense ( = columbaczense). In some areas, such as northern territories of Canada, outdoor activities are almost impossible at certain times of the year due to the intolerable numbers of biting simuliids. However, it is as disease vectors that blackflies are most important.

#### Onchocerciasis

The causative agent of human onchocerciasis, Onchocerca volvulus, or a morphologically very similar species, has been found in a monkey in Mexico and a gorilla in the Congo, but despite these discoveries and the fact that chimpanzees can be experimentally infected, there is no evidence that onchocerciasis is a zoonosis. The disease, commonly called river blindness, occurs throughout West Africa, Central and much of East Africa between latitudes of approximately  $15^{\circ}$  north to  $13^{\circ}$  south, the most heavily infected areas being savanna regions, especially those in West Africa. A small focus is present in south Yemen. The same parasite causes onchocerciasis in small localised areas in Central and South America such as in southern Mexico, Guatemala, Venezuela, Brazil and Colombia.

Blackflies are the only vectors of human onchocerciasis. Their habit of tearing and rasping the skin to rupture the blood capillaries to obtain a blood-meal makes them particularly suited to the ingestion of the skin-borne microfilariae of O. volvulus. Many of the microfilariae ingested during feeding are destroyed or excreted but some penetrate the stomach wall and migrate to the thoracic muscles where they develop into sausage-shaped stages, and undergo two moults. A few survive and elongate into thinner worms and pass through the head and down the short proboscis. The infective stages (about 550  $\mu$ m) in the proboscis penetrate the host's skin when females alight to feed. The interval between the ingestion of microfilariae to the time infective larvae are in the proboscis is about 6-13 days, depending on temperature.

African vectors of onchocerciasis Cytological studies have shown that the Simulium damnosum complex to be composed of at least 25 distinct taxonomic forms, variously regarded as species or cytotypes. The damnosum complex is widespread in tropical Africa and certain members of the complex are the most important vectors of onchocerciasis in Africa.

Adults of the damnosum complex are mainly black and can be recognised by the front tarsi being broad and flattened and having a conspicuous dorsal crest of fine hairs, and by the presence of a very broad white area on the first segment of the hind tarsus (basitarsus). In many, but not all, species of the damnosum complex the larvae are readily recognised by the presence on each of the middle segments of the body of two small and almost conical protuberances. An additional distinctive feature of S. damnosum larvae is the presence over almost the entire body of minute dark setae or scales (figure 7.3b). Larvae are found in the rapids of small or very large rivers in both savanna and forested areas of Africa. Adults frequently disperse far from their breeding sites and biting females can be encountered up to 100-250 km away.

The other, but less important, African vector is S. *neavei*, which is responsible for the transmission of onchocerciasis in both of the Congo Republics and Uganda, and formerly in Kenya where it is considered to have been eradicated by insecticidal control measures and bush clearing. Females are readily separated from S. *damnosum* by their larger size, completely black legs, and by their distinctly yellowish appearance which is due to the presence of fine golden-yellow hairs on the face, dorsal surface of the thorax and abdomen. It is a phoretic species, that is the larvae and pupae are found attached to other fresh water fauna, in this instance to crabs of the genus *Potamoniautes* which occur in small rocky, rather turbid, streams and rivers.

American vectors of onchocerciasis S. ochraceum is the principal vector in southern Mexico and Guatemala and is widely distributed in Central America and northern parts of South America. Adults are very small and are easily recognised by their dark brown legs, bright orange scutum and the yellow basal part of the abdomen which contrasts with the black apical part. Females oviposit while in flight, dropping their eggs on to floating vegetation. Larval habitats consist of trickles of flowing water and very small streams which are often concealed by bushes, vegetation and fallen leaves. Adults do not appear to disperse far. The main biting season is unusual in being in the drier months of the year.

S. metallicum occurs in Mexico through Central America to northern areas of South America. In Venezuela it appears to be the most important vector while in other areas such as in Mexico and Guatemala it is considered a minor vector. It is a black species, and has a broad white area on the first segment of the hind tarsus and superficially resembles S. damnosum of Africa. Larvae occur in small or large streams and rivers. Adults fly further from their breeding sites than do those of S. ochraceum.

Other species known or considered to be local vectors of onchocerciasis in the Americas include S. callidum and S. exiguum.

# Mansonella ozzardi type filariae

Mansonella ozzardi is a filarial parasite of man that is usually regarded as non-pathogenic, although it has been reported causing morbidity in Colombia and Brazil; it is transmitted in Central and South America by Culicoides species (chapter 9). In Brazil a filarial parasite indistinguishable from this species has been found in S. amazonicum. Although it seems most likely that it is M. ozzardi this has not yet been proved conclusively.

# Venezuelan equine encephalitis virus

This is normally transmitted to man, horses, rodents and birds by various mosquitoes (chapter 6), but the virus has been recovered from blackflies which may play a minor role in transmission.

# Control

There are few reports on the effectiveness of repellents against blackfly attacks, but some protection, usually lasting up to two hours, can be gained by use of repellents such as diethyltomuamide (DET), dimethyl phthalate (DMP), butyryl-tetrahydro quinoline.

Although insecticidal fogging or spraying of vegetation thought to harbour resting adult blackflies has occasionally been undertaken, this approach results in very temporary and localised control. The only practical method at present available for the control of blackflies is the application of insecticides to their breeding places to kill the larvae. Organochlorine or organophosphate insecticides need be applied only to a few selected sites on watercourses for some 15-30minutes, because as the insecticide is carried downstream it kills simuliid larvae over long stretches of water. The flow rates of the water and its depth are used to calculate the quantity of insecticide to be released. Applying DDT by this method has resulted in good control of S. damnosum in areas of Nigeria and Uganda. If treatment is not repeated at intervals throughout the year then gravid female adults dispersing into the area from untreated areas will probably result in recolonisation. In Kenya S. neavei has apparently been eradicated by the application of DDT to the relatively small streams in which this vector occurred, together with bush clearing.

In many areas ground application of larvicides is

difficult, either because of the enormous size of the rivers requiring treatment or because breeding occurs in a large network of small streams and watercourses. Under these conditions aerial applications from small aircraft or helicopters have been used. More experience has been gained in North America on the chemical control of blackflies than in other parts of the world, although much valuable information is presently being obtained in West Africa on the control of S. damnosum, under the auspices of the Onchocerciasis Control Programme in the Volta River Basin area. This programme is currently responsible for larviciding, with 20 per cent emulsifiable concentrate of temephos (Abate), all breeding places of S. damnosum in and around the Volta River Basin of seven West African countries. This vast internationally backed control scheme started spraying operations in 1975 and will probably continue for 20 years or more. If the vector population is reduced and held to a very low level then onchocerciasis transmission will be interrupted, but as the adult worms live in man for as long as 15 years, control must be extended over this period to allow the disease to die out in the human population.

# 8 Phlebotomine sandflies (Order Diptera: Family Psychodidae)

#### Species

There are some 600 species in five genera within the subfamily Phlebotominae. Species in three genera, *Phlebotomus, Lutzomyia* and *Sergentomyia*, suck blood from vertebrates, the former two are the more important medically as they contain disease vectors. A few species of *Sergentomyia* bite man, but they are not considered to be important in the transmission of diseases to man. Some previous classifications placed these five genera in a separate family the Phlebotomidae, but they are now regarded as being in the subfamily Phlebotominae of the family Psychodidae.

Adult flies are often called sandflies, but this can be confusing because in some parts of the world the small biting midges of the Ceratopogonidae (chapter 9) and blackflies (Simuliidae, chapter 7) are called sandflies. If the vernacular name is used it should be qualified and the flies referred to as phlebotomine sandflies. The most important vector species include *Phlebotomus papatasi*, *P. sergenti*, *P. major syriacus*, *P. argentipes*, *P. ariasi*, *P. perniciosus*, *Lutzomyia longipalpis* and species of the *Lutzomyia flaviscutellata* complex.

#### Distribution

Widely distributed in the tropics, subtropics and warm mainland regions, extending in some areas of the Old and New Worlds to almost 50°N.

The genus *Phlebotomus* occurs only in the Old World, especially in southern parts of the northern temperate areas such as the Mediterranean region. The genus also occurs in the Old World tropics, but there are not many species in tropical Africa, especially in West Africa. Most *Phlebotomus* species inhabit semiarid and savanna areas in preference to forests.

Sergentomyia species are also confined to the Old World, being especially common in the Indian subregion, but also occurring in other areas such as Africa and Central Asia. Lutzomyia species by contrast are found only in the New World tropics and subtropics, occurring mostly in forested areas of Central and South America.

#### Medical importance

In both the Old and New Worlds sandflies are vectors of leishmaniasis; the virus responsible for sandfly fever and a protozoan parasite causing a disease called Bartonellosis (Carrion's disease).

# Phlebotomine sandflies

# **External morphology**

#### Adults (figure 8.1)

No details are given for distinguishing between the adults of *Phlebotomus*, *Lutzomyia* and *Sergentomyia* because this requires specialised knowledge and detailed examination; but generally species biting man in the Old World will be *Phlebotomus* and in the New World *Lutzomyia*.

Adult phlebotomine sandflies can be readily recognised by their minute size (2-5 mm in length), their hairy appearance, their relatively large black eyes and their relatively long and stilt-like legs. The only other blood-sucking flies which are as small as this are some species of biting midges (Ceratopogonidae), but these have non-hairy wings and differ in many other details (chapter 9). Phlebotomine sandflies have the head, thorax, wings and abdomen densely covered with long hairs. The antennae are long and composed of small bead-like segments with short hairs and are similar in both sexes. The mouthparts are short and inconspicuous but are adapted for blood-sucking, consisting of a labrum, paired mandibles and maxillae, a hypopharynx and a fleshy labium. At their base are a pair of five-segmented maxillary palps which are relatively conspicuous and droop downwards.



Fig 8.1 (a) Adult male phlebotomine sandfly, (b) terminal abdominal segments of a male sandfly showing the very prominent claspers and other external structures of the genitalia and (c) the terminal abdominal segments of a female adult sandfly showing the small finger-like cerci.

Wings are lanceolate in outline and quite distinct from the wings of other biting flies. The Phlebotominae can be distinguished from very small non-biting flies of the family of Psychodidae, which they superficially resemble, by the wings. In sandflies the wings are held erect over the body when the fly is at rest whereas in non-biting psychodid flies they are folded roof-like over the body. The wing venation also differs. In phlebotomine sandflies, but not in the other subfamilies of Psychodidae, vein two branches twice, although this may not be apparent unless most of the hairs are rubbed from the wing veins (figure 8.2).

The abdomen is moderately long and in the female more or less rounded at the tip (figure 8.1c) but in males it terminates in a prominent pair of claspers which give the end of the abdomen an upturned appearance (figure 8.1a, b). Identification of adult phlebotomine sandflies to species is difficult and usually necessitates the examination of internal structures, such as the arrangement of the teeth on the cibarial armature, the shape of the spermatheca in females and in males the structure of the external genitalia (terminalia).

# Life-cycle

The minute eggs (0.3-0.4 mm) are more or less ovoid in shape, usually brown or black, and careful examination under a microscope reveals that they are patterned as shown in figure 8.3. Some 15-100 eggs are laid singly at each oviposition. They are deposited in small cracks and holes in the ground, at the base of



Fig 8.2 Wing of a phlebotomine sandfly with all scales removed to show wing venation and two alternative systems of numbering the veins; note that the second vein branches twice.



Fig 8.3 An egg of a phlebotomine sandfly with mosaic-type pattern on the shell.

termite mounds, in cracks in masonry, on stable floors, in poultry houses, amongst leaf litter and in between buttress roots of forest trees etc. The type of oviposition site varies greatly according to species.

Although eggs are not laid in water they require a moist microhabitat with high humidity. They are unable to withstand desiccation. They hatch after about 6-17 days under optimum conditions, but hatching may be prolonged in cooler weather. Larvae are mainly scavengers, feeding on organic matter, such as fungi, decaying forest leaves, semi-rotting vegetation, animal faeces and decomposing bodies of

arthropods. Although some species, especially of the genus *Phlebotomus*, occur in semi-arid areas, the actual larval habitats must have a high degree of humidity. Larvae can usually survive if their breeding places are temporarily flooded.

There are four larval instars. The mature larva is 4-6 mm long and has a well defined black head which is provided with a pair of small mandibles, the body is greyish or yellowish and segmented (figure 8.4). The first seven abdominal segments have small pseudopods, but the most striking feature of the larva is the presence on the head and all body segments of conspicuous thick bristles with feathered stems and which in many species have slightly enlarged tips. They are called matchstick hairs and identify the larvae as those of phlebotomine sandflies. In most species the last abdominal segment bears two pairs of conspicuous long hairs called the caudal bristles. The first-instar larvae have two single bristles not two pairs.

Larval development is completed after 21-60 days, the duration depending on species, temperature and availability of food. In temperate areas species may



Fig 8.4 Last instar larva of a phlebotomine sandfly showing the matchstick hairs and the paired caudal bristles.

overwinter as diapausing fully grown larvae. Prior to pupation the larva assumes an almost erect position in the habitat, the skin then splits open and the pupa wriggles out. The larval skin, however, is not completely cast off but remains attached to the end of the pupa. The presence of this skin with its characteristic two pairs of caudal bristles aids in the recognition of phlebotomine pupae. The pupal shape is as shown in figure 8.5. Adults emerge from the pupae after about



Fig 8.5 A pupa of a phlebotomine sandfly with the larval skin still attached.

7-14 days. The life-cycle from oviposition to adult emergence may be 30-100 days depending on species and temperature; during cooler periods of the year the duration of the life-cycle may be extended. In temperate areas, adults die off in late summer or autumn and the species overwinter as larvae and the adults emerge the following spring.

It is usually extremely difficult to find larvae or pupae of sandflies and relatively little is known about their biology and ecology.

#### Adult behaviour

Both sexes feed on plant juices and sugary secretions but females in addition suck blood from a variety of vertebrates, including domestic animals, dogs, urban and wild rodents, snakes, lizards, amphibians and a few species feed on birds. In the Old World many *Phlebotomus* species bite man, whereas most species of *Sergentomyia* feed mainly on reptiles and rarely bite man. In the tropical Americas *Lutzomyia* species feed on a wide variety of mammals including man. Biting is usually restricted to crepuscular and nocturnal periods but they may bite man during the day in darkened rooms, or in forests during overcast days. Most species feed out of doors (exophagic) but a few also feed indoors (endophagic). Adults are weak fliers and do not usually disperse more than a few hundred metres from their breeding places, consequently biting may be localised to a few areas. (Occasionally, however, such as in the Crimea they have been reported flying up to 1200–1500 metres.) Sandflies have a characteristic hopping type of flight so that there may be several short flights and landings before females settle on their hosts. Windy weather inhibits their flight activities and biting. Because of their very short mouthparts they are unable to bite through clothing.

During the day adult sandflies rest in sheltered, dark and humid sites, but on dry surfaces, such as on tree trunks, on ground litter and foliage of forests, in animal burrows, termite mounds, tree holes, rock fissures, caves, cracks in the ground and inside human and animal habitations. Species that commonly rest in houses (endophilic) before or after feeding on man are often referred to as domestic or peridomestic species. Examples are *Phlebotomus papatasi* in the Mediterranean area and *Lutzomyia longipalpis* in South America.

In temperate areas of the Old World sandflies are seasonal in their appearance and adults occur only in the summer months. In tropical areas some species appear to be common more or less throughout the year, but in other species there may be well marked changes in the abundance of adults related to the dry and wet seasons.

# Medical importance

#### Annoyance

Apart from their importance as disease vectors, sandflies may constitute a serious, but usually localised, biting nuisance. In previously sensitised people their bites may result in severe and almost intolerable irritation, a condition known in the Near East as Harara.

#### Leishmaniasis

This is a term used to describe a number of closely related diseases caused by several distinct species, subspecies and strains of *Leishmania* parasites. Phlebotomine sandflies are the only known vectors. Parasites are ingested by females with a blood-meal. They develop a flagellum, with which they attach themselves to the gut wall, and multiply within the insect's stomach and then migrate to the anterior part of the mid gut and from there to the oesophagus. After about 4-12 days the infective forms are found in the mouthparts from where they are introduced into a new

host during feeding. It appears that previous feeding by females on plant juices aids the survival of the parasite in the insect's gut.

Most types of leishmaniasis are zoonoses, the degree of involvement of man varies greatly from area to area. The epidemiology is complex and largely determined by the species or even strains of sandflies, their ecology and behaviour, and the availability of a wide range of hosts and also the species and strains of *Leishmania* parasites. In some areas, for example, sandflies will transmit the disease almost entirely amongst wild or domesticated animals, with little or no human involvement, whereas elsewhere animals may provide an important reservoir of infection for man. In India the disease may be transmitted between man by sandflies, with animals taking little if any part in its transmission.

Leishmaniasis occurs in two main forms, dermal (cutaneous) and visceral leishmaniasis. Old World dermal leishmaniasis (Oriental sore, cutaneous leishmaniasis) is caused by *Leishmania tropica*, and in Central Asia (mainly Russia) gerbils and ground squirrels are the principal animal reservoirs. In other areas such as western India, the Middle East, Mediterranean countries and North Africa, dogs are important reservoirs, but rodents may also be involved. Important vectors of dermal leishmaniasis include *Phlebotomus papatasi*, *P. caucasicus* and *P. longipes*.

American dermal leishmaniasis (including mucocutaneous forms = espundia) is found from Mexico down to Argentina and is caused by several Leishmania species such as the Le. braziliensis and Le. mexicana complexes which normally infect a wide variety of forest rodents, marsupials, armadillos, edentates, primates, sloths and also domestic dogs. The disease is spread to man by several species of Lutzomyia including the L. flaviscutellata complex, L. wellcomei, L. intermedia and L. umbratilis.

The visceral form of Leishmania in the Old World (kala-azar) is caused by Leishmania donovani in most areas of its distribution, such as asiatic Russia, India, China, Kenya and Sudan, but by Le. infantum in the Mediterranean area to the Central Asian area. In the Mediterranean region dogs and foxes are the most important reservoirs and the major vectors are *P. perniciosus*, *P. ariasi* and *P. major syriacus*, while in China dogs are also reservoirs and the important vector is *P. chinensis*. In India neither dogs nor any other animal appears to act as a reservoir and the disease is transmitted to man mainly by *P. argentipes*. In the Middle East *P. major syriacus* is a vector and wild and domestic dogs appear to be the most important reservoirs.

In South America both wild and domesticated dogs are reservoirs and the vector is usually *Lutzomyia longipalpis*.

## Bartonellosis

This disease is sometimes called Oroya fever or Carrion's disease and is encountered in arid mountainous areas of the Andes in Peru, Ecuador and Colombia, it is caused by a small rod-like organism named *Bartonella bacilliformis* that is likely a bacterium. It is transmitted by *Lutzomyia vertucarum* and possibly by other *Lutzomyia* species. Transmission is probably entirely by contamination of the mouthparts.

# Sandfly fever

Sometimes called papatasi fever or three-day fever this disease is caused by various strains of a virus transmitted primarily by *P. papatasi*. Females become infective six to ten days after an infected blood-meal. It appears that the infected females can lay eggs containing the virus and that these can eventually give rise to infected adults. This is an example of transovarial transmission, a phenomenon that is more common in the transmission of various tick-borne diseases (chapter 21). Sandfly fever occurs mainly in the Mediterranean region but extends up the Nile and also into India and probably China where, in the absence of *P. papatasi* other *Phlebotomus* species are likely involved in its transmission.

# Control

Leishmaniasis has not in the past been considered a sufficiently important disease to justify expenditure on controlling the insect vectors. Phlebotomine sandflies are, however, very susceptible to most insecticides and no cases of resistance have been reported. In nearly all areas where residual insecticides, particularly DDT, have been used to control *Anopheles* vectors of malaria there have been drastic reduction of sandfly populations followed by interruption of leishmaniasis transmission. With the cessation of spraying, sandflies usually return and transmission is renewed.

Dosages of 1 or 2 g/m<sup>2</sup> of DDT or 0.4 g/m<sup>2</sup> of HCH applied as a residual house-spray have given excellent control of *Phlebotomus* species. It seems probable that insecticidal fogging of outdoor resting sites of adults should give good, although most likely temporary, control.

Personal protection can be achieved by the use of efficient insect repellents such as diethyltoluamide, dimethyl phthalate or trimethyl pentanediol, or the use of very fine screens or sandfly nets. A disadvantage of using screens and nets with very small holes, is that they reduce ventilation causing it to be unpleasantly hot in screened houses or under sandfly nets over beds.

# 9 Biting midges (Order Diptera: Family Ceratopogonidae)

#### Species

The family Ceratopogonidae (= Heleidae) has over so genera belonging to four subfamilies – Leptoconopinae, Forcipomyinae, Dasyheleinae and Ceratopogoninae. Only Lasiohelea (usually recognised as a subgenus of the genus Forcipomyia) and the genera Leptoconops (= Holoconops), and most importantly Culicoides, of which over 800 species have been described, feed on vertebrates and therefore are considered to be of medical importance. The most important species are Culicoides milnei (=? austeni)\*, C. grahamii, C. furens and Leptoconops becquaerti.

#### Distribution

The family has a more or less world-wide distribution, the most widely distributed genus being *Culicoides* which is found in both tropical and subarctic areas.

#### Medical importance

In many parts of the world species of Culicoides, and in the Americas also Leptoconops, can constitute a serious biting problem. Culicoides milnei and C. grahamii are vectors of Dipetalonema (= Acanthocheilonema) perstans and C. grahamii and possibly C. milnei are vectors of D. (= Acanthocheilonema) streptocerca; C. furens is a vector of Mansonella ozzardi. These filarial parasites are usually regarded as non-pathogenic to man.

# Culicoides species

# External morphology

# Adults (figure 9.1)

A generalised description is given of the adults of *Culicoides*, followed by brief notes on *Leptoconops* and *Lasiohelea*.

Adult *Culicoides* are sometimes known as midges or biting midges, and especially in the Americas as 'nosee-ums' or 'punkies' and in Austrialia and other countries as sandflies. This latter name is unfortunate and should be avoided because phlebotomines (chapter 8) and occasionally simuliids (chapter 7) may also be referred to as sandflies. The most appropriate common name is biting midges; this terminology serves to distinguish them from other small non-biting flies which are often referred to as midges.

Adults are very small insects being only about 1.5-



Fig 9.1 An adult Ceratopogonidae of the genus Culicoides.

\* There has been considerable confusion concerning these two closely related species. Most older works refer to C. austeni but in many instances the species under consideration was probably C. milnei. In this book references are made to C. milnei not C. austeni.

5 mm long and with the phlebotomines constitute the smallest biting flies attacking man.

The small head bears a prominent pair of eyes and a pair of relatively long antennae. As in mosquitoes, males do not take blood-meals and have feathery or plumose antennae, whereas the blood-sucking females have non-plumose antennae. The biting mouthparts are very small and inconspicuous, they do not project forwards but hang down vertically from the head. They comprise a sharp labrum, a pair of maxillae and a pair of mandibles, a thin hypopharynx and a fleshy labium which does not penetrate the host's skin. The arrangement and structure of the mouthparts are very similar to those of simuliids (chapter 7). In many species the thorax is covered dorsally with very small but distinct black spots and markings. In addition to these dark markings a pair of black, small but elongated depressions known as the humeral pits are present in all Culicoides species on the dorsal surface of the anterior part of the thorax (figure 9.2). The



Fig 9.2 Head and thorax of a female Culicoides showing a pair of humeral pits and numerous scattered black thoracic spots.

presence of these pits distinguishes the genus *Culicoides* from *Lasiohelea* and *Leptoconops*. The wings are short and relatively broad, and apart from the first veins their venation is faint (figure 9.3). The wings lack scales but in many species are covered with minute hairs, but these are seen only under a dissecting microscope. In most *Culicoides* species the wings have contrasting dark and milky white spots or patches (figure 9.3) but in some species these markings are indistinct and difficult to see unless the light is from the correct angle or the wing is viewed under dark-field illumination. In life the wings are placed over the abdomen like the blades of a closed pair of scissors (figure 9.1). The legs are relatively short.



Fig 9.3 Wing of a typical Culicoides species showing pattern of pale milky white marks on a dark background and cross vein r-m.

The abdomen is dull grey, yellowish brown or blackish and in the female is more or less rounded at its tip, but in the male there is a small but conspicuous pair of claspers.

#### Leptoconops

Wings lack the contrasting light and dark markings found in most *Culicoides*, instead they are a milky white colour and contrast markedly with the black bodies of the adult. Wing venation differs from that of *Culicoides* (see figure 9.3) and *Lasiohelea* in that the small cross vein (r-m) is absent.

#### Lasiohelea

Wings are unpatterned but very hairy.

# Life-cycle

The eggs are brown or black, cylindrical or curved and banana-shaped, and are about 0.5 mm long (figure 9.4c). They are laid in batches of about 30–130 on the surface of mud, wet soil especially that near swamps and marshes including salt water marshes, on decaying leaf litter, humus, manure, or on plants and other objects near or partially submerged in water, in tree holes, in semi-rotting vegetation and in the cut stumps of banana plants (for example *Culicoides milnei* and *C. grahamii*). The type of oviposition site selected depends on the species.

Eggs usually hatch within about two to nine days, depending on temperature and species, but some temperate species overwinter as eggs. There are four larval instars and the fully grown larva is cylindrical, whitish and about 5-6 mm long. It has a very small yellowish, brown or black conical-shaped head which bears a small pair of eyes and a pair of minute antennae and mandibles (figure 9.4a). There are three thoracic and nine abdominal segments, all of which are very



Fig 9.4 (a) Larva of Culicoides with the two four-lobed 'gills' extending from the posterior abdominal segment, (b) terminal segments with 'gills' retracted inside the last segment and (c) eggs of Culicoides species.

similar in appearance and lack any conspicuous structures, except that the last segment terminates in two four-lobed retractile gill-like structures (figure 9.4a). These are not always readily seen in preserved larvae because they are often retracted within the last abdominal segment (figure 9.4b). *Culicoides* larvae are best recognised by the combination of a small dark head followed by a segmented body devoid of any obvious structures, such as hairs, bristles or protuberances, and, when they are extruded, by the presence of terminal 'gills'. When alive they can also be recognised by their serpentine swimming motions.

Larvae feed mainly on decaying vegetable matter and occur in many different types of habitats, including fresh or salt water marshes and swamps, edges of ponds, boggy and semi-waterlogged areas, and in specialised habitats such as horse and cow excreta, tree holes and cacti. When the water level in swamps and marshes rises the larvae of many species migrate towards the damp soil and mud at the edges to avoid becoming completely submerged. Some important pest species breed in sandy areas near the sea shore. Larvae are difficult to find and are rarely encountered unless special surveys are made to collect them.

In warm countries larval development is completed within 14-25 days, but in temperate regions many species overwinter and remain as larvae for seven months. Species occupying marshy habitats frequently migrate to the drier peripheral areas for pupation, but in species that are aquatic the pupae float at the water surface.

The pupa (figure 9.5) is 2-4 mm long and readily recognised by the following combination of characters: a pair of breathing trumpets on the cephalothorax which appear to be composed of two segments, abdominal segments bearing small but conspicuous tubercles ending in a fine hair, and a prominent pair of horn-like processes on the last segment. The pupal period lasts three to ten days.



Fig 9.5 Lateral and dorsal views of Culicoides pupae.

#### Adult behaviour

Adults of both sexes feed on naturally occurring sugar solutions. In addition females take blood-meals from man and a wide variety of mammals and birds. Adults bite at any time of the day and night but many species are particularly active and troublesome in the evenings and first half of the night. In contrast *Culicoides grahamii* is particularly active in the early mornings. Because of their short mouthparts biting midges are not as successful in biting through clothing as are mosquitoes, tabanids and tsetse flies, all of which have a longer proboscis. For this reason midges often appear in swarms or clouds around the head, biting the face especially the forehead and scalp, but they also bite other exposed parts such as the hands and arms, and the legs of people wearing shorts. Most species bite only out of doors, but a few including *C. milnei* and *C.* grahamii will enter houses to feed on man (endophagic). Adults normally fly only a few hundred metres from their larval habitats, but they may be dispersed considerably further by wind.

# **Medical importance**

# Annoyance

Biting midges are very small, but what they lack in size they can make up for in numbers, – as has been said, one midge is an entomological curiosity, a thousand sheer hell! In several areas of the world as dissimilar as the west coast of Scotland, the Caribbean and the sunny regions of California and Florida biting midges can be a serious economic threat to the tourist industry. The persistent biting of large numbers of midges can make outdoor recreational activities impossible, not only at dusk but often during much of the day. In some areas they have even prevented the continuation of harvesting and other outdoor work during the evenings.

Important pest species in southern areas of North America down to Brazil include *Culicoides furens* which breeds in salt marshes and other saline coastal habitats, while in North America *Leptoconops torrens* and *L. becquaerti* which also breed in sandy soils and coastal areas can be serious pests. In Europe *C. impunctatus* and many other species are troublesome biters, while in the Malagasy Republic, Seychelles and Brunei *L. spinosifrons* can cause a considerable biting problem.

# **Filarial infections**

A few Culicoides species are vectors of parasites to man. In Africa, especially West and Central, but also in parts of East Africa as far south as Zimbabwe–Rhodesia, Dipetalonema (= Acanthocheilonema) perstans is transmitted to man by Culicoides milnei (= austeni) and C. grahamii, while D. (= A.) streptocerca is transmitted in the rain forests of Ghana, Nigeria, Cameroun and Zaire by C. grahamii and possibly by C. milnei. These species breed in the rotting cut stumps of banana and plantain plants. D. perstans also occurs in Trinidad and South America where it is transmitted by other Culicoides species.

In Mexico, Panama, the West Indies and South America Mansonella ozzardi is transmitted by Culicoides species, mainly C. furens, but other species such as C. phlebotomus are also likely to be involved. (See chapter 7 regarding the possibility that this filarial parasite is transmitted also by Simulium species.) Microfilariae of these parasites are non-periodic and are ingested with a blood-meal and pass through a similar developmental cycle as do other filarial parasites in mosquitoes, that is they undergo morphological changes, invade the thoracic flight muscles, moult twice and then migrate to the head and after about 9-12 days pass down the proboscis. The infective thirdstage larvae are deposited on the skin of the host when the female takes a blood-meal. The salivary glands of Culicoides play no part in the transmission of these parasites. None of the three filarial parasites carried by midges appears to cause much harm to man, and they are usually regarded as non-pathogenic, although morbidity or allergic reactions may sometimes occur. In general the Ceratopogonidae are not considered as very important vectors of disease to man.

# Arboviruses

There have been reports of the isolation of Eastern Equine encephalitis virus from batches of *Culicoides* species collected in the U.S.A. and isolation of Japanese encephalitis from *Lasiohelea* species caught in China. There is, however, no evidence that they are biological vectors, these records appear to be more or less chance isolations. Both Eastern Equine and Japanese encephalitis viruses are normally transmitted by mosquitoes (chapter 6).

# Control

Because many of the major pest species breed in extensive and often diffuse habitats, such as fresh and salt water marshes and wet coastal sand, whose limits are usually difficult to define, it is usually very difficult to substantially reduce larval breeding. For effective control often large areas of land or marshy areas must be drained or sprayed with insecticides.

Larval habitats can be eradicated by draining or filling them in, but this is often costly and laborious and in many areas impractical. Sometimes semi-aquatic sites such as muddy or marshy areas can be impounded and flooded under 5-8 cm of water to ensure that the soil is never exposed, thus destroying suitable habitats. This type of environmental control can be effective against some species, but be ineffective against others. If maintained such methods have the advantage of giving permanent control. However, although they avoid contaminating the environment with insecticides they in themselves result in completely changing the habitat.

Insecticidal applications can sometimes be effective. The best and most lasting control is usually obtained by spraying breeding sites with organochlorine insecticides but heavy rainfall is needed to wash the insecticides through the surface vegetation to the underlying soil and mud harbouring the midge larvae. Dieldrin at rates of about 1-4 kg/ha has given good control in the U.S.A. against *Culicoides* and *Leptoconops* species. Diazinon at about 2 kg/ha has been successfully employed in the U.S.A. against *Leptoconops* species; chlorpyrifos (Dursban), another organophosphate insecticide, has also proved useful in midge control.

Thermal insecticidal aerosols or ultra-low-volume

(ULV) applications have sometimes been used to kill adults resting in vegetation, but the effects are very short-lived, and sprayed areas are soon invaded by midges flying in from unsprayed areas.

Limited personal protection can be achieved by the use of suitable repellents such as diethyltoluamide, dimethyl phthalate or trimethyl pentanediol. Mosquito nets and screening used to keep out houseflies and mosquitoes may not exclude the much smaller biting midges. To prevent them from passing through protective nets and screens a very small mesh size must be used, but a disadvantage is that this substantially reduces air flow and ventilation. Nets and screens can be treated with insecticides such as 6 per cent malathion or propoxur (Arprocarb). Such screens may remain potent for about one month.

# 10 Horseflies, deerflies and clegs (Order Diptera: Family Tabanidae)

#### Species

The family Tabanidae is divided into four subfamilies the Scepsidinae, Pangoniinae, Chrysopsinae and Tabaninae, the latter being subdivided into the three 'tribes' the Haematopotini, Tabanini and the Diachlorini. Neither the subfamilies Scepsidinae and Pangoniinae nor the tribe Diachlorini are of any medical importance. There are many genera of tabanids and over 3000 species, but the most important from the medical point of view are certain species of *Chrysops* (subfamily Chrysopsinae) and *Tabanus* and *Haematopota* (subfamily Tabaninae).

#### Distribution

The Tabanidae have a world-wide distribution and species of *Tabanus* and *Chrysops* are found in temperate and tropical areas, but *Haematopota* are absent from South America and Australia and are not common in North America.

#### Medical importance

Chrysops, mainly C. silacea and C. dimidiata, are vectors in West and Central Africa of Loa loa. In the U.S.A. C. discalis is a vector of tularaemia, caused by Pasteurella (= Francisella) tularensis. Tabanidae possibly play a very minor role in the mechanical spread of human and animal trypanosomiases; in South America they may be involved in the transmission of Trypanosoma vivax.

# The Tabanidae

# **External morphology**

#### Adults (figure 10.1)

A generalised description is presented of the Tabanidae, with special reference to the genera Chrysops, Tabanus and Haematopota.

Tabanids are medium to very large flies (5-25 mm). Many, especially of the genus Tabanus, are robust and heavily built, and this genus contains the largest biting flies, some attaining a wing span of 6.5 cm. The colouration of tabanids varies from very dark brown or black to lighter reddish-brown, yellow or greenish; frequently the abdomen and thorax have stripes or patches of contrasting colours. The head is large and viewed from above is more or less semicircular in outline (figure 10.2) and is often described as semilunar. It bears a conspicuous pair of compound eyes which in life may be marked with contrasting iridescent colours, such as greens and reds or even purplish hues, arranged in bands, zig-zags or spots. Adults can be sexed by examination of their eyes. In the female there is a distinct space on top of the head (vertex) between the eyes; this is known as a dichoptic condition (figure 10.2a). In females of some species this space between the eyes may be narrow, while in others, especially Chrysops, it is quite large. In the males, the eves are so large that they occupy almost all of the head and either touch each other on top of the head or are very narrowly separated, this being known as a holoptic condition (figure 10.2b).

The antennae are relatively small but stout. They consist of three segments; the last is subdivided by annulations into three or four small divisions. Unlike the Muscidae, Glossinidae and Calliphoridae there is no antennal arista. The size and shape of the antennae serve to distinguish the genera Chrysops, Haematopota and Tabanus (figure 10.3a, b, c). The mouthparts of female Tabanidae are stout and adapted for biting. They consist of a lower well developed labium which is grooved along the anterior face to provide a protective sheath for the other more slender mouthparts. The labium terminates in a pair of large labella which are provided with a series of small tubes called pseudotracheae. In this, but certainly no other respects, the mouthparts resemble those of the non-biting Muscidae, such as the common housefly (chapter 12). Blood flowing from a wound may be sucked up



Fig 10.1 Adult Tabanidae: (a) Chrysops species, (b) Haematopota species and (c) Tabanus species.





Fig 10.3 Antennae of adult tabanids: (a) Chrysops species, (b) Haematopota and (c) Tabanus.

Fig 10.2 Dorsal view of tabanid heads showing (a) dichoptic condition of eyes in a female and (b) holoptic condition in a male.

through these pseudotracheae, much as various fluids are sucked up by the housefly. The uppermost part of the proboscis consists of a relatively narrow but sharply pointed labrum. Below this are the needle-like hypopharynx traversed by a duct from the salivary glands, two pointed maxillae which are provided with strong rasp-like teeth at their tips, and two broader and pointed mandibles. These six structures are stylet-like in appearance and are used for piercing the skin of the host; they are collectively known as the biting fascicle. Tabanids differ from tsetse flies, mosquitoes and *Stomoxys* in that the biting mouthparts always point downwards from the head; they do not project forwards. Only female tabanids take blood-meals; males have no mandibles and are incapable of blood-feeding.

The thorax is stout and bears a pair of wings which have two submarginal and five posterior cells and a completely closed discal cell in about the centre of the wing (figure 10.4). Although the wing venation alone



Fig 10.4 Wing of an adult tabanid showing wing venation, in particular the discal cell, two submarginal cells and five posterior cells.

may not be sufficient to identify the Tabanidae from all other Diptera, it nevertheless serves as a useful guide when considered with other characters, such as the shape and structure of the antennae and biting mouthparts. The wings may be completely clear and devoid of colour or have areas of brown colouration, or may be distinctly banded or appear mottled or speckled due to the presence of greyish patches (figure 10.1a, b, c). When adults are at rest the wings are placed either like a pair of open scissors (figure 10.5b) over the abdomen (such as in houseflies) or at a roof-like angle completely obscuring the abdomen (figure 10.5a). The presence or absence of coloured areas on the wings and the way in which they are held over the body provides useful additional characteristics for distinguishing between Chrysops, Tabanus and Haematopota (see pp. 92-93).

The abdomen is composed of seven visible segments and is usually broad and stout, and in unfed flies characteristically flattened dorsoventrally. It may be a more or less uniformly dark brown, blackish, light brown, reddish-brown, yellowish or even greenish, or alternatively marked with contrasting coloured stripes or patches.



Fig 10.5 Tabanids at rest, showing the wings folded (a) roof-like over the body in Haematopota and (b) like an open pair of scissors in Tabanus and Chrysops species.

The presence of a fleshy-type of empodium between the tarsal claws and a hexagonal shaped discal cell in the wings and biting mouthparts identifies flies as Tabanidae.

# Life-cycle

Males lack mandibles and feed on naturally occurring sugary secretions. Females also feed on sugary substances but in addition they bite a wide variety of mammals such as domestic animals, especially horses and cattle, deer and many other herbivores, carnivores, monkeys, reptiles, amphibia and a few species even attack birds. They also feed on man.

Some 100–1000 eggs, the number depending on the species, are deposited by female tabanids on the underside of objects such as leaves, grassy vegetation, plant stems, twigs, small branches, stones and rocks overhanging or adjacent to their larval habitats, which are mainly muddy, aquatic or semi-aquatic sites (see below). The eggs which are firmly glued to the substrate in an upright position are covered with a coating that is impervious to water, that is they are waterproofed. They are usually arranged in more or less lozenge-shaped patterns (figure 10.6a). They are mostly creamy white but some species lay grey, blackish-brown or even orange coloured eggs. They measure 1-2.5 mm in length and are curved or approximately cigar-shaped. They hatch after about 5-14 days, the time depending on both temperature and species. After wriggling out of the eggs the young larvae drop down on to the underlying mud or water.

Larvae are cylindrical and rather pointed at both ends (figure 10.6c). They are creamy white, brown or even greenish and have a very small black head which can be retracted into the thorax. There are three well differentiated thoracic and eight abdominal segments. Larvae are readily recognised by the prominent raised tyre-like rings which encircle most body segments. The first seven abdominal segments, that is segments four to ten, but not the last, have one pair of lateral and two pairs of ventral (a total of six) conspicuous roundish protuberances called pseudopods. The presence of the prominent rings and these pseudopods readily identifies larvae of tabanids. The last abdominal segment bears dorsally a short siphon which can be retracted into the abdomen and a pyriform structure known as Graber's organ which is composed of 15 or less black globular bodies. This can be readily seen with the aid of a hand lens or microscope and is unique to tabanid larvae. This organ is well provided with nerves and seems to be sensory but its exact function is not clearly understood.

Larvae live in mud, rotting vegetation, humus,



Fig 10.6 Immature stages of tabanids, (a) a single egg and a mass of eggs glued to a piece of grass, (b) a pupa and (c) a larva.

damp soil, in shallow and often muddy water, at the edges of small pools, swamps, ditches or slowly flowing streams. In aquatic habitats larvae sometimes adhere to floating leaves, logs, or other debris. A few species breed in tree holes, some of which may be more than 7 m above the ground. Some species occur in brackish habitats, while larvae of certain Tabanus and Haematopota species are found in the relatively dry soils of pastures and in the earth near the bases of trees. Larvae breathe atmospheric oxygen through the short siphon at the posterior end of the body, and because they are poor swimmers aquatic species are usually found in shallow waters. They move rather sluggishly in their muddy, aquatic or semi-aquatic environment. In some, in particular many Chrysops species, the larvae are scavengers, feeding on detritus and a variety of dead and decaying vegetable and animal matter; while in other species they are predacious and consume a variety of small arthropods that occur in their larval habitat, including larvae of other species of Tabanidae. In a few species the larvae are sometimes cannibalistic.

Larval development is prolonged. In both temperate and tropical countries many species spend one to two years as larvae, and several temperate species may remain as larvae for as long as three years. Larvae, however, are not easily found and relatively little is known about the life-cycle of many species, but there appear to be four to nine larval instars. Depending upon the species mature larvae may be  $1-6 \text{ cm} \log 1$ . Prior to pupation mature larvae migrate to drier areas at the periphery of the larval habitat where they pupate. The pupa is partially buried in the mud or soil in an upright position and superficially looks like a chrysalis of a butterfly (figure 10.6b). It is 7-40 mm long, size depending on the species, distinctly curved and is usually brown. The head and thorax are combined to form a distinct cephalothorax which has a pair of lateral and relatively large ear-shaped spiracles. The abdomen is composed of eight well defined segments, the first seven are supplied with a pair of lateral spiracles, while segments two to six have an encircling row of small backwardly directed spines. A few spines are also present ventrally on segment seven. The short terminal eighth abdominal segment is provided with six lobes which bear spine-like processes known collectively as the caudal aster. The pupal period lasts about 5-20 days.

#### Adult behaviour

Only females take blood-meals. Most species feed during the daylight hours and are especially active in bright sunshine. They locate their prey mainly by sight, but a few species feed at night. Tabanids are powerful fliers and may disperse many kilometres.

Most tabanids inhabit woods and forests. Many

*Chrysops* species are common in low-lying marshy scrub areas or swampy woods; some species, however, are found in more open savanna and grassland areas. Adults do not usually enter houses to feed, but *Chrysops silacea* is an exception. Other species may be found in houses especially on windows, but often these have entered accidentally and are trying to escape.

Because of their large and rather broad mouthparts, bites from tabanids are deep and painful, sometimes especially so, and wounds inflicted by tabanids frequently continue to bleed after the female has departed. Due to their painful bites they are frequently disturbed when feeding on either man or other hosts. As a result several small blood-meals may be taken from the same or different hosts before the female has obtained a complete meal. This interrupted feeding behaviour increases their likelihood of being mechanical vectors of disease. Because of their preference for dark objects they often prefer to bite through coloured clothing when attacking Caucasians than exposed areas of pale skin, in this respect they behave like tsetse flies.

In both temperate and tropical areas the occurrence of adults is seasonal. In temperate countries adults usually die off at the end of the summer, and a new population emerges in the spring or summer of the following year, whereas in the tropics the flies may not completely disappear in the dry months but their numbers are normally much reduced. Maximum numbers of biting flies usually appear towards the beginning of the rainy season.

# Identification of adult Chrysops, Tabanus and Haematopota

# Chrysops species (deerflies, green heads, mangrove flies)

These are medium sized flies ranging in size from that of a housefly to a tsetse fly (figure 10.1a). In life most species have iridescent eyes, commonly with spots of red, green or purple. The mouthparts are relatively longer than those of *Tabanus* and *Haematopota*. The wings, which are held partially over the abdomen in an open scissor-like fashion, have one or more transverse bands of brownish colour (figure 10.1a). In the females the eyes are usually well separated dorsally. In many species the abdomen is blackish with orange or yellow patches or bands.

The most reliable method of distinguishing *Chrysops* species from *Tabanus* and *Haematopota* is by the antennae. In *Chrysops* they are long and the second segment is neither short nor bears a projection (figure 10.3a), while the third segment has four small sub-

divisions. The hind tibiae have apical spurs; these are absent from *Tabanus* and *Haematopota*.

Chrysops has a world-wide distribution.

#### Tabanus species (horseflies)

Medium to very large flies. The eyes are frequently brownish but they may be iridescent although often not to such an extent as those of *Chrysops* or *Haematopota*, and the iridescent markings are usually in the form of horizontal bands. The wings which are held over the body much as in *Chrysops* are often clear (figures 10.1a and 10.5b), but in some species there are dark markings.

Tabanus species are readily identified by the shape and size of the antennae. Both the second and third antennal segments have small but distinct projections on the upper surface (figure 10.3c), and the third segment has four small sub-divisions and is usually distinctly curved upwards. The antennae are much shorter than those of *Chrysops* species, and are therefore less conspicuous.

Tabanus has a world-wide distribution.

#### Haematopota (clegs or stouts)

Medium sized dark grey flies which are easily distinguished from *Tabanus* and *Chrysops* by the fact that in life the wings are folded roof-like over the abdomen (figure 10.5a), moreover in nearly all species the wings are dusty grey and are speckled or mottled (figure 10.1b). The eyes have zig zag bands of iridescent colours, and are widely separated in the females. The antennae are similar to those of *Tabanus*, but are usually longer and the third segment is straight not curved as in *Tabanus* and has only three, not four, small subdivisions, and does not bear a dorsal projection (figure 10.3b).

Haematopota species are not found in South America or Australia, and only very few species occur in North America. They are common in Europe, Asia, Africa, India and the Far East.

# Medical importance

Because females tend to be intermittent feeders and are often disturbed during feeding tabanids are paricularly liable to be mechanical vectors. Moreover, heir large fleshy labella traversed by pseudotracheae hold appreciable quantities of blood which can be asily conveyed, together with pathogens, to the next host. They are known to be mechanical vectors of nthrax and anaplasmosis, both of which can infect man. They are also mechanical vectors of *Trypanosoma*  evansi, a disease of camels, horses and cattle, often with fatal results; it is not a disease affecting man.

Because of their painful bites, tabanids may sometimes constitute a pest nuisance and make activities out of doors, whether recreational or work, difficult. Some people develop severe allergic symptoms, due to the large amount of saliva that is pumped into the wound to prevent blood clotting.

Two diseases are spread to man by the Tabanidae. The less important one is tularaemia (*Pasteurella* (*= Francisella*) tularensis) which is spread mechanically from horses, rabbits and other rodents. In North America Chrysops discalis is a vector, but other tabanids may also transmit it. The disease is also commonly spread by handling infected rodents, by ixodid tick bites and by eating insufficiently cooked food.

#### Loiasis

The only important disease transmitted to man by tabanids is loiasis, caused by the parasitic nematode Loa loa which undergoes a developmental cycle in the fly. This disease occurs principally in the equatorial rain forests of Sierra Leone westwards to Ghana and then from Nigeria across Central Africa, the southern Sudan and into western parts of Uganda. Diurnal periodic microfilariae are found in the peripheral blood of man and are ingested by tabanids with their bloodmeal. In Chrysops species, in particular Chrysops silacea and C. dimidiata, some, but not all, the microfilariae survive the process of blood digestion and penetrate the gut wall and migrate to the thoracic muscles. Here they grow, moult twice and develop into shorter and fatter larval forms (2 mm long), which after 10-12 days are able to migrate down the proboscis. Infective larvae may be quite numerous in the fly and are commonly found in the abdomen and thorax as well as in the head and proboscis, but when Chrysops feed on man the infective worms migrate from other parts of the body to the proboscis and enter the skin. They eventually develop into adult worms which live in the subcutaneous tissues of man. Microfilariae of Loa loa are more or less absent from the blood circulation of man at night but appear in it during the day, especially in the morning, and are therefore readily picked up by C. silacea and C. dimidiata species which bite during the day. In Central Africa the vector appears to be C. distinctipennis.

A similar parasite, which was previously considered to be Loa loa, but which may be a different but closely related species, subspecies or strain, occurs in some forest monkeys. The microfilariae appear in their peripheral blood at night and are picked up by C. centurionis and C. langi, species which are mainly crepuscular and nocturnal in their biting habits. It is possible, but not yet proved, that man can become infected with *Loa loa* parasites originating from monkeys.

# Control

There are very few practical control measures to combat tabanids. In theory efficient drainage to remove not only standing water but to dry out marshy and muddy areas might reduce the numbers of species breeding in these habitats, but the cost of both locating the larval habitats, and carrying out drainage obviates this approach. Similarly, because of the difficulty of locating breeding places and their often diffuse and large size it is usually impossible to achieve control by the application of insecticides. Moreover, because the larvae of many species live below the surface of the ground heavy dosage rates are needed for the insecticide to penetrate through the surface soil and vegetation to reach the larvae; these problems are somewhat similar to those encountered in the control of ceratopogonid larvae (chapter 9).

Recently trials in Britain have shown that darkcoloured 60 cm square plaster-board panels coated with a permanently sticky adhesive (polyisobutylene) and placed in fields attract and trap adult Haematopota pluvialis. It was concluded that it was possible to considerably reduce the local population of H. pluvialis by employing a number of these sticky panels. In the U.S.A. sticky traps baited with dry ice (solid carbon dioxide) have also been reported as reducing local populations of Tabanidae. It seems unlikely, however, that either of these methods would be of much practical use in Central and West Africa for reducing transmission of loiasis. Protection from the annoying bites of tabanids can be afforded by the use of suitable repellents such as diethyltoluamide and dimethyl phthalate.

# 11 Tsetse flies (Order Diptera: Family Glossinidae)

#### Species

There are thirty named species and subspecies of tsetse flies all of which belong to the genus Glossina. The most important vectors of human disease are Glossina palpalis<sup>\*</sup>, G. tachinoides, G. fuscipes<sup>\*</sup>, G. pallidipes, G. swynnertoni and G. morsitans<sup>\*</sup>.

#### Distribution

Restricted to tropical Africa from between approximately latitudes  $15^{\circ}$  north and  $20^{\circ}$  south, but extending to about  $30^{\circ}$  south along the eastern coastal area of Africa. Some species such as G. morsitans are found across West Africa to Central and East Africa, whereas others are more restricted in their distribution. For example, G. palpalis occurs only in the West African subregion, G. tachinoides only in West Africa and Ethiopia and G. swynnertoni and G. pallidipes only in East Africa. G. fuscipes occurs in Central and East Africa and is the counterpart of G. palpalis in West Africa. At the beginning of this century G. tachinoides was reported from southern Arabia, but there is no evidence that it exists there now.

#### Medical importance

Tsetse flies are vectors of both human and animal African trypanosomiasis, the disease in man being referred to as sleeping sickness.

# The tsetse fly (Glossina)

# External morphology

# Adults (figure 11.1)

A general description of tsetse flies, without special reference to any particular species, is as follows. Adults

are yellowish or brown-black robust flies that are rather larger (6-15 mm) than houseflies. In some species the six visible abdominal segments are uniformly coloured while in others there may be lighter coloured transverse stripes and a median longitudinal one. Tsetse flies are readily distinguished from all other biting flies and similar sized non-biting flies by the combination of a rigid and forwardly projecting proboscis and a characteristic wing venation. In between veins four and five there is a closed cell which with a little imagination looks like an upside down hatchet (that is, axe, cleaver or chopper) and is consequently often termed the hatchet cell (figure 11.2a). This one character serves to identify a fly as a tsetse. Tsetse flies also differ from most biting and many non-biting cyclorrhaphous flies such as houseflies and calliphorids in that the wings of the fly at rest are placed over the abdomen like the closed blades of a pair of scissors (figure 11.1a). In many other flies the wings remain apart like the open blades of a pair of scissors, thus revealing part of the abdomen.

A long pair of maxillary palps arise dorsally and very close to the proboscis and lie alongside it. They are difficult to distinguish except when the tsetse fly is feeding and the proboscis is swung downwards while the palps remain projecting forwards (figure 11.2b). The first two antennal segments are small and inconspicuous, but the third is relatively large and cylindrical and is somewhat banana-shaped, bearing near its base the arista. As in *Stomoxys calcitrans* the arista has hairs only on its upper surface, but unlike *Stomoxys* these hairs are branched giving the arista a feathery or plumose appearance (figure 11.2c).

The proboscis consists of a lower, relatively large, gutter-shaped labium, which has a bulbous base and terminates in a pair of labella which are furnished with fine rasp-like teeth. The narrower upper component of the proboscis is the labrum. The apposition of the labrum and labium results in forming a tube called the

<sup>\*</sup> Glossina palpalis, G. fuscipes and G. morsitans have each been divided into two or more subspecies, based mainly on their geographical distribution and small differences in their behaviour and /or morphology. This is really of concern only to the specialists and here they are referred to as three single species.



Fig 11.1 Tsetse fly with (a) wings folded like a closed pair of scissors over the body and (b) wings pulled out to display the abdomen and wing venation.



Fig 11.2 Adult tsetse fly, (a) wing showing the hatchet cell shaded, (b) lateral view of head to show palps, antenna and mouthparts and (c) antenna showing plumose branching of hairs on arista.

food channel. There are no mandibles or maxillae but a fine hypopharynx is located within the food channel. When a tsetse takes a blood-meal the rasp-like teeth on the labella of the labium grip the host's skin allowing the needle-like hypopharynx and sharply pointed labrum to be thrust into the skin. Saliva containing anticoagulants is pumped down a duct in the hypopharynx into the wound formed by the fly. Sometimes the teeth on the labella rupture the surface capillaries and blood is sucked up without the labrum and hypopharynx penetrating deeply into the tissues. The blood-meal flows up the food channel formed by the labrum and labium.

The dorsal surface of the thorax of the tsetse has a pattern of dark brown stripes and patches. In the Muscidae only four abdominal segments are visible dorsally but there are six in the tsetse fly. They may be uniformly dark brown or blackish, or have transverse stripes and a median one of a lighter brown or yellowish colour. Although as far as disease transmission is concerned it may not be important to distinguish between the sexes, because both take blood-meals, this can easily be done by examining the tip of the ventral surface of the abdomen. In the male tsetse fly there is a prominent raised almost circular knob-like structure called the hypopygium. This structure can be turned back with a dissecting needle and its distal part folded back again (figure 11.3a, b, c). Thus the whole structure has the appearance of a small distal plate hinged to a larger one which is hinged to the abdomen. The distal plate is composed mainly of a pair of claspers of the external male genital process. The shape of these claspers and associated structures are valuable in the specific identification of male Glossina. In the female fly there is no such knoblike protuberance.

# The alimentary canal

# Adults (figure 11.4)

A knowledge of the morphology of the alimentary canal and its associated salivary glands is essential for an



Fig 11.3 Diagram of ventral surface of the terminal segments of a male tsetse fly showing (a) the normal position of the knob-like protuberance and this structure (b) half unfolded and (c) fully unfolded to expose external genitalia.

understanding of the life-cycle of trypanosomes within the tsetse.

The food channel, formed by the apposition of the labrum and labium, leads to the pharynx and then to the oesophagus which has a slender duct leading to an oesophageal diverticulum commonly called the crop. Just behind the oesophagus there is an important bulbous structure termed the proventriculus. The distal end of the proventriculus marks the end of the



Fig 11.4 Diagrammatic representation of the alimentary canal of a tsetse fly and the thread-like salivary glands.

fore gut and beginning of the mid gut which in the tsetse fly is very long and convoluted. A peritrophic membrane which plays an important part in the cyclical development of sleeping sickness trypanosomes (*Trypanosoma brucei gambiense*, *T. brucei rhodesiense*) in the tsetse fly, is secreted by the epithelial cells in the anterior part of the proventriculus. The peritrophic membrane when first produced by the proventriculus is a very delicate soft almost fluid structure but as it passes back into the gut it hardens to form a thin but relatively tough sleeve, something like a sausage skin. This tube-like peritrophic membrane lines the entire length of the mid gut.

The junctions of the four Malpighian tubules separate the mid gut from the hind gut, which terminates in a small dilated rectum and opens to the exterior through the anus.

The slender paired salivary glands originating in the head of the tsetse are enormously long, very convoluted and stretch back to almost the end of the abdomen. Anteriorly, the ducts from both glands unite in the head to form the common salivary duct which passes down the length of the hypopharynx.

# Life-cycle

# Feeding and reproduction

Both male and female tsetse flies bite man and a large variety of domesticated and wild mammals, and also reptiles and birds. No species of tsetse feeds exclusively on one type of host, but most species show definite host preferences. For example, in East Africa Glossina swynnertoni feeds mainly on wild pigs and G. morsitans
on wild and domesticated bovids and wild pigs, but in West Africa G. morsitans feeds mainly on warthogs. In East Africa G. pallidipes feeds principally on wild bovids, while in West Africa G. palpalis feeds predominantly on reptiles and man, and G. tachinoides feeds on man and bovids, but in southern Nigeria predominantly on domestic pigs. They take bloodmeals about every two to three days, although this interval may be reduced in dry, hot weather or prolonged for about ten days in cool humid conditions. Feeding is restricted to daylight hours and vision plays an important part in host location, dark moving objects being particularly attractive. On paleskinned people, such as Caucasians, tsetse flies often prefer to bite through dark coloured clothing such as socks, trousers and shorts in preference to settling on the skin. During feeding blood is sucked up the proboscis and passes to the crop, but when the proboscis is withdrawn from the host the blood passes from the crop to the stomach where digestion proceeds.

The different types of flies so far described in this book lay eggs, but in marked contrast tsetse flies do not, instead they deposit larvae, one at a time. Adults of *Sarcophaga, Wohlfahrtia* and *Oestrus* also deposit larvae not eggs (chapters 13 and 14).

After a female tsetse fly has been inseminated by a male and after it has taken a blood-meal, a single egg in



Fig 11.5 Diagrammatic representation of the reproductive system of a female tsetse fly showing a full grown larva in the uterus.

one of the two ovaries completes maturation, passes down the common oviduct into the uterus where it is

fertilised by the release of spermatozoa from the spermathecae. The egg is creamy white in colour, has distinct reticulations and measures about 1.5 mm. It hatches within the uterus after about three to four days, and the empty egg shell passes out through the genital orifice (vagina). The uterus which is capable of considerable expansion is supplied with a conspicuous pair of branched secretary accessory glands which in tsetse flies are called the milk glands (figure 11.5). Fatty, nutrient fluid from these glands flows through a small duct to enter the uterus at its anterior end. The larva is orientated within the uterus so that its mouth is near the opening of the common duct of the milk glands; and the secretion of these glands provide the larva with all the food it needs for growth and development. The larva passes through three instars in the female, and the cast-off skin from each moult is passed out through the genital orifice. Regular blood-meals must be taken by the female for a continuous and adequate provision of nutrient fluid from the milk glands. If the fly is unable to feed the larva may fail to complete its development and as a consequence be aborted.

Larval development is completed after about four to five days, by which time the third and final instar larva is 8-9 mm long. It is creamy white in colour and composed of 12 visible segments, the last of which bears a pair of prominent dark protuberances called the polypneustic lobes (figure 11.6b). These bear supernumerary stigmata and are respiratory structures carrying out the functions of the posterior spiracles of muscid larvae. A female containing a fully developed larva is easily recognised because the fly's abdomen is enlarged and stretched, and the fly is obviously 'pregnant'. Furthermore, the black polypneustic lobes can be seen through her abdominal integument.

The mature third-instar larva wriggles out posterior end first from the genital orifice, thus birth can be termed a 'breech case'. Females always select shaded sites for larviposition. The larva is deposited on loose friable soil, sand or humus, frequently underneath bushes, trees, fallen logs, rocks, between buttress roots



Fig 11.6 (a) Puparium and (b) larva of a tsetse fly.

of trees, in sandy river beds, in animal burrows and even in rot holes in trees which may be formed some distance above the ground (4-5 m). Immediately the larva is deposited it commences to bury under 2-5 cmof soil. After about 15 minutes the third-instar larval skin contracts and hardens to form a reddish brown or dark brown, barrel-shaped puparium which is about 5-8 mm long and has distinct polypneustic lobes (figure 11.6a). Within this puparial case the larva pupates.

The duration of the pupal period is comparatively long, usually extending over four to five weeks but at high temperature may be completed within three weeks and conversely at low temperatures prolonged to thirteen weeks. After pupal development has been completed the fly emerges from the puparium, forces its way to the surface of the ground and after some 15-20 minutes takes flight.

During the development of the larva within the female the tsetse feeds several times, about every two to three days. The first larva is deposited about 16-20 days after the female has emerged from the puparium, thereafter if food is plentiful a larva is deposited about every 9-12 days. In the laboratory female tsetse flies have produced up to 20 offspring, but the average, is nearer five to eight. Breeding generally continues throughout the year but in very humid conditions reproduction may be diminished. Maximum population size is usually obtained at the end of the rainy season. The population diminishes in the dry season when suitable areas of refuge for adult flies and suitable larviposition sites may become restricted and localised.

#### Adult behaviour

Knowledge of certain aspects of the behaviour of tsetse flies is essential for an understanding of approaches to their control and also the part vector species play in the transmission of sleeping sickness.

Blood-engorged tsetse flies and unfed hungry flies waiting to feed on suitable hosts spend the nights and much of the daytime hours resting in dark and usually humid resting sites. During the day the favoured resting sites of most species are twigs, branches and trunks of trees and bushes. Flies are not found resting in sites in which temperatures rise above about  $36^{\circ}$ C. At night tsetse flies prefer to rest on the upper surfaces of leaves. Accurate knowledge of the actual resting sites may be required for control measures. For example, the height they rest on trees determines the height at which trees need to be sprayed with insecticides to kill resting adults. Most species in fact rest below 4 m; in Nigeria 50 per cent of G. palpalis and G. morsitans commonly rest between ground level and 30 cm.

Based on their morphology, ecology, karotype and

behaviour tsetses can be separated into the following three main groups.

Fusca group (forest flies) This group contains 14 species and subspecies of Glossina all of which are large (10.5– 15.5 mm). They are forest flies (except G. longipennis) and most are restricted to the equatorial forests of West and Central Africa, for example G. fusca occurs in relict forests of West and Central Africa and G. brevipalpis in secondary forests of East Africa.

The *fusca* group rarely feeds on man and none of the species is a vector of sleeping sickness.

Morsitans group (savanna flies) Seven species and subspecies are included within this group. They are medium sized insects (7.5-11 mm). They are typically flies inhabiting the savanna regions of Africa, which may extend from the coast or the edges of forests to dry semi-desert regions. G. morsitans occupies the savanna regions of West, Central and East Africa, whereas G. pallidipes and G. swynnertoni are restricted to the savannas of East Africa. The former two species occur in country ranging from wooded savanna at the edges of forests to the dry thicket vegetation of arid region while G. swynnertoni is mainly restricted to relatively dry thicket country.

All three species are vectors of sleeping sickness, the most important being G. morsitans.

Palpalis group (riverine and forest flies) Nine tsetse species and subspecies are found in this group, the smallest being about 6.5 mm in length and the largest 11 mm. They are essentially flies inhabiting wetter types of vegetation, such as forests, luxuriant scrub and vegetation growing along rivers and shores of lakes. G. palpalis inhabits riverine vegetation bordering rivers and lakes, mangrove swamps and forested areas, and occurs throughout most of West Africa, down the western part of the continent to Angola. G. fuscipes, which is closely related to G. palpalis, occurs mainly in Central Africa but extends its range to the western areas of East Africa. G. tachinoides is a riverine species found near streams and rivers in wet humid coastal areas, through wooded savanna regions to the riverine vegetation of very dry savanna areas. It is found mainly in West and Central Africa but also occurs in Ethiopia.

All these species are vectors of sleeping sickness.

# Medical importance

Probably any species of tsetse fly can transmit African trypanosomiasis to man, just as almost any species of triatomine bug can transmit American crypanosomiasis (*Trypanosoma cruzi*) to man (chapter 18). In practice, however, relatively few species of either tsetse flies or triatomines are natural vectors, because many species rarely if ever feed on man. It is the behaviour of the adult tsetse and the degree of fly-man contact, and in the case of Rhodesian sleeping sickness also the degree of vector contact with the reservoir hosts of the trypanosomes, that establishes whether a tsetse fly is a vector.

Sleeping sickness covers 10 million km<sup>2</sup> of land in Africa. There are two species of trypanosomes causing African sleeping sickness in man, *Trypanosoma brucei* gambiense and *T. brucei rhodesiense*, parasites that are morphologically indistinguishable but which produce different clinical symptoms in man and have different epidemiologies. The most important vectors of sleeping sickness are *G. palpalis*, *G. fuscipes*, *G. tachinoides*, *G. morsitans*, *G. pallidipes* and *G. swynnertoni*.

The cycle of development of Trypanosoma brucei gambiense and T. brucei rhodesiense in the tsetse is the same and is as follows - trypanosomes sucked up by male or female tsetse flies in a blood-meal from an infected man (or in the case of T. brucei rhodesiense mainly from a non-human reservoir) pass first through the oesophagus to the crop, and then after feeding has ceased into the peritrophic tube lining the mid gut. Blood is digested within the mid gut but the gambiense and *rhodesiense* trypanosomes are not destroyed but in fact multiply. Until recently it was considered that the only way in which they were able to infect the salivary glands was for them to travel round the end of the peritrophic membrane and migrate between it and the mid gut wall back to the proventriculus. Here the peritrophic membrane is soft and more or less fluid and this allows the parasites to penetrate it and pass to the oesophagus, from here they continue their journey through the pharynx down to the tip of the proboscis. They now migrate up the salivary duct in the hypopharynx to reach the paired salivary glands where the mature infective metacyclic forms (trypomastigotes) develop. These are injected into the host when the fly feeds. The interval between the time a tsetse fly engorges on an infected person to when its salivary glands contain infective trypanosomes varies between 18-34 days. More recently, however, it has been found that T. brucei rhodesiense parasites in Glossina morsitans can penetrate the gut cells and pass across into the haemocoel from where it seems they can migrate directly to the salivary glands. The importance of this more direct route of trypanosomal migration to the salivary glands is as yet unclear.

When establishing infection rates in tsetse flies by dissection any trypanosomes found in the gut or proboscis are ignored, as only those in the salivary glands can be ascribed with any degree of certainty to T. brucei gambiense or T. brucei rhodesiense, but there are complications. T. brucei brucei which does not cause

sleeping sickness in man but causes animal trypanosomiasis, commonly called nagana, undergoes a similar cyclical development in the fly. Consequently the presence of trypomastigotes (metacyclic forms) in the salivary glands does not necessarily indicate infection of trypanosomes infective to man.

Salivary gland infection rates in tsetse flies are low, nearly always being less than 1 per cent, even in areas of endemic sleeping sickness.

#### Gambian sleeping sickness

Glossina palpalis and G. tachinoides are the most important vectors in West Africa and G. fuscipes in Central and East Africa of Trypanosoma brucei gambiense, the causative agent of Gambian sleeping sickness. This disease is relatively chronic, with death often not occurring until after many years. It is usually considered that there are no natural reservoirs of the disease other than man, but recent studies in West Africa indicate that pigs sometimes have trypanosomes very similar to those of T. brucei gambiense and therefore it is possible that pigs may sometimes act as reservoirs, but this needs clarification.

The vectors of Gambian sleeping sickness are especially common at watering places, fords across rivers and along lake shores etc., in fact in places where people frequently visit to collect water or do their washing. As a consequence there may be limited and localised foci of transmission.

#### Rhodesian sleeping sickness

The causative agent, *T. brucei rhodesiense*, causes a more virulent disease than *T. brucei gambiense*, but it is not so widespread, being more or less restricted to Tanzania, Malawi, Zambia, Zimbabwe-Rhodesia, Mozambique and to the northern areas of Lake Victoria in Kenya and Uganda. The most important vectors are *G. morsitans*, *G. swynnertoni* and *G. pallidipes*, species which feed on a variety of game animals and domestic livestock, especially bovids, in preference to man. These flies often occur in savanna areas thinly populated by humans. Wild animals, especially a number of bovid species, are important reservoirs of *T. brucei rhodesiense*. The disease is a zoonosis.

# Control

Because the immature stages are so well protected, the larva being retained by the female for almost all of its life and the puparium being buried in the soil, control against tsetse flies is aimed at the adults.

Many control methods have been directed at tsetse

flies to combat human and animal trypanosomiasis. At one time there were campaigns in Zimbabwe-Rhodesia and some East African countries to kill in selected areas all game animals that might provide food for tsetses or be reservoirs of trypanosomiasis. The wide scale and often indiscriminate slaughter of animals is no longer acceptable in a world that is increasingly sensitive to the preservation of wild life.

The distribution and abundance of tsetse flies is largely determined by types of vegetation and microclimate, and their dependence on such requirements gave rise to another form of control. This consisted of clearing vegetation, especially that near rivers and lake shores, either completely or partially and often on a large scale so that habitats were opened up and made unsuitable for tsetses. The destruction of such vegetation has in the past achieved considerable success in controlling tsetse flies, but in many countries this method, at least on a large scale, is at present no longer ecologically or economically acceptable, but may in the future again play a part in control, especially in an integrated approach.

There are no problems of insecticide resistance with tsetse flies, and at present tsetse control relies almost exclusively on insecticides. Currently only the persistent organochlorine insecticides, such as DDT, dieldrin and endosulfan are used on a wide scale to control or eradicate tsetse. They are generally applied as either wettable powders or emulsions to vegetation harbouring resting adults. The success of such methods depends much on a detailed knowledge of the behaviour and movements of the adult flies. Insecticides are often sprayed on vegetation at concentrations of 1.5-5 per cent of active ingredient from knapsack sprayers or other insecticidal ground-based machines. The frequency of application depends much on local conditions, such as rainfall, other climatic factors and the growth and spread of fresh vegetation which can provide new resting surfaces free of insecticides.

Alternatively aerial applications of insecticides can be made, either as ultra-low-volume dosages of nonresidual insecticides from fixed-wing aircraft or as residual deposits applied from helicopters. Although aerial spraying, particularly of residual deposits, results in a greater coverage of vegetation by the insecticides than achieved by ground application techniques, it usually kills a greater number of non-target insects and animals, many of which may be directly or indirectly beneficial to man. Repeated insecticide applications, especially of non-residual insecticides like resmethrin and other synthetic pyrethroids, are necessary because the tsetse population is in the soil as puparia for four to five weeks or longer and is not affected by spraying. There is no doubt that indiscriminate or intensive and repeated sprayings of residual organochlorine insecticides can have disastrous effects on the local fauna, but it should be appreciated that the application rates of insecticides for tsetse control are often very low. It should also be realised that after tsetse flies have been eradicated from an area and spraying stopped, much if not all of the wild life may revert to their original numbers. Intensive bush clearance for tsetse control can also greatly diminish the numbers and variety of local fauna, but in this instance the change may be permanent, especially if local people occupy and farm areas that were originally scrub or forest.

# 12 Houseflies, related flies and stableflies (Order Diptera: Family Muscidae)

#### Species

The family Muscidae comprises three subfamilies, the Muscinae, Fanniinae and Anthomyiinae. In some classification these three subfamilies are considered to merit elevation to family status.

There are many genera and species of Muscidae. The most important from the medical aspect are the housefly (*Musca domestica*), the greater housefly (*Muscina stabulans*), the lesser housefly (*Fannia canicularis*), the latrinefly (*Fannia scalaris*) and the blood-sucking stablefly (*Stomoxys calcitrans*).

#### Distribution

Musca domestica, Fannia canicularis, Muscina stabulans, Fannia scalaris and Stomoxys calcitrans occur worldwide while other closely related species have a more restricted distribution.

#### Medical importance

The non-biting muscid flies are vectors of several cestodes, nematodes, faecal bacteria, protozoa and viruses, resulting in the spread of such enteric diseases as the dysenteries and typhoids. Some species may occasionally cause urinogenital myiasis in man. *Stomoxys calcitrans* can be a mechanical vector of trypano-somiasis.

# The common housefly (Musca domestica)

# External morphology

#### Adults (figure 12.1)

Houseflies have a world-wide distribution. They are medium sized non-metallic flies about 6-9 mm in

length, varying in colour from light to dark grey and have four broadish dorsal dark longitudinal stripes on the thorax (figure 12.1a). The head bears a prominent pair of brownish compound eyes which are closer



Fig 12.1 Musca domestica, (a) adult fly and (b) terminal tarsal segments showing paired claws, paired pulvilli and bristle-like empodium.

together and slightly bigger in males than females. Each antenna consists of three segments, the last and biggest of which is cylindrical and has a prominent hair, called an arista, arising from the dorsal surface near its base. The arista has small hairs on both sides, giving it a feather-like appearance (figures 12.2 and 12.4). The antennae lie concealed in a depression on the front of the face of the fly and are not easily seen unless the fly is carefully examined. The mouthparts (proboscis) of the housefly are complicated and specially



Fig 12.2 Musca domestica, (a) proboscis retracted and (b) proboscis extended for feeding.

adapted for sucking up fluid or semi-fluid foods. When not in use the mouthparts are partially withdrawn into the head capsule (figure 12.2a), but are extended vertically downwards in a telescopic fashion when the fly feeds (figure 12.2b).

Some explanation of the structure of the proboscis is necessary in order to understand the methods by which the fly feeds and spreads disease.

The basal part of the mouthparts is often termed the rostrum and is roughly pyramidal in shape and is more heavily sclerotised than other components of the proboscis (figure 12.3). The rostrum bears on the anterior surface a pair of small unsegmented maxillary palps. Just below these palps there is a small constriction in the proboscis which marks the end of the rostrum and beginning of the next part which is called the haustellum. The rostrum and haustellum together constitute the *labium*. The distal part of the labium, that is the haustellum, is deeply grooved along its anterior face and in this groove lie the small *labrum* and hypopharynx. The slender *labrum* is grooved on its posterior surface and is appressed against the bladelike hypopharynx which is grooved along its anterior face. The



Fig 12.3 Diagram of main parts of the proboscis of Musca domestica.

apposition of these two grooved mouthparts results in the formation of a food channel up which food is sucked into the pharynx. The section of the mouthparts distal to the haustellum is termed the oral disc, its most prominent structure being a pair of conspicuous fleshy labella. Saliva flows down a minute channel in the hypopharynx which opens at the junction of the labella and the main part of the labium. Surrounding this opening is a sclerotised structure called the discal sclerite which bears ten minute prestomal teeth. From this sclerite arise the pseudotracheae which traverse the two oval lobes of the labella. Along the length of these pseudotracheae are minute openings through which fluids and very small food particles are sucked into the mouth which is situated in the middle of the oral disc. Food is then drawn up the food channel, formed by the apposition of the labrum and hypopharynx, and passes to the pharynx and into the alimentary canal.

The thorax of Musca domestica has four well developed and rather broad dark grey or blackish longitudinal stripes, which are usually more clearly defined anteriorly than posteriorly (figure 12.1a). The wings of the housefly have vein four bending up sharply to join the costa close vein to three (figure 12.4). This is an important taxonomic character which can help distinguish Musca species from other rather similar flies such as species of Fannia and Muscina and also the blood-sucking Stomoxys calcitrans although this latter species is more readily recognised by its forwardly projecting proboscis. Each of three pairs of legs end in a pair of claws and a pair of fleshy pad-like structures called the pulvilli which are supplied with glandular hairs (figure 12.1b). These sticky hairs enable the fly to adhere to very smooth surfaces, such as windows, and are also responsible for the fly picking up dirt, filth and pathogens when it visits excreta, septic wounds, rubbish dumps etc.

The abdomen which is greyish and has a pattern of darker and lighter markings is usually partially obscured from view by the wings. It appears to be composed of only four segments, but there are other segments withdrawn into the abdomen. In the females these are modified to form a retractile segmented and tube-like structure which is extended from the abdomen when the fly lays her eggs.

#### Feeding methods of adult flies

Adult male and female houseflies feed on a great variety of substances, such as sugar, milk, almost all food of man, rotting vegetables and carcasses, excreta and vomit, in fact almost any organic material. In this respect, but not in the actual method of feeding, they resemble cockroaches. During feeding the mouthparts are extended downwards on to the food, but the method of feeding differs according to the physical state of the food (figure 12.5a, b, c). For example, for thin fluids, such as milk and beer the labella are closely appressed on to the food which is then sucked up through the small openings in the pseudotracheae (figure 12.5a). This is referred to as the 'filtering position' of feeding. On thicker fluids such as syrups, pus, serum and other viscous or semi-fluid substances the labella are arched up away from the surface, but the fluids still pass up the pseudotracheae. This is some-







domestica





Muscina stabulans









calcitrans Stomoxys

Fig 12.4 Wings and antennae of Musca domestica, Muscina stabulans, Fannia canicularis and Stomoxys calcitrans showing venation, especially curvature of vein four, and form of arista.



Fig 12.5 Diagrammatic representation of positions assumed by the proboscis of *Musca domestica* in feeding on (a) thin fluids ('filtering position'), (b) solids ('scraping position') and (c) thick fluids ('direct feeding position'). (Modified from Gordon and Lavoipierre, 1962.)

times referred to as the 'cupping position'. However, in practice there may not be very clear distinctions between these two feeding methods. When the flies feed on semi-solids like excreta, sputum, nasal discharge, a 'direct feeding' procedure is employed: the labella are completely everted and food is sucked up directly into the food channel (figure 12.5c). When flies feed on more solid materials such as sugar lumps, dried blood, cheese and cooked meats the labella are everted and the prestomal teeth surrounding the oral opening are exposed and scrape away at the solid food (figure 12.5b). The fly then moistens small particles with either saliva or the regurgitated contents of its crop, after which the food is sucked up. This latter type of feeding is clearly a method that is conducive to the spread of a variety of pathogens.

# Life-cycle

Fertilised females of Musca domestica which are ready to oviposit are attracted for egg laying to a variety of decomposing materials such as horse manure, poultry dung, urine-contaminated bedding, foodstuff, carcasses, decaying and decomposing organic materials found in rubbish dumps and amongst household garbage and waste foods from kitchens and hotels. Usually some 500 eggs, but occasionally up to 1000, are matured by females during their lifetime and these are laid during five to six separate ovipositions. During egg laying about 75-100 eggs are deposited together, or in separate batches, either in cracks and crevices or scattered over the surface. The eggs are creamy-white, about 0.8-1.0 mm long, distinctly concave dorsally and with two inconspicuous raised ridges; the anterior face is convex, so that the eggs look banana-shaped (figure 12.6a). Eggs can hatch after only 6-12 hours, but this period is extended in cool weather. Hatching is accomplished by the strip of egg shell between the parallel ridges on the dorsal concave surface lifting up

and partially detaching itself from the rest of the egg. Eggs cannot withstand desiccation and die if they dry out. Neither can they tolerate extremes of temperatures, most dying after exposure to temperatures below  $15^{\circ}$ C or above  $40^{\circ}$ C.



Fig 12.6 Musca domestica, (a) eggs, (b) larva, (c) posterior spiracles of larva and (d) puparium.

The larvae which hatch from the eggs are 12segmented, cylindrical, maggot-shaped and white to creamy white (figure 12.6b). They have no spicules. At the pointed head end they have attached to the cephalopharyngeal skeleton a pair of small curved mouthhooks which are unequal in size. This can be seen as a blackish structure situated beneath the integument of the head and first few thoracic segments. At the anterior and posterior ends of the body there are a pair of spiracles; the posterior ones are more conspicuous and are shaped like a letter D. Each posterior spiracle has a complete and thick peritreme and a distinct button surrounded by three very sinuous spiracular slits (figure 12.6c).

Larvae feed on liquid food resulting from decomposing and decaying organic material. There are three larval instars. The speed of larval growth and development depends on the abundance of food supply and temperature. During hot periods of the year the internal temperatures of decaying refuse may become too hot for the larvae which as a consequence migrate to the outer and cooler layers. If larval habitats dry out the larvae are killed, but if habitats become too wet the larvae drown. Mature larvae measure about 10-15 mm, the final size depending on environmental conditions, especially the abundance of available food. Development may be complete within only three days, but more often takes about five days and under less favourable conditions seven to ten days; in cool weather development may extend to about 24 days.

Prior to pupation the third-instar larvae may mi-

grate away from their larval habitats to drier ground, either adjacent to the breeding place or several metres away. Sometimes, however, the periphery of breeding places, such as rubbish dumps, may be sufficiently dry for larvae to pupate there, pupation may also occur in the dry soil underneath larval habitats or in dry heaps of animal manure. Pupation begins with the larval skin contracting, hardening and turning dark brown, afterwhich a barrel-shaped structure measuring about 6 mm, called the puparium, is formed. Close examination shows this is segmented (figure 12.6d). The puparium is often referred to as the pupa but technically this is incorrect because the actual pupa is formed within the protective shell of the puparial case. (A puparium is also formed by tsetse flies, see chapter 11.) The puparial pupal stage lasts about three to five days in warm weather, but may be prolonged to 7-14days during cooler periods.

Developmental time from egg to adult is about 49 days at 16°C, 21 days at 20°C, 16 days at 23°C, 9–11 days at 30°C and eight days at 35°C. Occasionally the period can be less than seven days. In temperate areas a varying, but usually small, proportion of houseflies may survive throughout the winter as puparia, but more frequently they overwinter as hibernating adults. Occasionally there is a very prolonged but continuous larval development during the winter months.

The adult fly escapes from the puparium by pushing off its anterior end. It wriggles out, forces its way to the surface of the soil and, after its wings have straightened out and hardened, takes flight. As described under the section devoted to feeding techniques the adults are omnivorous, feeding on a variety of fresh or putrid animal or vegetable substances.

Adults of Musca domestica generally avoid direct sunlight, preferring to seek shelter in buildings inhabited by man or his animals. Houseflies and related flies, and the calliphorid flies discussed in the next two chapters, are often called domestic or synanthropic flies, because of their close association with man and his home. M. domestica adults are most active during the cooler part of the day, but at temperatures below 10°C they become inactive. This behaviour contrasts with that of houseflies of the Musca sorbens complex, which are active mostly in the hotter parts of the day, and which prefer sunlit to dark situations. Houseflies defaecate at random and frequently regurgitate their foods at intervals resulting in unsightly 'fly spots'. Adults frequently fly 3-4 km from their emergence sites, and may travel as far as 34 km.

# Other species of houseflies

There are about 60 species of Musca, all of which, except M. domestica which is widespread in its distri-

bution and *M. autumnalis* which occurs in both the Old and New Worlds, are confined to the Old World. The only medically important species belong to the *M.* domestica and *M. sorbens* complexes, although *M.* autumnalis, sometimes called the facefly, may also be a nuisance. Many of the forms within these complexes, such as *M. vicina*, *M. nebulo*, *M. curviforceps* and *M.* calleva of the *M. domestica* complex, have been named as distinct species, subspecies or varieties, but it is still uncertain what taxonomic status most of these forms merit.

The Musca sorbens complex is abundant in the tropics and subtropics of the Old World, including Australia, where it complements M. domestica in its biology and behaviour. Members of the M. sorbens complex are found indoors more rarely than M. domestica, they prefer light to shaded places and have a greater tolerance to high temperatures. Adults are more attracted to the human body than M. domestica. They are especially common on the face around the eyes, on sores and on any discharges. Females mature up to about 80 eggs at a time and these are laid in one or several batches, especially on human excrement, but excreta of other animals such as pigs, cows and dogs and also carcasses provide suitable larval habitats. At 25-28°C the life-cycle from egg to adult takes about nine days.

# Medical importance

Houseflies can transmit a large number of diseases to man owing to their habit of visiting almost indiscriminately faeces and other unhygienic matter and then the food of man, in addition they vomit during feeding and frequently defaecate on food. Over 100 species of pathogens have been recorded as being carried by houseflies. In nearly every instance transmission is mechanical, that is the fly acts as a physical carrier, irrespective of whether pathogens are transmitted by (1) the flies' feet and body hairs, (2) their mouthparts, (3) ingestion and deposition of faecal spots or (4) regurgitation of their vomit. None of the pathogens undergoes obligatory cyclical development in the fly. However, it should be remembered that larvae which have fed on contaminated foods can produce infected adult flies.

Houseflies can transmit viruses such as poliomyelitis, trachoma, Coxsackie virus, infectious hepatitis, rickettisae such as Q fever (*Coxiella burneti*), and numerous bacterial diseases, but mainly enteric ones such as bacillary dysentery (*Shigella*), cholera, the typhoids and paratyphoids (*Salmonella*), a variety of streptococci and staphylococci, conjunctivitis, yaws, and more rarely even tuberculosis, leprosy and anthrax. They may also be vectors of protozoan parasites such as the amoebic dysenteries (*Entamoeba*, *Giardia*) and eggs of a variety of tapeworms, for example, *Taenia* spp. including *T. solium*, *Hymenolepis nana*, *H. diminuta*, *Dipylidium caninum*, *Diphyllobothrium latum* and several others, also nematodes such as *Necator*, *Ancylostoma*, *Thelazia californiensis*, *Tricocephalus* (= *Trichuris*) trichiurus, Enterobius vermicularis and Ascaris lumbricoides. In addition, they can be carriers in the tropical Americas of the eggs of Dermatobia hominis a myiasisproducing fly (see chapter 14).

Larvae of houseflies have occasionally been recorded in cases of urinogenital and traumatic myiasis and more rarely in aural and nasopharyngeal myiasis. If food infected with fly maggots is eaten then these may be passed more or less intact in the excreta, often causing considerable alarm and surprise, but there is no true intestinal myiasis in man (chapter 13).

It has long been recognised that flies, because of their dirty habits, are potential vectors of many pathogens to man but it is very difficult to assess their relative importance in the transmission of most diseases. Much information of their real role in the spread of disease to man is circumstantial, for example, seasonal increase of fly abundance is often closely correlated with outbreaks of diarrhoeal diseases. The classic demonstration of the association between flies and disease was in Texas in 1946 and 1947, when it was convincingly shown in two towns that the reduction of flies by insecticides was accompanied by a reduced incidence of Shigella infection, and a marked decrease in the number of deaths in children due to diarrhoea. A similar association between the incidence of diarrhoea and housefly abundance has been observed in Palestinian refugee camps.

# Control

Control methods can be divided conveniently into three categories – (1) physical and mechanical, (2) environmental sanitation and (3) insecticidal. A useful account of fly control as practised in the U.S.A. is found in the publication issued by the Center for Disease Control, Atlanta, written by Pratt, Littig and Scott (1975), the complete reference is given at the end of the book.

# Physical and mechanical control

In some situations flies and other obnoxious insects can be prevented from entering buildings by screening doorways, windows, openings, air vents etc. Screens should be made of non-corrosive material, such as plastic, copper or aluminium gauze and have a mesh size of about 16 strands per inch. This will exclude relatively large insects such as houseflies from buildings, without unduly decreasing air circulation or light. Such screening will not prevent the entry of small insects such as biting midges and small mosquitoes. Screening can be costly, but may be worthwhile employing in hospitals and restaurants. It is usually unnecessary to screen windows higher than the fourth floor of buildings. Screening should be periodically inspected and any tears mended. Screening can reduce fly nuisance but does not solve the problem, for flies will continue to breed locally and to enter unscreened houses.

The establishment in doorways of an air current, such as the air barriers found in the entrances of some shops, and fans mounted over doorways may help to reduce the number of flies entering premises. The old and well known practice of placing in doorways curtains made of many vertical, often coloured, strips of plastic or beading also helps to keep out flies. Electrocution methods are also used. For example, a not uncommon method, especially in restaurants and food stores, is to mount an ultraviolet light trap on a wall. Flies attracted to the trap are killed on entering it by contact with an electric grid. In some large dairies and food processing plants the metal screening on windows and doors is electrified by using a low amperage but high voltage (about 35 000 volts). This kills flies which come into contact with the screens but does not harm humans or large animals. This latter method has very limited application.

# Environmental sanitation

This method usually aims at drastically curtailing housefly populations by reducing their breeding places. For example, all domestic refuse and garbage should be placed in either strong plastic bags and the openings tightly closed or in dustbins with tight-fitting lids. When possible there should also be regular refuse collections, preferably twice a week in warm countries, to prevent any eggs laid amongst the garbage developing to adults. If household refuse cannot be collected it should be burnt or buried.

In many towns and villages throughout the world there are unsightly and unhygienic rubbish dumps providing ideal breeding places for houseflies – they should be abolished. Refuse should be placed in pits which are covered, daily if possible, with a 15 cm layer of earth, when the pits are more or less full they must be finally covered with 60 cm of compacted earth. This depth of final fill is required to prevent rodents being attracted to buried decomposing organic material and burrowing into the rubbish pits.

It is also important to organise efficient disposal of household and industrial sewage and sanitary wastes. There should also be efficient latrines which prevent the breeding of houseflies and allied flies. In some areas manure from farm animals and poultry provide additional breeding places. Manure should not be allowed to accumulate in piles but spread thinly over the land as fertiliser, burnt, composted with soil or buried in pits.

#### Insecticidal control

Many different types of insecticides and procedures have been used to reduce fly nuisances. Commercially available small aerosol dispensers are commonly used in homes as a space-spray to kill flies. Most of these aerosols contain knock-down insecticides such as 0.5 per cent dichlorvos (DDVP) or 0.1–0.2 per cent pyrethrins or allethrin synergised with piperonyl butoxide. Synthetic pyrethroids such as resmethrin, used as a 0.1 per cent spray, need no synergists and are 20 times more toxic to houseflies than natural pyrethrins. Aerosol sprays have no, or very little, residual effect and consequently must be repeatedly used to achieve control; they do little to alleviate the source of the fly nuisance, and moreover they can be costly.

The outdoor application of insecticidal aerosols or mists from special spraying machines can give effective control in certain situations, such as in and around dairies, farms and poultry sheds and this method can also give good local control of the blood-sucking stablefly (Stomoxys calcitrans). Organophosphate insecticides such as malathion, diazinon, fenthion (Baytex), naled (Dibrom) and dichlorvos (DDVP) have been successfully used in outdoor aerosol applications. Flies may also be controlled by spraying the indoor walls, ceilings, doors etc. with residual insecticides such as 3 per cent malathion or 1 per cent dimethoate emulsions, 0.5-1 per cent fenchlorphos (Ronnel) or 1-2 per cent tetrachlorvinphos (Rabon) wettable powders or 1 per cent propoxur (Arprocarb) emulsion or wettable powder. These residual insecticides should remain effective for one to two months, but much depends on local circumstances and whether walls are washed. The outside walls of houses, cattle sheds etc. may also be sprayed with residual insecticides, but their duration of effectiveness will depend on several factors, such as whether the surface deposit is washed off by rain or rubbed off.

Alternative methods employ strips of cord or rope soaked in such insecticides as diazinon or parathion. The latter is extremely poisonous and great care must be exercised in handling it – rubber gloves should be used. Any treated cord should be dyed, preferably red, to alert people that it is impregnated with insecticides. Flies are killed when they rest on these cords hung up in dairies and houses. Dichlorvos resin strips are also commonly used to control flies in houses, restaurants, hotel kitchens, dairies etc. These impregnated strips kill flies by dissemination of the dichlorvos vapour. They may remain effective for two to three months, but this depends on the temperature and degree of ventilation in the rooms in which they are used. In some countries their use has been restricted because of the possible danger of prolonged exposure to DDVP.

Larviciding, that is the use of insecticides to kill the maggots of houseflies, has had mixed success. As practised in the control of the calliphorid flies (chapter 13) insecticidal emulsions or wettable powders can be sprayed on the insides and outsides of dustbins and on walls adjacent to them. Such applications can undoubtedly reduce breeding in these situations. Another method is to fix a dichlorvos strip on to the inside of the dustbin lid, or to scatter paradichlorobenzene crystals in the dustbin every one to two weeks. Refuse and garbage heaps, manure piles and other large breeding sites can be sprayed with a variety of insecticides, but the main problem is that there is usually insufficient penetration of insecticide into these breeding places to give effective control. However, regular spraying of such piles of breeding media may deter flies from settling on them and ovipositing, and may even kill some adult flies. The most successful larvicides are emulsions of 0.5 per cent diazinon, 1-1.5 per cent dimethoate, 0.5 per cent malathion and 0.5 - Iper cent dichlorvos.

Attractant solid or liquid fly baits have also been used to control houseflies. Sugar mixed with an inert carrier such as sand or bran and treated with 1-2 per cent insecticides provides an attractive solid bait. Liquid baits commonly comprise 10 per cent sugar dissolved in water plus 0.1-0.2 per insecticides. The most commonly used insecticides in both dry and liquid baits are malathion, diazinon, dichlorvos, naled (Dibrom), fenchlorphos (Ronnel), or trichlorphon either alone or in combination. In some countries commercially prepared dry or liquid fly baits are available. Dry baits can be scattered or placed in wooden, plastic or metal trays. Liquid baits can be placed in a glass bottle which is inverted over a saucerlike receptacle so that as the bait evaporates in the receptacle more flows in from the reservoir, as in automatic water feeders for poultry. Attractant baits can very quickly produce spectacular reductions of flies in buildings, but solid baits must be replaced about every two days.

# Insecticide resistance

In most areas of the world houseflies have developed resistance to the more common organochlorine insecticides so that DDT, HCH and dieldrin are often of little use in control. Many populations of flies have also developed resistance to several organophosphate insecticides, but not to the natural pyrethrins or synthetic ones such as bioresmethrin or permethrin. If fly control is not proving effective despite efficient application methods then arrangements should be made to test the local fly population for their susceptibility to the insecticides being used.

# The lesser housefly (Fannia canicularis) and latrinefly (Fannia scalaris)

# External morphology

#### Adults

Flies of the genus Fannia resemble houseflies but are generally a little smaller (6-7 mm). Both F. canicularis and F. scalaris have a world-wide distribution. They are most readily distinguished from houseflies and other Musca species and from Muscina by their wing venation and antennae (figure 12.4). In Fannia vein four of the wing is more or less parallel to vein three, whereas in Musca it bends upwards and almost touches vein three at the wing apex, while in Muscina vein four also bends upwards but not to such an extent. The sixth vein is also much shorter than in Musca and Muscina. Careful examination with a hand lens or dissecting microscope shows that the arista arising from the third antennal segment is completely devoid of hairs (figure 12.4), whereas in both Musca and Muscina it bears branches on both sides. In most other respects the adult flies are similar to houseflies. Two species of Fannia are commonly encountered in houses, namely Fannia canicularis (the lesser housefly) which has three longitudinal brown stripes on the thorax and F. scalaris (the latrinefly) which has two such stripes.

#### Life-cycle of Fannia canicularis

About 50-100 eggs, which resemble those of the common housefly, are laid, usually on food of man, but also in urine-soaked bedding of man and animals, compost heaps, decaying piles of grass, human and animal excreta and in poultry litter. The eggs hatch after one to two days. The larvae are quite distinct from the maggot-shaped larvae of *Musca* and are unlikely to be confused with the larvae of any other medically important fly (figure 12.7a). They are flattened dorsoventrally and have many thin but conspicuous fleshy processes arising from the body segments which bear small spiniform secondary pro-



Fig 12.7 Larvae of (a) Fannia canicularis and (b) Fannia scalaris.

cesses. Larval development takes about one to two weeks, but may be prolonged if the habitat starts to dry out. Prior to pupation larvae migrate to drier areas. The puparium is brown in colour and is similar to the shape of the larva. After one to two weeks the adult fly emerges. The life-cycle often lasts about one month which is considerably longer than in *Musca domestica*, but may under favourable conditions be completed within 18-22 days. The feeding mechanism of the adult is similar to that of *M. domestica*, but although adults commonly enter houses they do not settle on man or on his food so much as houseflies.

#### Life-cycle of Fannia scalaris

The life-cycle of this species is similar to that of Fannia canicularis, but with a few minor differences. Eggs, which are also similar to those of Musca domestica, may be deposited on food of man and carcasses, but the most favoured oviposition sites are human and animal faeces, hence the name latrinefly. Larvae closely resemble those of F. canicularis but are differentiated by the fleshy processes which arise from the segments being a little larger and thicker, moreover the secondary processes are also thicker, thus giving them a feathery appearance (figure 12.7b). The duration of larval development is similar to that of F. canicularis. As with F. canicularis, adults frequently enter houses, but because of their oviposition preferences they may be especially common near latrines. They resemble F. canicularis in that they rest on man and his food less commonly than the housefly.

# Medical importance of Fannia species

All pathogens transmitted by the housefly can be transmitted by *Fannia* species, although they are generally considered less important vectors than houseflies, mainly because they have less contact with man's food. There is, however, little real evidence to support this. They have been incriminated in cases of urinogenital myiasis, and larvae are sometimes found in stools, but as previously stressed true intestinal myiasis does not occur in man. *Fannia canicularis* acts as intermediate hosts for the nematode *Thelazia californiensis*, a parasite of domestic animals and rarely man.

# Control

The same methods apply to species of Fannia as to Musca but particular attention should be given to the prevention and eradication of Fannia scalaris breeding in latrines.

# The greater housefly (Muscina stabulans)

# **External morphology**

# Adults

Muscina stabulans has a world-wide distribution and is commonly referred to as the greater housefly or the false stablefly. Adults are about 8-9 mm long and so slightly larger than houseflies. They can be distinguished from both Musca and Fannia because vein four of the wing curves slightly but distinctly upwards towards vein three (figure 12.4), and from Fannia, but not Musca, by having hairs on both the upper and lower sides of the arista (figure 12.4). M. stabulans has four dark brown-black longitudinal stripes on the thorax.

# Life-cycle

Females scatter about 150-200 eggs more or less indiscriminately over the surface of decaying matter such as rotting fruits, vegetables and fungi, on cooked and raw meats especially if putrified, carcasses and also on human and animal excreta. The eggs hatch after one to two days and the resultant larvae resemble the maggot-shaped larvae of the housefly, but can be readily distinguished by the structure of the posterior spiracles. In *M. stabulans* the spiracular plate is almost



Fig 12.8 Posterior spiracles of larvae of (a) Muscina stabulans and (b) Stomoxys calcitrans.

circular, not D-shaped as in *Musca domestica*; the peritreme, which is heavily sclerotised and very wide, encircles three crescent-shaped spiracular slits (figure 12.8a). No conspicuous button is present. The two mouthhooks, unlike those of *M. domestica*, are of equal size. First-instar larvae are omnivorous scavengers, as are larvae of *Musca* and *Fannia*, but towards the end of the larval period they become predacious, feeding on any other fly larvae in the breeding places. When fully grown they migrate to drier ground to pupate. The brown puparium is similar in shape to that of *M. domestica* and its duration is about one to two weeks. Consequently, like *Fannia* species, but not *M. domestica*, the life-cycle is about four to five weeks, although in warm weather it may be reduced to 20-25 days.

Adults enter buildings and feed and behave much as do adults of *M. domestica*.

# Medical importance

Adults of *Muscina stabulans* can transmit the same diseases as houseflies, but like *Fannia canicularis* and *F. scalaris* they are not so closely associated with man and are therefore considered to be less important in the spread of diseases. Larvae like those of *Fannia* and also *Musca* have occasionally been recorded in cases of accidental intestinal myiasis.

# Control

The control measures are very similar to those applied to the housefly.

# The stablefly (Stomoxys calcitrans)

# External morphology

# Adults (figure 12.9)

Stomoxys calcitrans has a world-wide distribution and is commonly known as the stablefly, biting housefly or

dogfly. Adults are dark grey with four brown-black longitudinal stripes on the thorax and are about the same size (5-6 mm) as houseflies which they superficially resemble. They are, however, easily separated from *Musca*, *Fannia*, *Muscina* and other related genera of the family Muscidae by a conspicuous forwardly projecting, rigid and non-retractile proboscis (figure 12.9). The wing venation resembles that of *Muscina* in



Fig 12.9 Head of Stomoxys calcitrans showing forwardly directed proboscis.

that vein four curves gently towards vein three, hence on wing venation alone *Stomoxys* can be separated from *Musca* and *Fannia* (figure 12.4), but not *Muscina*. The arista of the third antennal segment differs from *Musca*, *Fannia* and *Muscina* in having hairs arising from only the upper side (figure 12.4). This character is less easily seen than are the diagnostic features of the proboscis and wing venation.

Stableflies are distinguished from biting Muscidae of the genus Haematobia (= Siphona = Lyperosia) by their larger size and the shorter length of the palps at the base of the proboscis. In Africa adults might at first glance be confused with tsetse flies which also have a forwardly projecting proboscis, but Stomoxys calcitrans is a smaller fly and when at rest its wings are not placed completely over the body in a closed scissor-like fashion as in tsetse flies, but are kept apart as in houseflies. The wing venation and antennae are also different. In the tsetse fly there is an enclosed cell in the wing called the hatchet cell (figure 11.2a) which is absent from stableflies, and the hairs on the arista in tsetse flies are feathered, not simple as in the stablefly.

# Life-cycle

Both males and females take blood-meals from a variety of wild and domesticated animals, including cattle, horses, pigs and dogs; they also feed on man, especially if their more preferred hosts are absent or scarce in the area. Their bites can be painful and most are on the legs of man and cattle. When feeding the forwardly projecting proboscis is swung downwards, and the skin is penetrated by the rasp-like action of the fine teeth on the labella at the end of the labium of the proboscis. In hot weather flies digest their blood-meals within 12–24 hours and feed about every one to three days, but in cooler conditions blood digestion is prolonged to two to four days or more and refeeding occurs every five to ten days. Biting is restricted to the daylight hours and occurs both in bright sunshine and cloudy overcast weather. Most biting occurs out of doors but stableflies will also enter houses and other buildings to feed. They are mostly encountered in and around farms or where horses are kept, such as near riding stables. They are not usually common in towns, being essentially rural insects.

The eggs are creamy white and about 1 mm long and resemble those of houseflies. They are usually deposited in batches of less than 20, but sometimes as many as 50–100 may be laid together. They are frequently laid in horse manure which is mixed with straw and hay but also in compost pits, decaying and fermenting piles of vegetable matter, weeds, cut grass or hay. In coastal areas they may also lay their eggs on seaweed and other vegetation washed up on the shore. Stableflies very rarely lay their eggs in human or animal faeces, unless it is liberally mixed with hay or straw.

The eggs hatch within one to four days and the resultant larvae are creamy coloured maggots which when fully grown are about 12 mm long. They resemble larvae of the housefly but can be readily separated by the arrangement of two posterior spiracular plates which are widely separated (figure 12.8), thus differing from the more closely positioned spiracular plates of Musca and Muscina. They are approximately round in outline, lack a peritreme, and the S-shaped spiracles are widely separated from each other. There is also a poorly defined central button. Larvae prefer a high degree of moisture for development and therefore are found mostly in wet mixtures of manure and soil or straw, and in vegetable matter in advanced stages of decay. Under optimum conditions the larval period lasts about six to eight days, but in cooler weather or when there is a shortage of food larval development can be prolonged to four to five weeks or more.

Like housefly maggots the larvae of stableflies migrate to drier areas and bury themselves in the soil prior to pupation. The puparium is dark brown and resembles that of the housefly, but can be distinguished from it by having the posterior spiracles widely separated. The puparial stage lasts 5-26 days. The life-cycle from egg laying to adult emergence may last from 12-58 days, the time depending mainly on temperature.

In tropical areas stableflies breed continuously

throughout the year, but in more temperate climates they pass through the cooler months as larvae or puparia. Sometimes adults survive winters in warm stables or buildings, feeding intermittently during the cooler months.

# Medical importance

Because of the painful bites of both sexes *Stomoxys* calcitrans can sometimes be a serious pest of man and cattle. Adult flies are vectors of several protozoal and helminthal diseases to cattle and wild animals, but although they have been suspected of being capable of transmitting diseases to man, including poliomyelitis, there is no real evidence for this. Since they rarely visit excreta or festering wounds they are therefore much less likely to spread pathogens picked up by their feet or body hairs. However, under certain conditions they can be mechanical vectors of human and animal trypanosomiasis, but their role in spreading trypanosomiasis *is minimal*. In the tropical Americas eggs of *Dermatobia hominis*, a myiasis-producing fly (chapter 14) are sometimes attached to adult stableflies.

# Control

Many of the control methods aimed at houseflies can be applied with some modification to control stableflies. For example, source reduction of their breeding places, such as not allowing piles of manure, grass cuttings or decaying vegetable matter to accumulate. Manure and grass cuttings should be thinly spread over the soil. Straw and bedding material can be burnt to prevent the formation of potential breeding sites.

Breeding places can be sprayed with organochlorine or organophosphate insecticides but as noted in the section on housefly control it is difficult to get deep insecticidal penetration into the breeding place where most larvae are found. Insecticidal spraying of horse stables, animal shelters, barns and other farm buildings can help reduce their numbers. The use of insecticidal aerosols and mists to fog areas, such as small woods where adult flies may be resting, can sometimes also result in effective control, but usually for only relatively short periods.

Stableflies have not developed resistance and therefore a variety of insecticides can give control if efficiently applied.

# 13 Myiasis-producing flies, blowflies, bluebottles, greenbottles and fleshflies (Order Diptera: Families Calliphoridae and Sarcophagidae)

# **Myiasis**

Myiasis can be defined as the invasion of organs and tissues of man or vertebrate animals with *dipterous* larvae, which for at least a period feed upon the living, necrotic or dead tissues, or as in the case of intestinal myiasis, on the host's ingested food. Different terms can be used to describe myiasis that affects different parts of the body, for example (I) cutaneous, dermal or subdermal myiasis, (2) urinogenital myiasis, (3) ophthalmic myiasis, (4) nasopharyngeal myiasis and (5) intestinal or enteric myiasis. When larvae burrow just under the surface layers of the skin this is sometimes called creeping eruption or creeping myiasis, when boil-like lesions are produced the term furuncular myiasis may be used and when wounds become infested this is often referred to as traumatic myiasis.

Myiasis may be obligatory or facultative. In obligatory myiasis it is essential for the fly larvae to live on a live host for at least a certain part of their life; whereas in facultative myiasis the larvae are normally freeliving, often attacking carcasses, but under certain conditions they may infect living hosts.

There is no obligatory intestinal myiasis of man. When man has maggots (larvae) in his intestinal tract this is most likely due to accidental swallowing of eggs or larvae on food. Although the maggots may be able to survive for some time in the intestine there is no species of fly that is specially adapted to cause intestinal myiasis in man. In contrast obligatory intestinal myiasis does occur in animals, for example *Gasterophilus* larvae in horses. The presence of larvae in man's intestine may nevertheless cause considerable discomfort, abdominal pain and diarrhoea, which may be accompanied by discharge of blood and vomiting. Living larvae may be passed with the excreta or vomit. Occasionally facultative urinogenital myiasis occurs in man, this usually involves larvae of *Musca* or *Fannia*  species. It seems that ovipositing flies are attracted to unhygienic discharges and lay their eggs near genital orifices. When these eggs hatch the minute larvae enter the genital orifice and work their way up the urogenital tract. Much pain may be caused by larvae obstructing these passages, and mucous and blood, and eventually larvae, may be discharged with the urine.

Several types of fly, including species of Calliphora, Lucilia, Phormia and Sarcophaga, that normally breed in meat or carrion may cause facultative cutaneous myiasis in man by infecting festering sores and wounds. In marked contrast larvae of a few species, such as Cordylobia anthropophaga, Cochliomyia (= Callitroga) hominivorax, Chrysomya bezziana, Dermatobia hominis, species of Oestrus, Hypoderma, Gasterophilus and Wohlfahrtia are obligatory parasites of man and other vertebrates.

When larvae occur in wounds, sores and dermal or subdermal tissues their removal under aseptic conditions is usually a relatively simple procedure, but when they are more deeply imbedded in the underlying tissues or when they have penetrated the mucous membranes, frontal sinuses or cavities, their removal is more difficult. Major and irreversible damage may be done by the larvae.

The biologies and medical importance of the principal types of flies causing facultative and obligatory myiasis in man are discussed in detail in this and the following chapter.

# Calliphoridae

#### Species

There are many species in this family, the principal ones of medical importance being Auchmeromyia luteola (Congo floor-maggot fly), Cordylobia anthropophaga (tumbu fly), Cochliomyia ( = Callitroga) hominivorax (New World screw-worm), Chrysomya bezziana (Old World screw-worm), and various species belonging to the genera Lucilia (greenbottles), Calliphora (bluebottles or blowflies) and Phormia (small bluebottles).

#### Distribution

Auchmeromyia luteola and Cordylobia anthropophaga are found only in Africa, Cochliomyia hominivorax only in North, Central and South America and the Caribbean islands, and Chrysomya bezziana in the Old World tropics and subtropics. Species of Lucilia and Calliphora occur world-wide, but are more common in temperate regions than the tropics. Phormia species are confined to northern temperate regions of America, Europe and Asia.

#### Medical importance

All the above flies have larvae that are either obligatory parasites of living tissues of man, and as a consequence cause primary (obligatory) myiasis, or infest sores and wounds attacking mainly dead tissues and giving rise to secondary (facultative) myiasis. Because some of these flies, such as blowflies and greenbottles, frequent carrion, decomposing bodies, decaying refuse and excreta they are suspected of being mechanical vectors of a number of pathogenic organisms.

# The non-metallic Calliphoridae

# Cordylobia anthropophaga

# External morphology

#### Adults (figure 13.1)

This species is known as the tumbu or mango fly and is found only in Africa, occurring from Ethiopia in the north through West and East Africa to Natal and Transvaal in the south.

Adults are robust, relatively big flies, about 9-12 mm long, dull yellowish to light-brown in colour but with two dark grey and poorly defined dorsal longitudinal stripes on the thorax. There are four visible abdominal segments which are more or less equal in length (compare *Auchmeromyia luteola* in which the second abdominal segment is markedly longer than the others). The posterior segments are a darker brownblack colour than the anterior ones. The wings are slightly brownish.

# Life-cycle

Females lay up to 200-300 eggs in batches on dry soil and sand in shaded places, especially when contaminated with the urine or excreta of either man or a variety of animals, such as rats, small rodents, dogs and monkeys. Females may oviposit on underclothes or soiled nappies (diapers) of babies placed on the ground to dry. The eggs are white and curved along one side giving them a banana shape. They hatch after about one to three days. A newly emerged larva attaches itself to a suitable host, including man, and by means of its powerful hook-like mouthparts penetrates the skin. It buries itself completely except for its posterior spiracles situated at the tip of the abdomen which remain in contact with the air. Newly emerged larvae can live as long as 9-15 days on the ground in the absence of a suitable host before they die. They may attach themselves temporarily to washed clothing placed on the ground to dry, and so get transferred to man if the clothing is not ironed before wearing.

Within the subcutaneous tissues the larvae undergo two moults, so that there are three larval instars. The first-instar larvae are typically maggot-shaped but the second-instar are club-shaped. After eight to ten days the third and last instar larvae are fully grown. They are rather fat, broadly oval-shaped maggots, about 11-15 mm long, yellowish-white in colour, and covered with numerous small spines which are often, but not always, grouped into three or more transverse rows per segment (figure 13.2a). The posterior spiracles lack a sclerotised peritreme, the button is indistinct and the three spiracular slits rather sinuous (figure 13.2b). Mature larvae wriggle out of the boillike swellings and fall to the ground where they bury themselves and turn into puparia. Adult flies emerge some ten days later and readily enter houses, especially mud huts, where they may lay their eggs on the mud floors especially if children have urinated on them.

An extremely similar fly is Cordylobia rodhaini, sometimes placed in the genus Stasisia. This insect, which is known sometimes as 'Lund's fly', is widespread in tropical rain forests of Africa where its usual hosts are antelopes and rats. It is a rarer parasite of man than C. anthropophaga. Adults are more or less indistinguishable from those of the tumbu fly, but the larvae can be separated because the spicules of C. rodhaini are bigger and never arranged in distinct rows. Moreover, the spiracular slits are much more sinuous than in the tumbu fly.

# Medical importance

The larvae of *Cordylobia* cause boil-like (furuncular) swelling on almost any part of the body. Although



Fig 13.1 Adults of Cordylobia anthropophaga and Auchmeromyia luteola showing the differences in depth of the second abdominal segment.

these swellings may become sore and inflamed, and even quite hard and exude serous fluids, they do not usually contain pus. The standard method of extracting a larva is to cover the small hole in the swelling with medicinal liquid paraffin. This prevents the larva from breathing through its posterior spiracles with the result that it wriggles a little further out of the swelling to protrude the spiracles. In so doing it lubricates the pocket in the skin, and the larva can then usually be extracted by gently pressing around the swelling.

Infections are prevented by wearing shoes and ensuring that clothes, bed linen and towels are not spread on the ground to dry.

#### Auchmeromyia luteola\*

#### External morphology

#### Adults (figure 13.1)

These flies, commonly known as Congo floor-maggot flies, although not strictly speaking producing myiasis are described here because the adults are often confused with those of *Cordylobia anthropophaga*. They occur throughout Africa south of the Sahara and also in the Cape Verde islands. There are five species of *Auchmeromyia*, and they mostly occur in and around burrows of wart-hogs and ant-bears; only one, *A. luteola*, attacks man.

Adults are very similar to *C. anthropophaga* but are readily distinguished from it by the shape of the second abdominal segment which is about twice as long as any of the others (figure 13.1), whereas in the tumbu fly all segments are about equally long.

\* It has recently been shown that the correct name is A. senegalensis



Fig 13.2 Cordylobia anthropophaga, (a) final instar larva and (b) posterior larval spiracles.

# Life-cycle and medical importance

Eggs are laid in batches of about 50 on the dry sandy floor of mud huts. They hatch after one to three days and the larvae hide away in cracks and crevices in the hut floor, especially under beds and sleeping mats. At night the larvae crawl out from these daytime refuges and take blood-meals from sleeping people within the hut. After taking a full blood-meal the now pinkishred larvae return to their hiding places. Larvae may feed about four to five times a week, but can withstand starvation for long periods in the absence of suitable hosts. There are three larval instars each requiring at least one blood-meal. Under optimum conditions larval development is completed within three to four weeks but this period may be prolonged by several weeks if the larvae fail to obtain regular feeds. The fully developed third-instar larvae, unlike those of



Fig 13.3 Auchmeromyia luteola, (a) final instar larva and (b) posterior larval spiracles.

Cordylobia anthropophaga, are not covered with small spines and the spiracular plates at the end of the abdomen are widely separated, lack a distinct peritreme and have three parallel spiracular slits (figure 13.3b). Larvae cannot climb, and therefore people will not be attacked if they sleep on beds raised from the floors by only short legs. Mature larvae pupate in cracks or directly on the surface of the mud floor of huts. Adults emerge from the puparia after about two weeks.

The Congo floor-maggot used to be not uncommon in certain parts of Africa, but due to changes in lifestyle of most Africans it is becoming increasingly rare and these days is not much of a problem.

# Metallic Calliphoridae

# Cochliomyia hominivorax

# External morphology

# Adults (figure 13.4)

The New World Screw-worm, Cochliomyia homini-

vorax, previously known as Callitroga americana, occurs from Illinois and South Dakota in the southern U.S.A. through Central America down to Argentina.

Adult flies are 8-10 mm long, metallic green to bluish-green in colour and have three distinct dark longitudinal stripes on the dorsal surface of the thorax. The dorsal bristles on the thorax, like those of *Chrysomya*, are poorly developed, and examination under a microscope shows that the squama of the wing is covered with fine hairs on the dorsal surface (figures 13.4 and 13.5a). The face of the fly is orange, yellow or reddish with the eyes a deeper red. (See the description of adult *Chrysomya bezziana* for the separation of this species from *C. hominivorax.*)

A closely related species, Cochliomyia macellaria, whose range extends from Argentina further north than C. hominivorax (to Maine and Quebec) and which also occurs on Ascension Island, may also cause myiasis



Fig 13.5 Thoraces and bases of the right wing to show presence or absence of well developed bristles on dorsal surface of thorax and fine hairs on squama of wing, (a) Cochliomyia species, note three dark thoracic stripes, (b) Chrysomya species, note only two dark thoracic stripes, (c) Calliphora species, note presence of well-developed thoracic bristles and hairs on squama, and (d) Lucilia species, note thoracic bristles are well developed but squama is without hairs.



Cochliomyia

Lucilia

Fig 13.4 Adult flies of the genera Cochliomyia and Lucilia.

in man and animals. It is not, however, an obligatory parasite of living tissues, larvae usually live in wounds and scavenge on dead tissues. It is not considered to be a serious pest, and is therefore sometimes called the secondary New World screw-worm fly. Both larvae and adults very closely resemble those of the primary screw-worm fly, C. hominivorax.

# Life-cycle

Females of C. hominovorax lay about ten to almost 400 eggs on the edges of wounds, scabs, sores or even small scratches or pimples, on dried blood clots, on diseased and even healthy mucous membranes such as nasal passages, the mouth and vagina. In new-born babies eggs are also laid in the umbilicus. Eggs hatch within 12-24 hours and the active larvae bury deeply into the living tissues and feed gregariously. There are three larval instars and the third-instar larvae, which are formed after two to three days, are about 15-17 mm long, typically maggot-shaped, but readily distinguished from housefly maggots by the presence of distinct bands of small spines encircling the anterior margins of all body segments (figure 13.6a). The peritreme of the posterior spiracles is incomplete at the button (figure 13.6b).

After four to eight days the larvae reach maturity and wriggle out of the wounds or passages they have excavated by devouring living tissue and drop to the ground where they bury in the soil and pupate. In warm weather the puparial stage lasts about seven to ten days, but in cooler weather may be prolonged for many weeks or even months. Under optimum con-



Fig 13.6 Larvae of metallic calliphorids, (a) final instar larva of Cochliomyia hominivorax, (b) posterior larval spiracles of C. hominivorax, (c) anterior spiracles of C. hominivorax showing eight finger-like processes, (d) anterior spiracles of Chrysomya bezziana showing five finger-like processes, (e) final instar larva of Lucilia species (Calliphora larvae are very similar), (f) posterior spiracles of larva of Phormia species, and (g) posterior spiracles of larva of Phormia species.

ditions the life-cycle from egg to egg is about 20 days. Larvae are very similar in appearance to those of the Old World screw-worm (*Chrysomya bezziana*) but differ in having eight minute finger-like processes, not five, on the inconspicuous anterior spiracles (figure 13.6c,d). In practice, however, the easiest way to separate the two species is by their geographical distribution, – screw-worms of the Americas belong to the genus *Cochliomyia* while those of the Old World belong to the genus *Chrysomya*.

# Chrysomya bezziana

# **External morphology**

#### Adults

The genus Chrysomya contains many species and in the tropics largely replaces the other metallic calliphorid genera Calliphora and Lucilia. About ten species are known to cause myiasis in man, but only Chrysomya bezziana is important because its larvae are obligatory parasites of living tissues, whereas the larvae of the other species are not, and often develop in carrion and decomposing matter. C. bezziana occurs throughout tropical Africa and most of Asia, ranging from India to the Philippines, Celebes, New Guinea and China, but the fly is absent from Australia.

Adults are 8-12 mm long, their colour ranging from metallic green, bluish-green to almost purplishblue. The thorax has two indistinct dark longitudinal stripes (figure 13.5b) and the dorsal bristles of the thorax, like those of Cochliomyia, are poorly developed, thus distinguishing screw-worm adults from both Calliphora and Lucilia which have well developed bristles (figure 13.5c,d). Also, as in the New World screw-worm, the squama of the wing is covered with fine hairs on the dorsal surface, thus distinguishing adults from Lucilia in which the squama lacks hairs. Superficially they resemble adults of Cochliomyia hominivorax, but never have three distinct stripes on the thorax as does this species. The species are best distinguished, however, by their different distributions, screw-worms in the Old World being Chrysomya while those in the Americas are Cochliomyia.

# Life-cycle

Basically the life-cycle is very similar to that of Cochliomyia hominivorax. Some 150-600 eggs are laid in wounds, open sores, scabs, ulcers, gums, scratches or on mucous membranes especially those contaminated with discharges. The eggs hatch within 8-24 hours and newly emerged larvae burrow through the skin to the underlying tissues where they commonly remain congregated together. Larvae tend to penetrate deeply into tissues so that infections near the eyes, nose and mouth can cause considerable destruction of these areas, often accompanied by putrid smelling discharges and ulcerations. The larvae can reach the third-instar within as little as two to three days. They resemble the common housefly maggot but differ in having rows of small spines encircling the anterior margins of all body segments. The peritreme of the posterior spiracles is incomplete at the button. Larvae are very similar to those of the New World screw-worm (Cochliomyia hominivorax) but can be distinguished by the inconspicous anterior spiracles which in C. bezziana bear five minute finger-like processes (figure 13.6d), whereas in C. hominivorax there are eight such processes.

Larvae complete their development in five to six days and then wriggle out of the wounds and drop to the ground where they bury themselves and pupate. The puparial period lasts about seven to ten days in warm weather, but is prolonged to several weeks or even months during cold weather. The life-cycle from egg to egg under ideal conditions is about 20 days.

Adults of both the New and Old World screwworms are frequently found feeding on decomposing corpses, decaying matter, excreta and flowers.

# Medical importance of Old and New World screw-worms

Larvae of both Chrysomya bezziana and Cochliomyia hominivorax are obligatory parasites of living tissues and cause human myiasis, which can be very severe resulting in considerable damage and disfigurement especially if the face is attacked. When larvae invade natural orifices, such as the nose, mouth, eyes or vagina they can cause excruciating pain and misery. In one patient suffering from a nasal infection 385 larvae of C. hominivorax were removed over a nine day period! Larvae of both species may eat their way through the palate and as a result impair speech.

All myiasis cases should be treated immediately because their very rapid larval development can soon cause permanent damage.

*C. bezziana* seems to cause more cases of myiasis in people in India and other parts of Asia than it does in Africa.

Both screw-worm species cause myiasis in cattle, horses, goats, sheep and other animals. In the U.S.A. prior to 1958 the livestock industry lost an estimated 120 million dollars a year due to *C. hominivorax*. Because of such heavy economic losses much effort has been devoted to the control of the New World screwworm. One method involves the mass rearing and weekly release of up to 150 million C. hominivorax previously sterilised by irradiation or by chemosterilants. Released sterilised males compete with fertile males of the wild population for mates, and if they are successful numerous sterile matings should occur. This in fact did happen and resulted in the reduction and virtual elimination of the screw-worm population from the project area in Texas. These measures were very successful for a number of years and were frequently quoted as a classical example of genetical control of an insect pest, but setbacks to the programme developed in 1972 due to screw-worm reinvasions from Mexico and the reduced vigour of laboratory reared flies.

# Lucilia species

# External morphology

#### Adults (figure 13.4)

There are several species of greenbottles within the genus Lucilia and although the genus has a world-wide distribution most species occur in northern temperate regions. They are mostly metallic or coppery green in colour usually a little smaller (about 10 mm long) and a little less bristly than species of Calliphora (bluebottles). As in Calliphora prominent bristles are present on the dorsal surface of the thorax (figure 13.5d) but whereas the squama of the wing is hairy dorsally in bluebottles it is without hairs in Lucilia (figure 13.5d). Lucilia (= Phaenica) sericata is the commonest species, occurring in the Americas, Europe, Asia, Africa, Australasia and in most other areas of the world. Other common species include L. illustris which is Holarctic in its distribution, and L. cuprina, which occurs mainly in Africa, Asia and Australia.

# Life-cycle

Female greenbottles normally lay their eggs on meat, fish, carrion and decaying or decomposing carcasses, but they will also oviposit on or near festering and foul-smelling wounds of man or animals, and on excreta and decaying vegetable matter. Eggs hatch within about eight hours. Larvae are typically maggotshaped (figure 13.6e), do not bear spines as do larvae of the screw-worms, and therefore resemble larvae of the common housefly, from which they can be separated by the form of the posterior spiracles which are not D-shaped and have a peritreme that surrounds the button (figure 13.6f).

The larval period lasts about four to eight days. Mature larvae migrate to, or fall on to, the ground, bury in the loose soil and pupate, the puparial period lasts about 6-14 days. Larvae can be distinguished from those of *Calliphora* by the shape of the cephalopharyngeal skeleton.

Adult flies frequently visit carrion, excreta, general refuse, decaying material, sores and wounds. They are particularly common around unhygienic places and situations where meat or decaying animals are present; they are nearly always abundant near slaughter houses and piggeries. They commonly fly into houses, where they are particularly troublesome because of their noisy buzzing flights. Adults are strong fliers and can disperse several (2-5) kilometres. The most common species infesting wounds of humans are *Lucilia sericata* and *L. cuprina*.

# Calliphora species

# **External morphology**

#### Adults

These flies are usually known as bluebottles or blowflies. Although the genus *Calliphora* has a worldwide distribution and there are several species, bluebottles are commoner in the northern temperate regions than in the tropical or southern temperate regions; for example there is only one endemic species (*C. croceipalpis*) in Africa.

Adults are mostly dull metallic bluish or bluishblack in colour, and the abdomen is more shiny than the thorax. They are about 8-14 mm long, robust and as in *Lucilia* have well developed dorsal bristles on the thorax (figure 13.5c), but the squama of the wing is hairy on the dorsal surface whereas in *Lucilia* it lacks hairs.

# Life-cycle

Larvae look very similar to those of Lucilia, and the life-cycle is also very similar to that described for Lucilia.

# Medical importance of Lucilia and Calliphora species

The dirty habits of greenbottles and bluebottles

(blowflies) of alighting and feeding on excreta, decaying material and virtually all common foods of man make them potential mechanical vectors of a number of pathogens. It has been suggested that *Lucilia sericata* (and *Phormia regina*) may aid the transmission of poliomyelitis, transfer of the virus occurring when flies feed on carrion and then wounds. However, their medical importance is usually associated with myiasis.

Larvae of both Lucilia and Calliphora have been found in many parts of the world developing in foulsmelling wounds and ulcerations, especially those producing pus, both in man and in animals such as sheep and cattle. They have also been recorded in hospitals underneath the bandages and dressings of patients, especially when these have become contaminated with blood and pus. Such infection do not usually cause any serious damage or harm since the larvae feed mainly on pus and dead tissues. In fact until comparatively recently they were sometimes used to cleanse septic wounds, and help prevent osteomyelitis. Very occasionally maggots which have infected wounds invade healthy tissues, and even more rarely infections with these maggots have caused a hand or leg to be amputated.

Occasionally intestinal myiasis is reported. This is usually caused by eating uncooked food contaminated with larvae of *Lucilia* or *Calliphora*, but usually the larvae are killed within the human alimentary canal and no serious harm is done. As emphasised on page 113 there is no obligatory intestinal myiasis in man.

# Phormia species

# External morphology

# Adults

Adults of *Phormia* resemble greenbottles or bluebottles, but are usually a little smaller (7-11 mm) and have more numerous short bristles on the dorsal surface of the thorax than those of other flies. In *Phormia terraenovae* the thorax and abdomen are black or purplish-black with a metallic lustre, and adults are sometimes referred to as black blowflies, whereas in *P. regina* the thorax is metallic green or greenish-blue and the abdomen is either a similar colour or rather more greenish-purple. Species of *Phormia* are distinguished from bluebottles (*Calliphora*) by the absence of hairs on the squama of the wings. They are not found in the tropics (except Hawaii), but occur in the northern temperate regions of North America, Europe and Asia, they are also found in Australia and Hawaii.

# Life-cycle and medical importance

Eggs are usually laid on meat, fish or decaying carcasses. Larvae are typically maggot-shaped, do not possess spicules on the body and differ from larvae of *Calliphora* and *Lucilia* in the morphology of the posterior spiracles (figure 13.6g). In *Phormia* the larval peritreme is incomplete and therefore does not surround the button as in the other two genera. Prior to pupation the third-instar larva usually buries in the soil. The puparial stage lasts some 6-14 days, and the lifecycle from egg to adult takes about 10-25 days.

In North America, but not apparently in the Old World, larvae have occasionally been found in suppurating wounds and sores, feeding mainly on pus and necrotic tissues. They have also been recorded as causing enteric myiasis, presumably due to larvae being ingested with uncooked food. They are not, however, important myiasis-producing flies.

It has been suggested that carrion feeders, such as *Phormia regina* (and *Lucilia sericata*) may transmit poliomyelitis virus by feeding on carrion and wounds.

# Control of bluebottles, greenbottles and blowflies

The principal breeding places of bluebottles, greenbottles and blowflies in both towns and villages include domestic refuse, rubbish tips, dustbins, offal and other wastes of slaughter houses and meat packing factories and also food such as meat and fish left out in the sun to dry. Any methods which reduce the accumulation of these potential breeding sites are welcome. Dustbins and garbage cans should have tight-fitting lids and be emptied once or twice a week. The outside of such bins and both sides of the lid can be sprayed with 5 per cent DDT water dispersable powder or 0.5 per cent HCH every seven to ten days, and the adjacent walls and fences sprayed every two weeks. This will deter flies from ovipositing in these sites.

See also chapter 12 for control measures used against houseflies and related flies many of which are applicable to the Calliphoridae.

# Sarcophagidae

#### Species

Only the genera *Sarcophaga* and *Wohlfahrtia*, both of which have several species, are of any medical importance. They are sometimes called fleshflies.

#### Distribution

Sarcophaga and Wohlfahrtia occur world-wide, although several species within these genera may have a more restricted distribution.

#### Medical importance

The flies cause myiasis in man, and possibly act as mechanical vectors of pathogens.

# External morphology of the Sarcophaga species

# Adults (figure 13.7)

These are large and hairy non-metallic flies, about 11 - 15 mm long and greyish in colour. They have three prominent black longitudinal stripes dorsally on the thorax. The abdomen is sometimes distinctly, but



S. carnaria

W. magnifica

Fig 13.7 Adults of Sarcophaga carnaria and Wohlfahrtia magnifica showing differences in abdominal markings.

other times indistinctly, marked with squarish dark patches on a grey background giving it a chequerboard (chess-board) appearance. A few species, however are not greyish but are more brownish or brownish-yellow, but the dark thoracic stripes are always present.

# Life-cycle and medical importance

Adults do not lay eggs but deposit first-instar larvae, as do *Wohlfahrtia*, *Oestrus* and tsetse flies. The larvae are deposited in batches of 20-40, usually on decaying carcasses, rotting food and human and animal excreta, but sometimes in wounds. They are primarily scavengers. Larvae are typically maggot-shaped, with spicules on the body, and have the three spiracular slits of the posterior spiracles converging towards the button, the peritreme is incomplete (figure 13.8a, b). They are readily distinguished from larvae of the Calliphoridae because the posterior spiracles are situated in a deep pit (figure 13.8a), and are thus difficult to see. Larvae of *Sarcophaga* are not easily distinguished from those of *Wohlfahrtia*.

Larval development is rapid, lasting in hot weather in the presence of a nutritious food supply only three to



Fig 13.8 Sarcophaga carnaria, (a) final instar larva showing pit at posterior end of body in which (b) the posterior spiracles are situated. (Larvae of Wohlfahrtia are very similar in appearance.)

four days, at the end of which time they bury in the soil and pupate. The puparial stage lasts about 7-14 days.

Although larvae are normally deposited in carrion they very occasionally occur in wounds, but usually they cause little damage as they feed mainly on necrotic tissues. They have more commonly been incriminated with accidental intestinal myiasis, causing considerable discomfort and pain before the larvae are passed out with the faeces. The most common species is *Sarcophaga haemorrhoidalis*, which is widely distributed in the Americas, Europe, Africa and Asia. Because adults frequent festering wounds, excreta and decaying animal matter they may be mechanical vectors of various pathogens.

# External morphology of the Wohlfahrtia species

#### Adults (figure 13.7)

These are hairy flies about as large, or a little larger, than bluebottles, they are greyish and like Sarcophaga have three distinct black lines on the dorsal surface of the thorax. The dark markings on the abdomen, however, are not in the form of a chess-board pattern as in Sarcophaga species but are usually present as roundish lateral spots and triangular-shaped dark markings along the midline. There is, however, considerable variation and sometimes the dark marks are so large as to be more or less confluent, making the abdomen appear mainly black.

# Life-cycle and medical importance

As with Sarcophaga, Oestrus and tsetse flies, adults of Wohlfahrtia deposit larvae not eggs. These are deposited in batches of 50-70 in scratches, wounds, sores and ulcerations on man and animals; W. vigil will also deposit its larvae on unbroken skin. Wohlfahrtia larvae have the spiracles situated in a deep pit and are very similar in appearance to those of Sarcophaga. Larval development takes 7-12 days, mature larvae drop to the ground and bury amongst loose soil and then pupate. Adults emerge from the puparia after 7-14days. The following three species of Wohlfahrtia have been identified as causing myiasis in man. Wohlfahrtia magnifica occurs in southern Europe, Asia, North Africa, but not south of the Sahara; its larvae are obligatory parasites of mammals, including occasionally man. They never develop in carrion or decomposing materials. They are most frequently deposited in the ear, nose or eyes of man, and in these sites they can cause considerable pain and extensive damage, and may even rarely cause death. A great number of domestic pets and farmyard animals are also attacked by the larvae. W. nuba occurs from Senegal in West Africa across to Karachi in Pakistan. Larvae are obligatory parasites of animal tissues, and behave similarly to those of W. magnifica.

*W. vigil* occurs in Northern America from Alaska as far south as Iowa, Ohio and Pennsylvania. Larvae are obligatory parasites, and may be deposited on *intact* unbroken skin if it is soft and tender, such as in babies and very young children, which are much more commonly attacked than adults. Larvae remain in the dermal tissues and therefore do not cause such extensive damage and discomfort as those of the other two species, which bury deeper into the tissues. They are also parasites of dogs, rabbits, cats and a variety of wild animals.

# 14 Myiasis and warble and botflies (Order Diptera: Families Cuterebridae, Oestridae and Gasterophilidae)

# Cuterebridae

#### Species

Dermatobia hominis is the only species of medical importance.

#### Distribution

Found in Central and South America, from Mexico down to northern Argentina, it also occurs in Trinidad but is apparently absent from all other West Indian islands.

#### Medical importance

Larvae cause myiasis in man.

# External morphology of Dermatobia hominis

# Adults (figure 14.1)

These flies are sometimes known as human botflies, or by a variety of local names such as 'bernefly' and 'ver macaque'.

Adults are a little larger (12-18 mm) than bluebottles (*Calliphora* spp.), but have a similar dark blue metallic-coloured abdomen, dark bluish-grey thorax and a mainly yellowish head. They are readily separated from bluebottles by the absence of any prominent bristles or hairs on the thorax, by the different shaped head and by the widely separated compound eyes (figure 14.1a), and are distinguished from all other flies of medical importance by the mask-like flap that hangs down from the head and hides the vestigial mouthparts (figure 14.1b).

# Life-cycle

The fly occurs primarily in lowland forests, being especially common along woodland paths and at the margins of forest and scrub areas, but it has occasionally been recorded at altitudes up to about 900 m. These flies have an interesting and remarkable lifehistory. Females glue about 15-25 eggs to the lateral or ventral margins of the abdomen, or sometimes the thorax, of other arthropods, such as ticks or various Diptera, many, but not all, of which are blood-sucking flies. The most commonly involved insects are daybiting mosquitoes especially those belonging to the genus Psorophora, but stableflies (Stomoxys calcitrans) also often have eggs firmly attached to their bodies. Embryos within the attached eggs mature into fully developed first-instar larvae within about seven days. They do not hatch until the insects carrying the eggs settle on sheep, goats, dogs, cats, pigs, monkeys, man or some other warm-blooded animal, or even birds to take a blood-meal or, as in the case of houseflies, feed on sweat. The larvae then emerge from the eggs, which are still attached to the insect carrier. They fall on to the host's skin where they almost immediately proceed to burrow through the epidermis to the subcutaneous tissues. Eggs may also occasionally be deposited on leaves of forest plants. In this instance larvae emerge from them when forest animals brush past.

The first-instar larvae are 1-1.5 mm long, more or less cylindrical in shape (figure 14.2a), and have the anterior half of the body covered with numerous spines of two different sizes. The second-instar larvae are a completely different shape, being enlarged anteriorly but with the posterior half of the body distinctly narrower, giving the appearance of a bottle with a long neck. Relatively large thorn-like spines encircle the middle segments (figure 14.2b). The third and final instar larvae are again a different shape. They are about



Fig 14.1 Dermatobia hominis, (a) dorsal view of adult and (b) lateral view of head showing flap-like mask.



Fig 14.2 Larvae of Dermatobia hominis, (a) first-instar larva, (b) second-instar larva and (c) third-instar larva.

18-25 mm long, more or less oval and have relatively small spines on the anterior segments (figure 14.2c). A pair of very distinct flower-like spiracles are present anteriorly, while less conspicuous slit-like spiracles are situated in a small concavity of the last abdominal segment. A small but stout pair of curved mouthhooks are also present ventrally.

Larval development is completed in a small pocket excavated in the subdermal layer of the host, and lasts some 5-12 weeks. Mature larvae wriggle out of the skin and drop to the ground where they pupate just under the surface of the soil. Adult flies emerge from the puparia after about three to four weeks, but are rarely seen.

# Medical importance

Larvae of Dermatobia hominis invade the subcutaneous

tissues of man on various parts of his body, including the head, arms, abdomen, buttocks, thighs, scrotum and axillae. They produce boil-like swellings which suppurate and this may attract other myiasisproducing flies to the host. They may cause a lot of discomfort and considerable pain. Because of the long duration of the larval life (up to 12 weeks) infected persons may be encountered in almost any part of the world.

Treatment consists of the surgical removal of the larvae under sterile conditions, frequently a local anaesthetic is necessary.

# Oestridae

#### Species

Several species belonging to several genera infect sheep, goats, cattle and deer and a few such as Oestrus ovis, Hypoderma bovis and H. lineatum occasionally cause myiasis in man. Hypoderma species are sometimes placed in a separate family the Hypodermatidae.

#### Distribution

The Oestridae occur almost world-wide, but certain genera and species have a more restricted distribution.

#### Medical importance

Larvae may cause myiasis in man.

#### Oestrus ovis

# External morphology

#### Adults (figure 14.3)

Commonly known as the sheep nostril, sheep warble or botfly. Adult flies are a little bigger (12-14 mm long) than the common housefly (*Musca domestica*). The head is large, broad, squat and pale yellowishbrown, with small round pits containing black tubercles. The thorax is pale yellow to greyish and covered



Fig 14.3 Adult of Oestrus ovis.

dorsally with numerous small black tubercles. The abdomen is variegated with dark brown or blackish marks and light greyish or almost yellow marks. The legs are a pale yellow or a yellowish brown in colour.

# Life-cycle

The females are viviparous (larviparous), that is they do not lay eggs but deposit larvae as do tsetse flies, *Sarcophaga* and *Wohlfahrtia* species. As many as 500 larvae may be flicked into the nostrils of sheep and



Fig 14.4 Larvae of Oestrus ovis, (a) lateral view of final instar larva, (b) posterior spiracular plate of larva and (c) ventral view of final instar larva.

goats. These first-instar larvae migrate to the nasal cavities and frontal sinuses and undergo further development. After about 8-12 months mature larvae migrate back through the nasal passages and drop from the nostril to the ground where they pupate. Mature third-instar larvae are cylindrical (figure 14.4a), have large conspicuous mouthparts, and have the segments covered with small spines which are mostly confined to the ventral surface (figure 14.4c). Larvae grow to about 25 mm in length. The posterior spiracular plate does not have spiracular slits but, as in *Hypoderma* species, is covered with numerous small holes (figure 14.4b). Adults emerge from the puparia after some three to six weeks.

# Hypoderma bovis

# External morphology

# Adults

Commonly known as cattle warbleflies or cattle grubflies. Adults are about 15 mm long and are quite distinct from the sheep warblefly (O. ovis) in being more heavily built. They superficially resemble small bumblebees (Hymenoptera), but being Diptera they have only one pair of wings. In Hypoderma bovis the anterior thoracic hairs are mainly yellow and the posterior ones black, and the apical abdominal hairs yellow. In H. lineatum the thoracic hairs are brownishblack and white and are arranged more uniformly, and there are also four prominent lines on the dorsal surface of the thorax, the apical abdominal hairs are reddishorange. Adults of Hypoderma are rarely seen.

# Life-cycle

Female cattle warbleflies glue their eggs on to the hairs of the legs or belly, or those near the tail, of cattle. Larvae hatch from these eggs after seven days, penetrate the skin near the base of the hairs and invade the subcutaneous tissues. They finally migrate to the skin on the back adjacent to the spine and a small distinct swelling, termed a warble, appears. This is caused mainly by the reaction of the host's tissues to the presence of a larva beneath the skin. A small perforation is produced in the skin by the larva to serve as a breathing hole. Larvae are rather similar to those of *Oestrus ovis* but are more squat and bullet-like, the small spines covering the body are smaller and the mouthhooks are less well developed. As in *O. ovis* the posterior spiracular plates do not have spiracular slits



Fig 14.5 Spiracular plate of a larva of Hypoderma bovis.

but have numerous small holes, but whereas in O. ovis the plate is more or less round in outline, in Hypoderma species it is invaginated in the middle making it somewhat resemble a thick letter C (figure 14.5). After about 8–11 weeks the larva reaches maturity, squeezes itself out from the warble and falls to the ground, then it buries itself and pupates; some four to five weeks later the adult fly emerges.

# Gasterophilidae

#### Species

There are several genera and species within this family, the most important species medically belong to the genus *Gasterophilus*, such as *G. intestinalis* and *G. pecorum*.

# Distribution

Almost world-wide, but some species of Gasterophilus are restricted to the Old World.

#### **Medical** importance

Larvae may cause myiasis in man.

# Gasterophilus intestinalis

# External morphology

#### Adults (figure 14.6)

Adults are commonly called botflies. They superficially resemble hivebees or small bumblebees both in size (9-12 mm) and colouring, but can easily be separated from bees because they have only a single pair of wings. Adults look rather like those of *Hypoderma* but the ovipositor of the female is usually protuberant, giving the abdomen a pointed appearance (figure 14.6a, b).

# Life-cycle

Female flies lay about 700-1000 eggs either on the hairs of animals, usually on horse's legs, or as with *Gasterophilus haemorrhoidalis* on or near the horse's lips. In warm weather the eggs hatch after about five days and the newly emerged larvae get transferred to the horse's mouth either by its tongue during self-



Fig 14.6 Gasterophilus intestinalis, (a) lateral and (b) dorsal view.

grooming, or else they actively crawl into the mouth. After reaching the mouth the larvae burrow into the mucous membranes of the tongue or other tissues and then migrate to the oesophagus or stomach where the second and third-instar larvae attach themselves and



Fig 14.7 Gasterophilus species, (a) final instar larva and (b) spiracular plate of larva of Gasterophilus.

remain for many months, often until the following spring or summer. The mature larvae then pass out with the faeces, bury in the soil and pupate. Adult flies emerge from the puparia some five to seven weeks later.

The full grown larva is cylindrical about 16–20 mm long, and has well developed rather large spines on the first seven to eight segments, and often a few spines on the following segments (figure 14.7a). The posterior spiracular plate has three distinctly curved slits (figure 14.7b) clearly distinguishing the larva from those of *Hypoderma* and *Oestrus* species.

The life-cycle of other Gasterophilus species differs from that of G. intestinalis in minor details, for example eggs of G. pecorum are laid on grass and other vegetation and hatch when ingested with the horse's food. The number and arrangement of larval spines also differs according to species; in G. haemorrhoidalis the terminal segments of the adults are reddish.

# Medical importance of Oestrus, Hypoderma and Gasterophilus species

Man is not the primary host of any species belonging to these three genera, but he is occasionally infected with their larvae which cause myiasis, although usually of a temporary nature. People most likely to become infected are those working closely with the flies' natural hosts, such as shepherds and those looking after cattle and horses.

On man Oestrus ovis usually deposits her larvae in the eyes. The minute larvae, about I-I.5 mm long, cause intense irritation and discomfort and produce inflamed eyes, but this lasts only for a short time because the larvae cannot undergo any further development in such an unnatural site on an atypical host. Larvae are also occasionally deposited on the mucous membranes of the nose and mouth.

The cattle warblefly, Hypoderma bovis, occasionally lays her eggs on man and in such cases the myiasis produced is more serious than that caused by O. ovis. The first-instar larvae migrate beneath the skin and wander around leaving a tortuous thin red inflamed line. (This skin reaction is very similar to that produced by some helminth larvae and referred to as cutaneous 'creeping eruptions' or 'larva migrans'.) Many of the first-instar larvae wandering around beneath the skin in man die, but some survive to later instars which penetrate a little deeper into the tissues, especially on the back, head and neck, to cause boil-like swellings or abcesses. Larvae may cause considerable pain and discomfort, and this may cause sleepless nights. Local paralysis may occur in extreme cases. Larvae also sometimes invade the eyes and have in rare instances caused their complete destruction. There is a record of larvae of Hypoderma lineatum invading the brain of a person and causing death.

On rare occasions man becomes infected, especially on the hands, feet or face, with larvae of Gasterophilus either due to close contact with horses, or in the case of G. pecorum by contact with wet grass upon which the eggs are laid. The first-instar larvae burrow just beneath the surface of the skin resulting in meandering linear eruptions which are indistinguishable from those caused by Hypoderma species and by certain helminths. Precise identification can be made only by dissecting out the causative agent. Invasion of the human eye by young larvae (0.5-1 mm) has also been reported. In man larval development never proceeds further than the first-instar larvae and infections therefore last for only short periods.

# 15 Fleas (Order Siphonaptera: Families Pulicidae, Leptopsyllidae and Ceratophyllidae)

#### Species

There are some 3000 species of fleas belonging to about 200 genera, but only relatively few are important pests of man. About 94 per cent of known species bite mammals and the remainder are parasites of birds. There are several families of fleas; the most important medically are the Pulicidae which includes the genera *Xenopsylla, Pulex, Tunga* and *Ctenocephalides*, the Leptopsyllidae containing *Leptopsylla* and the Ceratophyllidae which includes the genus *Nosopsyllus*.

#### Distribution

Fleas are found throughout most of the world, but many species and genera have a more restricted distribution, for example the genus *Xenopsylla*, which contains important plague vectors, is confined to the tropics and subtropics.

#### Medical importance

Xenopsylla species, such as X. cheopis, X. astia and X. brasiliensis, are vectors of plague (Yersinia (= Pasteurella) pestis), X. cheopis and Nosopsyllus fasciatus are vectors of flea-borne endemic typhus (Rickettsia mooseri (= typhi)), Ctenocephalides canis, C. felis felis, Xenopsylla and Nosopsyllus species are intermediate hosts of cestodes (Dipylidium caninum, ?Hymenolepis nana, H. diminuta). Fleas may also be vectors of tularaemia (Pasteurella (= Francisella) tularensis), while the chigoe or jigger flea (Tunga penetrans) burrows into the feet of man.

# External morphology of fleas

# Adults (figure 15.1)

Adults are relatively small (1.0-8.5 mm), and more or

less oval insects, compressed laterally and varying in colour from light to dark brown. Although there are no distinct demarcations or constrictions that separate the head, thorax and abdomen from each other, these three regions are easily identified. Wings are absent, but there are three pairs of powerful and well developed legs, the hind pair of which are specialised for jumping. The legs and also much of the body are covered with bristles and small spines.

The head is roughly triangular in shape, bears a pair of conspicuous black eyes (a few species are eyeless) and short three-segmented more or less club-shaped antennae which lie in depressions behind the eyes. The mouthparts which point downwards consist of a pair of four-segmented maxillary palps, a pair of labial palps which vary both in length and the apparent number of segments, a single pointed epipharynx (the labrum is present as a minute structure at its base), a finely serrated pair of maxillae (maxillary lacinia), and a pair of maxillary blades (figure 15.2). The epipharynx and paired maxillae form three stylets which pierce the host's skin during blood-feeding. In some species a row of coarse, well developed and tooth-like spines, collectively known as the genal comb or genal ctenidium, is present along the bottom margin of the head capsule (figures 15.1 and 15.3d, e).

The thorax has three distinct segments, the pro-, meso- and metathorax. The arrangement of the sclerites or plates that cover the flea's body is complicated, but some knowledge is required for the recognition of important vector species. Each thoracic segment has a conspicuous dorsal plate or sclerite, the pro-, meso- or metanotum and a more ventral sclerite (although appearing to be lateral) called the pro-, meso- and metasternum, the latter is divided into the episternum and a lower metepisternum (figure 15.1). The posterior margin of the pronotum may bear a row of teeth-like coarse spines forming the pronotal comb or pronotal ctenidium (figure 15.3c, d, e). Some genera of fleas lack both the pronotal and genal combs and are



Fig 15.1 Lateral view of an adult female flea showing some of the principal taxonomic features:- **apy**, antepygidial bristle; **epi**, episternum; **prn**, pronotum; **prs**, prosternum; **mep**, metepimeron; **msn**, mesonotum; **mtn**, metanotum; **mts**, metepisternum. The meral rod is located on the sclerite called the mesosternum (mesopleuron). The episternum (**epi**) and metepisternum (**mts**) combined form the metasternum.



Fig 15.2 Frontal view of the head of an adult flea showing maxillary palps and mouthparts.

referred to as combless fleas (figure 15.3a, b, f), whereas in some other genera both combs are present

(figure 15.3d, e). The mesosternum (mesopleuron) is located above the middle pair of legs and is of value in the recognition of *Xenopsylla*. In several genera, including *Xenopsylla* which contains important plague vectors, this sternite is clearly divided into two parts by a thick vertical rod-like structure termed the meral rod or mesopleural suture. The presence of a meral rod, combined with the absence of both genal and pronotal combs, indicates the genus *Xenopsylla* (figure 15.3a). However, it must be stressed that the presence of a meral rod by itself does not identify fleas as a species of *Xenopsylla*, because several other genera which have combs also have a meral rod.

Each thoracic segment bears a prominent and well developed pair of legs, the hind pair are a little longer than the other two pairs. The trochanter and femur of all legs are broad and flattened and the tarsi are distinctly five-segmented and terminate in a pair of large claws.

The abdomen consists of eight well defined and



Fig 15.3 Diagram of the head and first three thoracic segments of adult fleas of the genera (a) Xenopsylla, (b) Pulex, (c) Nosopsyllus, (d) Ctenocephalides, (e) Leptopsylla and (f) Tunga, showing the presence or absence of meral rods and genal and pronotal combs.

easily recognised segments, each divided into a dorsal tergum and a ventral sternum, and in addition much smaller, modified, and less obvious ninth and tenth segments. On the posterior margin of the eighth tergum is a conspicuous pin-cushion-like structure which bears numerous short hairs and is termed the pygidium (sensilium). The sex of a flea is easily determined by examination of the tip of the abdomen. In males it has an upturned appearance due to the presence of copulatory structures. These consist of a pair of claspers (parameres) which may have several lobes, a pair of narrow rod-like structures (which represent the ninth sternite) and internally the aedeagus (penis) (figure 15.4b). In the female the tip of the abdomen is more rounded than in the male and lacks all these features, but lying internally in about the position of the sixth to eighth abdominal segments is a distinct brownish spermatheca (figure 15.4a), which because its shape and size varies greatly according to species is of considerable diagnostic value. It is not always important to separate the sexes as both take bloodmeals and can be vectors of disease.

The above characters of the adult flea are best seen in fleas that have had the soft internal organs and tissues dissolved in 10 per cent solution of potassium or sodium hydroxide. The flea is placed in hydroxide solution in a test tube and preferably left at room temperature for one to two days; alternatively the tube



Fig 15.4 Terminal abdominal segments of an adult (a) female and (b) male flea.

can be placed for about five minutes in a water bath of boiling water.

# The alimentary canal of adult fleas (figure 15.5)

For a better understanding of the role fleas have in the transmission of plague it is necessary to describe the alimentary canal and the method of blood-feeding.

The host's skin is pierced by the epipharynx and the paired finely toothed maxillae (figure 15.2). Saliva is injected and the host's blood is sucked up through the food channel formed by the apposition of the grooved epipharynx and maxillae. The blood-meal then passes



Fig 15.5 Diagrammatic representation of the alimentary canal of an adult flea showing the backwardly projecting spines in the proventriculus.

through the spindle-shaped pharynx and thin oesophagus into the bulbous proventriculus. This is provided internally with numerous backwardly projecting stiff spines which when pressed together prevent the regurgitation of the blood-meal into the oesophagus (figure 15.5). (The proventriculus is important in the mechanism of plague transmission.) Finally, the blood-meal enters into a relatively large stomach (mid gut) where it is digested. The distal end of the stomach is connected to the hind gut, the junction being easily identified by the four Malpighian tubules. The hind gut is continuous with a small dilated rectum which has prominent rectal papillae which extract water from the faeces, so that it passes out through the anus in an almost dry state.

# Life-cycle (figure 15.6)

Both sexes of fleas take blood-meals and are therefore equally important as vectors of disease. The life-cycles of fleas which are more or less permanently attached to their hosts (for example the chigoe or jigger flea of man and the stick-tight fleas of poultry) differ considerably from the life-cycle of more usual fleas. However, of these types of fleas only the chigoe (*Tunga penetrans*) is a parasite of man and its life-cycle is described separately. The present account is a generalised description of the life-cycle of fleas that may occur on man or animals, such as dogs, cats and commensal rats.

A female flea that is ready to oviposit may leave the host to deposit her eggs in debris that accumulates in the host's dwelling place, such as rodent burrows or nests. With species that occur on man or his domestic pets, such as cats and dogs, females often lay their eggs in or near cracks and crevices on the floor or amongst dust, dirt and debris. Sometimes, however, eggs are laid while the flea is still on the host and these usually, but not always, fall to the ground. The eggs are very small and only just visible without a hand lens. They are oval or roundish in shape, white or yellowish and do not have any sculpturing or pattern. They are thinly coated with a sticky substance which usually results in



Fig 15.6 Diagram of the life-cycle of a flea.

them becoming covered with dirt and debris. Adult fleas may live for up to 6–12 months, or possibly two years or more, and during this time a female may lay 300–1000 eggs, mostly in small batches of about 15– 30 a day.

Eggs hatch within about 2-14 days depending on the species of flea, temperature and also humidity. A minute legless larva emerges from the egg (figure 15.6). It has a small blackish head with a very small pair of antennae, followed by 13 pale brown distinct and more or less similar segments, the first three of which represent the thorax. Each segment bears a circle of setae near the posterior border. The last segment ends in a pair of finger-like ventral processes termed the anal struts. The presence of these struts combined with the setae on the body distinguish larval fleas from all other types of insects of medical importance.

Larvae are very active. They avoid light and seek shelter in cracks and crevices and amongst debris on floors of houses or at the bottom of nests and animal burrows. Sometimes, however, larvae are found amongst the fur of animals, and even on people who have unclean habits and dirt-laden clothes, and occasionally in beds. Larvae feed on almost any organic debris including the host's faeces and partly digested blood evacuated from the alimentary canal of adult fleas; in a few species feeding on expelled blood seems to be a nutritional requirement for larval development. In some species larvae are scavengers and feed on small dead insects or dead adult fleas of their own kind. There are usually three larval instars, but in some species there are only two instars. The larval period may last as little as 10-21 days, but this varies greatly according to species, and may be prolonged more than 200 days by unfavourable conditions such as limited food supply and low temperatures. Mature larvae are about 5 mm long. Unlike adult fleas, larvae cannot tolerate large extremes in relative humidity and they die if humidities are either too low or too high.

At the end of the larval period the larva spins a whitish cocoon from silk produced by the larval salivary glands. Because of its sticky nature it soon becomes covered with fine particles of dust, organic debris and sand picked up from the floor of the host's home. Cocoons camouflaged in this way are very difficult to distinguish from their surroundings. About two to three days after having spun a cocoon around itself the larva pupates within the cocoon. Adults emerge from the pupa after about 7-14 days, but this period depends on the ambient temperature. After having emerged from the pupa the adult flea requires a stimulus, usually vibrations such as caused by the movements of the host within its home, burrow or nest, before it escapes from the cocoon. If, however, animal shelters or houses are vacated, then adult fleas fail to escape from their cocoons until their dwelling

places are reoccupied. In some species carbon dioxide emitted from hosts or a seasonal increase in humidity stimulate emergence. Adults may remain alive in their cocoons for about a year. This explains why people moving into buildings that have been vacated for many months may be suddenly attacked by large numbers of very blood-thirsty fleas, these being newly emerged adults seeking their first blood-meal.

The life-cycle from egg to adult emergence may be as short as two to three weeks for certain species under optimum conditions, but frequently the life-cycle is considerably longer.

Fleas avoid light and are therefore usually found sheltering amongst the hairs or feathers of animals, or on man under his clothing or in his bed. If given the opportunity many species of fleas feed several times during the day or night on their hosts. While feeding, fleas eject faeces composed at first of semi-digested blood of the previous meal and then excess blood taken in during the act of feeding. This mixture of partially digested and virtually undigested blood often marks clothing and bed linen of people heavily infested with fleas.

Although most species of fleas have one or two favourite species of hosts, they are not entirely hostspecific, for example, cat and dog fleas (Ctenocephalides felis felis and C. canis) will readily feed on man, especially in the absence of their normal hosts. Human fleas (Pulex irritans) feed on pigs, and rat fleas of the genus Xenopsylla will attack man in the absence of rats. Most fleas will in fact bite other hosts in their immediate vicinity when their normal hosts are absent or scarce. However, although feeding on less acceptable hosts keeps fleas alive, their fertility can be seriously reduced by continued feeding on such hosts. Fleas rapidly abandon dead hosts to seek out new ones, behaviour which is of profound epidemiological importance in plague transmission. Fleas can withstand both considerable desiccation and prolonged periods of starvation, for example six months or more when no suitable hosts are present. On their host fleas move either by rapidly crawling or by jumping, but off the host they tend to jump more than crawl in their search for new hosts. Fleas can jump about 18 cm vertically and 35 cm horizontally.

# Medical importance

#### Flea nuisance

Although certain species of fleas may be important vectors of disease the most widespread complaint about them concerns the annoyance caused by their bites, which in some people leads to considerable discomfort and irritation. The three most widespread and common nuisance fleas are the cat and dog fleas, *Ctenocephalides felis felis* and *C. canis* and to a lesser extent the so-called human flea<sup>\*</sup>, *Pulex irritans*. In some areas other species such as the European chicken flea (*Ceratophyllus gallinae*) and the Western chicken flea (*C. niger*) may be of local importance.

Fleas frequently bite people on the ankles and legs, but at night a sleeping person is bitten on other parts of the body. In many people the bite is felt almost immediately, but irritation usually becomes worse sometime after biting. In sensitised people intense itching may result. Because fleas are difficult to catch this tends to increase the annoyance they cause, and people attacked by fleas frequently spend sleepless nights alternately scratching themselves and trying to catch the fleas. There is evidence that children under ten years generally experience greater discomfort than older people.

#### Plague

Plague is caused by Yersinia (= Pasteurella) pestis and is primarily a disease of wild animals, especially rodents, not man. Over 220 rodent species have been shown to harbour plague bacilli. The cycle of transmission of plague between wild rodents, such as gerbils, marmots, voles, chipmunks and ground squirrels, is termed sylvatic, campestral, rural or endemic plague. Many different species of fleas bite these rodents and maintain plague transmission amongst them. When people, such as fur trappers and hunters, handle these wild animals there is the risk that they will get bitten by rodent fleas and become infected with plague.

An important form of plague is urban plague. This describes the situation when plague circulating amongst the wild rodent population has been transmitted to commensal rats, and is maintained in the rat population by fleas such as Xenopsylla cheopis (Europe, Asia, Africa and the Americas), X. astia (Asia) and X. brasiliensis (Central Africa). When rats are living in close association with man, such as rat infested slums, fleas normally feeding on rats may turn their attention to man. This is most likely to happen when rats are infected with plague and as a result rapidly develop an acute and fatal septicaemia. On death of the rats the infected fleas leave their more normal hosts and feed on man. In this way plague is spread by rat fleas to the human population. The most important vector species is X. cheopis but some 29 other species of fleas have been found to transmit plague, including Xenopsylla astia, X. brasiliensis, and more rarely Nosopsyllus fasciatus and Leptopsylla aethiopica, species which are normally reluctant to bite man, and also the cat and dog fleas. In

\* In western and southern parts of North America Pulex simulans which is closely related to P. irritans and has similar habits is often referred to as the human flea.

addition to man becoming infected by the bite of fleas that have previously fed on infected rats the disease can also be spread from man to man by fleas such as *Xenopsylla* species, *Pulex irritans* and *Pulex simulans*, feeding on a plague victim then on another person. This latter method, however, appears to play a minor role in the transmission of plague.

It is important to understand the methods by which fleas transmit plague. Plague bacilli sucked up with the blood-meals of male and female fleas are passed to the stomach where they undergo so great a multiplication that they extend forwards to invade the proventriculus (figure 15.5). In some species, especially those of the genus Xenopsylla, further multiplication in the proventriculus results in it becoming partially, or more or less completely, blocked. This prevents the proventriculus from functioning normally and results in fleas regurgitating some of the blood-meal during later feeds. Thus, plague bacilli obtained from a previous feed are passed down the flea's mouthparts into the host. In the case of completely blocked fleas, blood is sucked up with considerable difficulty about as far as the proventriculus, where it mixes with the bacilli and is then regurgitated back into the new host. Blocked fleas soon become starved and repeatedly bite in attempts to get a blood-meal and are therefore potentially very dangerous. Even if there is no blockage of the alimentary canal, plague transmission can nevertheless occur by direct contamination from the flea's mouthparts.

Another, but less important, method of infection is by the flea's faeces being rubbed into abrasions in the skin or coming into contact with mucous membranes. Plague bacilli can remain infective in flea faeces for as long as three years. Occasionally the tonsils become infected with plague bacilli due to crushing infected fleas between the teeth.

In pneumonic plague the bacilli occur in enormous numbers in the sputum. This form of plague is highly contagious due to the ease by which bacilli are transmitted from patients to others by coughing, and the inhalation of droplets; insects are not involved in the spread of pneumonic plague.

# Flea-borne endemic typhus

Flea-borne or murine typhus is caused by *Rickettsia* mooseri (= typhi) which is ingested by the flea with its blood-meal. Within the gut the rickettsiae multiply, but unlike plague bacilli they do not cause any blockage of the proventriculus or stomach. Infection is caused by infected faeces being rubbed into abrasions or coming into contact with delicate mucous membranes, and by the release of rickettsiae from crushed fleas. Faeces may remain infective under ideal conditions for as long as four to nine years. Murine typhus is essentially a disease of rodents, particularly rats and especially Rattus norvegicus. It is spread amongst rats and other rodents by Xenopsylla species, especially X. cheopis, but also by Nosopsyllus fasciatus, Leptopsylla segnis and by a few ectoparasites that are not fleas such as the rat louse Polyplax spinulosa (possibly also Hoplopleura spp.) and maybe by the tropical rate mite Ornithonyssus bacoti. Man becomes infected mainly through Xenopsylla cheopis, but occasionally Nosopsyllus fasciatus, Ctenocephalides canis, C. felis felis and Pulex irritans may be involved. Leptopsylla segnis does not attack man, but it is possible that murine typhus is sometimes spread to man by an aerosol of infective faeces of this species.

#### Cestodes

Dipylidium caninum is one of the more common tapeworms of dogs and cats and occasionally occurs in children, while Hymenolepis diminuta infects rats and mice and occasionally man. These tapeworms can be transmitted by fleas to both rodents and man. Eggs of these parasites are passed out with the excreta of rats and domestic pets and may be swallowed by larval fleas feeding on excreta. Larval worms hatching from the ingested eggs penetrate the gut wall of the larval flea and pass across into the body cavity (coelom). They remain trapped within this space and pass on to the pupa and finally to the adult flea where they encapsulate and become cysticercoids (infective larvae). Animals can become infected by licking their coats during grooming and thus swallowing the infected adult fleas. Similarly, young children fondling and kissing dogs and cats can become infected with D. caninum by swallowing cat and dog fleas, or by being licked by dogs which have crushed infected fleas in their mouths thus liberating the infective cysticercoids. Adult rat fleas (Xenopsylla, Nosopsyllus) occasionally get mixed with food or drink and swallowed by man, who may then become infected with Hymenolepis diminuta.

Another tapeworm of man, *H. nana*, is spread through contaminated food or water, and possibly also by fleas but their role in transmission is unclear because a morphologically identical parasite, *H. fraterna* of rats and mice, has fleas, especially *Xenopsylla* species, as intermediate hosts. *H. nana* is known to be capable of developing in insects, and it is possible that fleas and rodents sometimes serve as intermediate hosts and reservoirs of infection, respectively.

#### Less important diseases

In addition to the above diseases and parasites spread by fleas these insects may play some small part in the transmission of *Pasteurella* (=*Francisella*) *tularensis*,
Erysipelothrix rhusiopathiae, Rickettsia conori, Rickettsia pavlovskyi and Coxiella burneti. It must be stressed, however, that their role in the spread of most of these diseases is little more than speculation.

# External morphology of Tunga penetrans

#### Adults (figure 15.7)

These fleas are found in the tropics and subtropics having a distribution stretching from Central and South America, the West Indies across Africa to the Malagasy Republic. They have occasionally been reported in persons in India returning from overseas, mainly Africa, but the flea is not indigenous in India or elsewhere in Asia. They are sometimes referred to as chigoe or jigger fleas, which is an unfortunate terminology because this frequently leads to confusion with the rather similarly pronounced name chiggers, which is commonly applied to *Leptotrombidium* mites, vectors of scrub-typhus. *Tunga penetrans* does not transmit any disease to man but is a nuisance because females burrow into the skin.



Fig 15.7 Adults of *Tunga penetrans*, (a) a non-gravid (immature) female and (b) a gravid female with enormously enlarged abdomen containing eggs embedded in the skin of a host, with the tip of the abdomen projecting from the skin to the exterior.

Adults of both sexes are exceedingly small, only about 1 mm long (figure 15.7a). They have neither genal nor pronotal combs and are easily separated from other fleas of medical importance by their very compressed thoracic segments, (figure 15.3f) and the paucity of spines and bristles on the body.

## Life-cycle and medical importance

The eggs are dropped on to the floor of houses or on the ground outside. They hatch within about three to four days and the larvae inhabit dirty and dusty floors or dry sandy soils, especially in areas frequented by hosts of the adult fleas. Under favourable conditions larval development is completed within about 10-14 days, the pupal period lasts about 5-14 days and the complete life-cycle can be as short as about 18 days.

Newly emerged adults are very agile and jump and crawl about on the ground until they locate a suitable host, which is usually a man or pig. Both sexes feed on blood, but whereas the male soon leaves the host after taking a blood-meal the female, after being fertilised, burrows into the skin where it is soft, such as between the toes or under toe-nails. Other areas of the foot including the sole may also be invaded by the females. In people habitually sitting on the ground, such as beggars or infants the buttocks may often be infected, and particularly heavy infestations have been recorded from leprosy patients. In heavily infected individuals the arms, especially on the elbows, may also be attacked, and occasionally the females burrow into the soft skin around the genital region. Burrowing into the skin appears to be accomplished by the flea's sharp and well developed mouthparts. The result is that the entire flea, with the exception of the tip of the abdomen bearing the anus, genital opening and large respiratory spiracles, becomes completely buried in the host's skin. In this embedded position she continues to feed. The area surrounding the embedded flea becomes very itchy and inflamed, and secondary infections may become established, resulting in ulcerations and the accumulation of pus. While the blood-meal is being digested, the abdomen distends to a relatively enormous size and the flea attains both the shape and size (6 mm) of a small pea (figure 15.7b). This expansion is accomplished in some eight to ten days. Towards the end of this period of abdominal enlargement the ovaries are composed of thousands of minute eggs. For the next seven to ten days about 150-200 eggs a day are passed out of the female genital opening, most of which eventually fall to the ground and hatch after about three to four days.

When female fleas die they remain embedded within the host and this frequently causes inflammation and may, in addition, result in secondary infections, which if ignored can lead to loss of the toes, tetanus, or even gangrene. Male fleas cause no such trouble as they do not burrow into the skin.

These fleas are most common in people not wearing shoes, such as children. Because these fleas are feeble jumpers, wearing shoes is a simple, but in some communities relatively costly, method of reducing the likelihood of infection. Females embedded in the skin should be removed with fine needles under aseptic conditions, and wounds caused by their extraction sterilised and dressed. They are best removed within the first few days of their becoming established, as they are difficult to extract when they have greatly distended abdomens containing numerous eggs without rupturing them, and this increases the risk of infections.

Pigs, in addition to man, are often commonly invaded by *Tunga* and they may provide a local reservoir of infection. Other animals such as cats, dogs and rats are also readily attacked.

#### Echidnophaga gallinacea

Another flea that may on rare occasions attack man and also dogs, cats and horses is the stick-tight flea, Echidnophaga gallinacea, a pest of poultry and wild birds in most tropical and subtropical countries. They are very small fleas (1.0-1.5 mm). Adults of both sexes bury their mouthparts more or less permanently into the head and neck of poultry, or on man almost anywhere. Ulcers may develop, and the female fleas either lay their eggs in these or else allow them to drop to the floor. Larvae that hatch from eggs oviposited in ulcers drop to the ground to complete their development. These fleas although capable of transmitting plague and murine typhus and acting as an intermediate host for Dipylidium caninum are not considered to be of any importance as vectors. Their main nuisance is as ectoparasites of man. They can be carefully removed under aseptic conditions.

## Control

Cat and dog fleas (*Ctenocephalides felis felis and C. canis*) can most easily be detected by examination of the fur round the neck, or on the belly of the hosts. Proprietary insecticidal powders containing I per cent HCH, 2-5per cent malathion, 3-5 per cent carbaryl (Sevin) or I per cent pyrethrum can be applied to the coat of an animal. A simple, but not always very efficient, procedure is to place a proprietary plastic collar impregnated with 20 per cent dichlorvos (DDVP) round the necks of dogs (except greyhounds) and cats (except Persian varieties). However, an important consideration is that most fleas are found away from the host not on it. For example, it has been said that a typical colony of cat fleas consists of only about 25 adult fleas on the cat, but that on the floor and bedding there may be 500 adult fleas, 500 cocoons and as many as 3000 larvae and 1000 eggs. Clearly control measures should not be restricted to the cat but applied to the total environment. Flea cocoons are not very susceptible to insecticides, consequently insecticidal treatments should be repeated about every two weeks for about six months. Beds, kennels, or other places where pets sleep or spend much of their time should be either treated with insecticidal powders or lightly sprayed with solutions containing 0.5 per cent HCH, 2 per cent malathion, 0.5 per cent diazinon or 2 per cent dichlorvos (DDVP) to kill both adult and larval fleas.

For more general control of fleas 5-10 per cent DDT, 1.0 per cent HCH or 0.5 per cent dieldrin can be liberally applied to floors of houses, runways of rodents, or dusts blown into their burrows. In many parts of the world, however, *Xenopsylla cheopis* and *Pulex irritans* have developed resistance to DDT, HCH and dieldrin. In such cases organophosphate or carbamate insecticides such as 2 per cent diazinon, 2 per cent fenthion (Baytex), 5 per cent malathion, 2 per cent fenitrothion (Sumithion), 2 per cent iodofenphos or 3-5 per cent carbaryl (Sevin) can be used. In India *X. cheopis* has developed resistance to malathion and recent trials in that country have shown carbaryl (Sevin) to be the most effective insecticide.

Insecticidal fogs or aerosols containing 2 per cent malathion or 2 per cent Ronnel have sometimes been used to fumigate houses harbouring fleas.

For the control of fleas in urban outbreaks of plague or murine typhus, extensive and well organised insecticidal operations may be necessary. At the same time as insecticides are applied rodenticides such as the anticoagulants for example, warfarin and fumarin, can be administered to kill the rodent population, but if fast-acting 'one-dose' rodenticides such as zinc phosphide, sodium fluoroacetate or strychnine are used then it is essential to apply these several days after insecticidal applications. Otherwise, the rodents will be killed but not their fleas, which will then bite other mammals including man and this may result in increasing disease transmission.

Insecticidal repellents such as dimethyl phthalate, diethyltoluamide or benzyl benzoate may afford some personal protection against fleas.

## 16 Lice (Order Anoplura: Families Pediculidae and Phthiridae)

## Species

Blood-sucking lice of man belong to three species and comprise the pubic or crab louse (*Phthirus pubis*\* of the family Phthiridae\*), the body louse (*Pediculus humanus* = *P. corporis*) and the head louse (*Pediculus capitis*), both of the latter belonging to the Pediculidae. Morphologically it is very difficult to separate head and body lice, in fact the two can interbreed in the laboratory, and the body louse has often been considered to represent a subspecies of the head louse.

#### Distribution

All three species have a more or less world-wide distribution, but are often commoner in temperate areas.

#### Medical importance

Body lice are vectors of louse-borne typhus (Rickettsia prowazeki), trench fever (Rickettsia (Rochalima) quintana) and louse-borne relapsing fever (Borrelia ( = Treponema) recurrentis).

#### The body louse (Pediculus humanus)

## External morphology

#### Adults (figure 16.1)

Adults are small, greyish and wingless insects, with a soft but rather leathery integument; they are flattened dorso-ventrally. Males measure about 2.5-3.5 mm and females about 3.5-4.5 mm. The head bears a pair

of inconspicuous eyes and a pair of short fivesegmented antennae. The three pairs of legs are stout and well developed. The short thick tibia has a small thumb-like spine on its inner side at the apex, while the short tarsus has a large curved claw (figure 16.2a). Hairs of the host or his clothing are gripped between this spine and claw. A single pair of spiracles is present on the thorax and a prominent pair on the first six visible abdominal segments, but not the last (seventh) segment (figure 16.1a).

The mouthparts of the louse are different from those of most other blood-sucking insects in that they do not constitute a projecting piercing proboscis. They cor sist of a flexible, sucking, almost tube-like mouth, called the haustellum, which is armed on the inner surface with minute teeth which grip the host's skin during feeding. Dorsal and ventral thin stylets are contained within a stylet sac below the pharynx (figure 16.3), and when a louse takes a blood-meal these are thrust forwards to pierce the skin. Saliva is then injected and blood is sucked into the mouth and passes into the stomach for digestion. With this type of feeding arrangement there are no projecting external mouthparts.

The lateral margins of the abdominal segments are sclerotised and much darker than the rest of the segments. In female lice the tip of the abdomen is bifurcated, and careful examination of the ventral surface shows a pair of small gonopods which grip fibres of clothing (figure 16.1c). A small genital opening is located between these gonopods. In the males the abdomen is not bifurcated and there are no gonopods, but a small distinct penis is present (figure 16.1b). However, as both sexes suck blood and are thus equally important in disease transmission, it is not usually important to be able to differentiate the sexes.

\* Occasionally generic and family names are spelt *Pthirus* and Pthiridae, because in the original published description of the pubic louse the spelling was given as *Pthirus*, due it is thought to a printer's error. The correct classical derivation from the Greek should be *Phthirus*.



Fig 16.1 Pediculus humanus (body louse), (a) dorsal view and (b) terminal abdominal segments of a male showing the penis, and (c) ventral view of a female.





- Fig 16.3 Diagrammatic representation of the longitudinal section through the head of a louse showing the stylet-sac ventral to the pharynx containing the piercing dorsal and ventral stylets.
- Fig 16.2 Body and head lice: (a) leg of P. humanus showing tarsal claw and tibial spine, (b) unhatched egg of P. humanus glued to fibres of clothing, (c) two unhatched and one hatched egg of P. capitis glued to a hair from the head; three eggs are shown here very close together for convenience, but in practice they are never this close together.

## Life-cycle

Both sexes take blood-meals and feeding occurs at any time during the day or night. Both the adult and immature stages live permanently on man, clinging mainly to hairs of his clothing and usually only on to body hairs during feeding. Female lice glue about six to nine eggs per day very firmly on to the hairs of clothing, especially to those along the seams of underclothes, such as vests and pants, but also on shirts and occasionally on body hairs. The egg, commonly called a nit, is oval and white, and has a distinct operculum containing numerous small perforations which give the egg the appearance of a miniscule pepper pot (figure 16.2b). The intake of air through these holes not only supplies the tissues of the developing embryo with oxygen but aides hatching in the following way. Just prior to hatching, the fully developed louse within its egg shell swallows air which distends the body against the egg shell thus building up a back pressure causing the head of the louse to be pushed up against the operculum and forcing it off. Female lice may live for one month and lay 200-300 eggs.

The duration of the egg stage is normally about six to nine days, but eggs on discarded clothing away from the warmth of the body may not hatch until two to three weeks. Eggs, however, cannot survive for as long as four weeks, consequently there is little danger of infestation with body lice from clothing that has not been worn for a month.

Lice have a hemimetabolous life-cycle. The louse which hatches from the egg is termed a nymph and resembles a small adult louse; it takes a blood-meal from man and passes through three nymphal instars, and after about 7-14 days becomes an adult male or female louse. The duration of the nymphal stage depends much on whether or not clothing is worn all the time. If it is discarded at night this may result in subjecting the nymphs to lower temperatures, thus slowing down their development.

The body louse is a true ectoparasite of man. Unfed lice die within about two to five days if kept away from man and without a blood-meal, but blood-fed individuals may survive for eight to ten days. Lice are very sensitive to changes in temperature. They quickly abandon a dead person, owing to cooling down of his body, to seek out new hosts; they also leave a person having a high temperature. They are unable to feed at temperatures above about 40°C.

A very heavily infested person may have 400-500 lice on his clothing and body. There is one record of an estimated 10 000 lice and 10 000 eggs from a single shirt! Usually, however, less than 100 lice are found on people, and many have considerably less than this.

Body lice are spread by close contact and are especially prevalent under conditions of overcrowding and in situations where people rarely wash or change their clothes. They are therefore commonly found on people in primitive jails, refugee camps and in trenches during wars, and also after disasters such as floods or earthquakes when people are forced to live in very overcrowded, and usually insanitary, conditions. Infestation may reach a peak in cold weather when several layers of woollen underclothes are worn and are rarely changed.

## Medical importance of body lice

#### Pediculosis

The presence of body, head or pubic lice on a person is sometimes referred to as pediculosis. The skin of people who habitually harbour large numbers of body lice may become pigmented and tough, a condition known as vagabond's disease or sometimes as *morbus errorum*.

Because lice feed several times a day saliva is repeatedly injected into people harbouring lice, and toxic effects may lead to weariness, irritability or a pessimistic mood, the person feels lousy. Allergies such as severe itching may be caused by repeated inoculation of saliva, and if inhaled the faeces may produce symptoms reminiscent of hay fever.

#### Louse-borne epidemic typhus

The rickettsiae of louse-borne typhus, Rickettsia prowazeki, are ingested with the blood-meal taken by both male and female lice and also their nymphs. They invade the epithelial cells lining the stomach of the louse where they multiply enormously and cause the cells to become greatly distended. About four days after the blood-meal the gut cells rupture and release the rickettsiae back into the lumen of the insect's intestine. Due to these injuries to the intestinal wall the blood-meal may seep into the haemocoel of the louse giving the body an overall reddish colour. The rickettsiae are passed out with the faeces of the louse, and man becomes infected when these are rubbed or scratched into abrasions, or come into contact with delicate mucous membranes, such as conjunctiva. Infection can also be caused by inhalation of the very fine powdered dry faeces. Alternatively, if a louse is crushed, such as by persistent scratching because of the irritation caused by its bites, the rickettsiae in the gut are released and may cause infection through abrasions etc. The rickettsiae may remain alive and infective in dried lice faeces for at least 90 days.

Man therefore becomes infected with typhus either by the faeces of the louse or by crushing it, not by its bite. An unusual feature of louse-borne epidemic typhus is that it is a disease of the louse as well as of man. The rupturing of the epithelial cells of the intestine, caused by the multiplication of the rickettsiae, frequently kills the louse after about eight to ten days. This may explain why people suffering from typhus are sometimes found with no, or remarkably few, lice on their bodies or clothing. Man is usually considered to be the reservoir of the disease. Asymptomatic carriers remain infective to body lice for many years. Recrudescences as Brill-Zinsser's disease, many years after the primary attack, may occur in a person and lead to the spread of epidemic typhus.

#### **Trench** fever

This is a relatively uncommon and non-fatal disease that was first noticed during World War I (1914-18) amongst soldiers in the trenches, and then reappeared again in eastern Europe during World War II (1939-45).

It is caused by *Rickettsia quintana* which is ingested by the louse during feeding on man. These rickettsiae are attached to the walls of the gut cells where they multiply; they do not penetrate the cells as do typhus rickettsiae and consequently they are not injurious to the louse. After five to nine days the faeces are infected. Like typhus, the disease is conveyed to man by either crushing the louse, or by its faeces coming into contact with skin abrasions or mucous membranes. The rickettsiae persist for a long time in dried louse faeces and it is suspected that infection may commonly arise from inhalation of the dust-like faeces. The disease may be contracted by those who have no lice but are handling louse infected clothing contaminated with faeces.

#### Louse-borne epidemic relapsing fever

The causative agent Borrelia ( = Treponema) recurrentis) is ingested with the louse's blood-meal from a person suffering from epidemic relapsing fever. Within about 24 hours all spirochaetes have disappeared from the lumen of the gut. Many have been destroyed, but the survivors have succeeded in passing through the stomach wall into the haemocoel, where they multiply greatly, reaching enormous numbers by the tenth to twelth days. The only way in which man can be infected with louse-borne relapsing fever is by the louse being crushed and the released spirochaetes entering the body through abrasions or mucous membranes. The habit of some people to crush lice between the fingernails, or the even less desirable habit of killing them by cracking them with the teeth, is clearly dangerous if the lice are infected with relapsing fever or rickettsiae.

This method of transmission must make it very rare for more than one person to be infected by any one infected louse, hence epidemics of louse-borne relapsing fever will rarely occur unless there are large louse populations.

#### Flea-borne endemic typhus

Flea-borne or murine typhus (*Rickettsia mooseri* (=ty-phi)) is usually transmitted to man by various fleas, although lice of the genus *Polyplax* may be involved in maintaining the rodent cycle (chapter 15). There is, however some evidence to suggest that *Pediculus humanus* may occasionally be involved in transmitting the disease amongst people.

## Control

The most obvious way to eradicate body lice from a person is by changing and washing the clothing in water hotter than 60°C, preferably followed by ironing. In epidemic situations, however, such measures may be impractical and immediate reinfestation may occur, hence insecticides are usually used for louse control.

Ten per cent DDT dust mixed with an inert carrier (talc) can be blown, at about the rate of 30 g per person, between the body and underclothes. If DDT resistant lice are present then 1 per cent HCH powder can be used. If the lice are also resistant to this insecticide 1 per cent malathion dust should be tried. DDT resistance has developed in many countries and lice resistant to HCH are also quite common; malathion resistant lice have also been reported. If lice are resistant to all these compounds alternative insecticidal dusts are 2 per cent temephos (Abate), 5 per cent carbaryl (Sevin), 1 per cent propoxur (Arprocarb) or 0.2 per cent pyrethrum. Since insecticidal dusts come into close and prolonged contact with people it is essential that they have very low mammalian toxicities.

## The head louse (Pediculus capitis)

## External morphology

#### Adults

There are only minor morphological differences separating body and head lice. For example, the head louse is usually slightly smaller and usually darker than the body louse, but the colour of the head louse is variable, also the head louse usually has the antennal segmentation less distinct, and the tibiae of the mid legs are smaller than in the body louse. However, in practice these differences are not very important because lice found on clothing or on the body are invariably body lice, while those on the head are nearly always head lice. In this book these two lice are regarded as distinct species although under laboratory conditions they can interbreed, and they are often regarded as subspecies.

## Life-cycle

The life-cycle is very similar to that of the body louse but the eggs (nits) are not laid on clothes but are cemented to the base of hairs of the head (figure 16.2c), especially behind and above the ears and back of the neck. The distance between the scalp and the furthest egg glued to a hair provides an approximate estimate of the duration of infestation. Only very occasionally are eggs laid on hairs elsewhere on the body. Most individuals harbour only 10-20 head lice, but in very severe infestations the hair may become matted with a mixture of nits, nymphs, adults and exudates from pustules resulting from bites of the lice. In such cases bacterial and fungal infections may become established and an unpleasant crust formed on parts of the head, underneath which are masses of head lice. Empty, hatched eggs remain firmly cemented to the hairs of the head. A female lays about six to eight eggs per day, amounting to about 300 eggs during her life-span, which is a little over one month. Eggs hatch within seven to ten days and the duration of the nymphal stages is about seven to eight days.

As with body lice dissemination of head lice is only by close contact, such as children playing together and with their heads frequently touching, or when people are crowded together such as in prisons or refugee camps. Use of other people's hats, scarves, combs, hair brushes etc. has not been proved to spread head lice. The occasional head louse found on such articles or on the backs of chairs are moribund individuals that will not be able to survive and infect someone else. Head lice are often rarer in men than in children or women of all ages. There is no correlation between hair length and infestation rates.

## Medical importance

There is no evidence that head lice are vectors of any of the diseases transmitted by body lice, although under laboratory conditions they appear to be capable of transmitting both rickettsiae and spirochaetes. They can, however, transmit impetigo, the bacteria are ingested with the blood-meal and pass out unharmed with the faeces.

## Control

Regular washing with soap and warm water may reduce the numbers of nymphs and adults on the hair, but will have no effect on the eggs which are firmly glued to the bases of the hairs. Before the advent of efficient insecticides lice and nits were removed by a steel comb, which had very closely-set fine teeth. Alternatively all hair was shaved from the head. Regular combing with an ordinary comb, although not removing the eggs, may reduce the number of nymphs and adults.

Insecticidal formulations for louse control include dusts, emulsions and lotions, the choice of formulation and insecticide depends on the availability of proprietary brands, preference of patients, degree of residual action required and costs. Although dusts (for example 10 per cent DDT and 1 per cent malathion) are efficient they are not acceptable to most people because they give the head a greyish appearance and reveal that the person has lice. Preparations such as shampoos which are applied and then washed off after a few minutes are not usually very effective and are not recommended.

Emulsions of 2-5 per cent DDT or 0.1 per cent HCH can be used, but if head lice are resistant to these compounds then emulsions containing 0.1 – 0.3 per cent pyrethrins, 0.5 per cent malathion, propoxur (Arprocarb) or carbaryl (Sevin), or dusts such as 1 per cent malathion or 5 per cent carbaryl can be applied. Some proprietary shampoos contain 10 per cent benzyl benzoate or 5 per cent isobornyl thiocyanoacetate, but as already mentioned shampoos are usually much less effective than treatments which leave the insecticide on the head.

A second washing with an insecticidal preparation after an interval of seven to ten days is required with formulations of DDT or HCH as they have no or little effect on the eggs. If, however, malathion or carbaryl are used only a single treatment is required because this kills eggs as well as nymphs and adults. None of the compounds will remove eggs cemented to the hairs, but these can be removed with a louse comb.

## The pubic louse (Phthirus pubis)

## External morphology

#### Adults (figure 16.4)

The pubic louse is generally smaller (1.25 - 2.0 mm) than *Pediculus* and is easily distinguished from it. In the pubic louse there is less differentiation between the thorax and abdomen, together they are almost round



Fig 16.4 Dorsal view of Phthirus pubis (pubic louse) showing the very large tarsal claws on the mid and hind legs.

so that the body is nearly as broad as long. Whereas all three pairs of legs are more or less equal in size in the body and head louse, in the pubic louse the front pair, although as long as the other two pairs, is much more slender and has smaller claws. In marked contrast the middle and hind legs have massive claws (figure 16.4). The presence of a broad squat body, very large claws on the middle and hind legs, together with the characteristically more sluggish movements, have resulted in the pubic louse being aptly called the crab louse.

## Life-cycle

The life-cycle is very similar to that of *Pediculus*, eggs take about six to eight days to hatch and the nymphal stages last about ten days. Females lay a total of about 150–200 eggs which are slightly smaller than those of the body or head louse, and are cemented to the coarse hairs of the genital and peri-anal regions of the body. In any infestations pubic lice may be found on other areas of the body having coarse and not very dense covering of hair, for example beard, moustache, eyelashes, underneath the arms and occasionally on the chest. They are very rarely found on the head. Pubic lice are considerably less active than *Pediculus*. The life-cycle, from egg laying to formation of the adult, is about 17 days.

Infestation with crab lice is usually through sexual intercourse, but it is wrong to suspect that this is the only method. Young children sleeping with parents can catch crab lice, and infestations can arise from discarded clothing, infested bedding, or even rarely from lavatory seats.

## Medical importance

Although under laboratory conditions pubic lice can transmit louse-borne typhus, there is little evidence that under natural conditions they spread any disease, although there was a suggestion that they have been responsible for typhus outbreaks in China. In some individuals, however, severe allergic reactions develop to their bites due to the injection of saliva and the deposition around the feeding sites of faeces. Small characteristic blue spots (maculae cerulae) may appear on the infested parts of the body. Infestations of pubic lice are sometimes known as pediculosis pubis.

## Control

Originally control involved shaving pubic hairs from the body, but this method has been replaced by the applications of insecticidal emulsions and lotions as used for head lice control. In addition, 10 per cent DDT, 2 per cent HCH or 0.5 per cent malathion powders can be used, but powders are usually less effective than emulsions or lotions. If DDT or HCH has been used then a second treatment should be given seven to ten days later as organochlorine insecticides will not kill the eggs, and in some infestations a third treatment may be required. Formulations containing malathion or carbaryl (Sevin) are usually better, and a single efficient application should kill eggs as well as nymphs and adults.

It may be advisable to treat all hairy areas of the body below the neck. Insecticidal shampoos are ineffective for controlling pubic lice.

Some insecticides, may cause irritation and dermatitis due to the sensitivity of the genital regions, in such cases shaving may remain a relatively simple and effective remedy.

## 17 Bedbugs (Order Hemiptera: Family Cimicidae)

There are two species of bedbugs both of which commonly feed on man; Cimex lectularius which has a widespread distribution and C. hemipterus (= rotundatus) commonly called the tropical bedbug and which is essentially a species of the Old and New World tropics but which also occurs in temperate regions. A few other Cimicidae occasionally feed on man, such as Leptocimex boueti, which is primarily an ectoparasite of bats in West Africa and Cimex columbarius in Europe which normally feeds on pigeons. It is not always easy to separate the two common bedbugs, but in C. lectularius the prothorax is generally about two and a half times as wide as long whereas in C. hemipterus it is only about twice as wide as long and its margins are not so flattened; also in C. hemipterus the abdomen is not so roundish as in C. lectularius.

#### Distribution

Bedbugs have a more or less world-wide distribution (see above).

#### Medical importance

Bedbugs are not known to transmit any disease to man but they can nevertheless constitute a public health problem because of their persistent biting at night which may lead to sleeplessness. In India the repeated feedings by large numbers of bedbugs on infants has been reported to cause iron deficiency.

## Cimex lectularius

## External morphology

## Adults (figure 17.1)

The adults are oval, wingless insects which are flattened dorsoventrally. They are about 4-5 mm long and

when unfed a pale yellow or brown colour, but after a full blood-meal they become a characteristically darker 'mahogany' brown, and also become rather fatter and even a little longer. The head is short and broad and has a pair of prominent compound eyes, in front of which are a pair of four-segmented antennae. The proboscis is slender, three-segmented (although it may appear to be composed of four segments) and is normally held closely appressed along the ventral surface of the head and prothorax, but when the bug takes a blood-meal it is swung forward and downwards (figure 17.2a). The prothorax is much larger than the meso- and metathorax and has distinct wing-like expansions. The rudimentary and non-functional wings, termed the hemielytra, appear as two more or less oval pads overlying the meso- and metathorax. The three pairs of legs are slender but well developed.

The abdomen is divided into eight visible segments. In adult males the tip of the abdomen is slightly more pointed than in the females and careful examination show that there is a small well developed and curved penis (figure 17.1a). When viewed ventrally a small incision is seen on the left side in the fourth abdominal segment of females (figure 17.1b), this opens into a special pouch called the mesospermalege or the organ of Berlese or Ribaga which serves to collect and store sperm. Since both male and female bugs bite man it may not, however, be very important to distinguish the sexes.

## Life-cycle

Both sexes take blood-meals and are equally important as pests. Feeding usually occurs on sleeping persons during the night, especially just before dawn, but if the bedbugs are starving, they will feed during the day in very dark rooms or even light ones. Bedbugs, unlike fleas and lice, do not stay long on man but visit him



Fig 17.1 The bedbug, Cimex hemipterus, (a) dorsal view of an adult male showing curved penis and (b) ventral view of an adult female showing opening into organ of Berlese (Ribaga).



Fig 17.2 Bedbugs, (a) diagram of adult with proboscis swung forward in a feeding position, (b) three unhatched and one hatched egg, and (c) a first-instar nymph.

only to take blood-meals. During the day both adults and nymphs are inactive and hide away in a variety of dark and dry places, such as cracks and crevices in furniture, walls, ceilings or floor boards, underneath seams in wallpaper and between mattresses and beds. Bedbugs are gregarious and are frequently found in large numbers.

Females lay about two to three eggs a day which are deposited in the same places where the bugs hide, such as in cracks and crevices of buildings and furniture. The eggs are about 1 mm long, pearly or yellowish white, covered with a very fine and delicate mosaic pattern, and characteristically slightly curved anteriorly (figure 17.2b). Some 500 or more eggs can sometimes be found more or less together cemented on rough surfaces such as walls or deposited in cracks. Females live several weeks to many months, and during this time may lay 150 to several hundred eggs.

The eggs usually hatch after about 8-11 days, but within less than a week if temperatures are about 27°C, but if, however, temperatures in houses are low hatching may be delayed for several weeks. At low temperatures eggs can survive for up to three months, but hatching will not occur below about 14°C. During hatching the small operculum is pushed up from the anterior end of the egg, but often remains partially attached. Empty egg shells usually remain cemented in place after hatching. The newly hatched bedbug (nymph) is very pale yellow and resembles an adult but is much smaller (figure 17.2c). The life-cycle is hemimetabolous and there are five nymphal instars (figure 2.14), each of which takes one or more bloodmeals. The nymphal period commonly lasts five to eight weeks, but this period may be greatly extended in cool conditions or if regular blood-feeding is prevented by lack of hosts. In the absence of man, bedbugs will attempt to feed on a variety of mammals,

including rabbits, rats, mice and bats, and even poultry and other birds. They can withstand long periods of starvation, up to 550 days; survival is dependent on both temperature and humidity. Under laboratory conditions adults have lived as long as four years.

The method of mating in bedbugs is unique among insects. The penis is not introduced into the genital opening, but penetrates the integument and enters the organ of Berlese (organ of Ribaga or mesospermalege) situated on the ventral surface of the female. This organ serves as a copulatory pouch into which spermatozoa are introduced. After one to two hours spermatozoa leave this 'pouch' and pass into the haemocoel of the female, and then migrate to the bases of the oviducts and ascend to the ovaries where fertilisation occurs.

Bedbugs in houses can be detected by the presence of live bugs, the cast-off skins of the nymphs, and hatched and unhatched eggs, all of which may be found in cracks and crevices. In addition small dark brown or black marks may be visible on bed sheets, walls and wallpaper, these are the bedbug's excreta and consist mainly of excess blood ingested during feeding. Houses with heavy infestations of bedbugs may have a characteristic sweet and rather sickly smell, but in practice this may not be apparent because the weak odour is masked by stronger insanitary smells. At night male and female adults and nymphs crawl from their daytime resting places to feed on sleeping people, after which they return to their resting sites to digest their blood-meals. Bedbugs can move quite rapidly when disturbed.

Because bedbugs do not have wings they have limited powers of dispersal. Occasionally they may crawl from one building to another, but they usually spread to new houses by being introduced with furniture and bedding or more rarely with clothing and hand baggage. Buying second-hand furniture can result in the introduction of bedbugs into houses.

## Medical importance

Bedbugs are not known to transmit any diseases to man. However, in some areas, especially those with dilapidated buildings and low standards of hygiene bedbug infestations can cause considerable distress. Reaction to their bites is variable. Some people show little or no reaction while others may suffer severe reactions and sleepless nights. In India repeated feedings of large numbers of bedbugs has been reported as responsible for iron deficiency in infants.

## Control

Floors and walls of infested houses together with as much furniture as possible should be sprayed with 5 per cent DDT emulsion. If, however, bedbugs are resistant to DDT then 0.5 per cent HCH should be tried, and if the bugs are also resistant to this compound, other insecticides such as 1-2 per cent malathion, 0.5 per cent diazinon or 0.5 per cent dichlorvos (DDVP) can be used. The addition of 0.1 - 0.2 per cent pyrethrins or synthetic pyrethroids such as bioresmethrin to sprays is useful because it helps flush out bedbugs from their hiding places and thus increases their contact with the insecticide.

Bedding and mattresses can be lightly sprayed with insecticides but should be aired afterwards to allow them to dry out completely before being reused. Insecticidal dusts can also be applied to mattresses and bedding. Infant's bedding should not be treated with insecticides. Commercially available insecticidal smoke generators (usually containing HCH or DDT) can be useful for fumigating infested premises.

## 18 Triatomine bugs (Order Hemiptera: Family Reduviidae, Subfamily Triatominae)

#### **Species**

The blood-sucking species of reduviid bugs belong to the subfamily Triatominae which comprises 15 genera and almost 100 species and subspecies. Principal species of medical importance are *Rhodnius prolixus*, *Pan*strongylus megistus and species of *Triatoma* such as *T.* infestans, *T. dimidiata*, *T. brasiliensis* and *T. maculata*. They are commonly called cone-nose bugs, assassin bugs or kissing bugs.

#### Distribution

Most Triatominae occur in the Americas, ranging from the southern States in the U.S.A. to Argentina, but a few species are found in the Old World tropics. All medically important species are confined to the southern U.S.A., Central and South America. Some species such as *P. megistus* and *T. brasiliensis* have a restricted distribution (Brazil), others such as *T. maculata* and *T. infestans*, *R. prolixus* and *T. dimidiata* occur more widely (see p. 148).

#### Medical importance

Vectors of Chagas' disease (Trypanosoma cruzi) in Central and South America, also of Trypanosoma rangeli an apparently non-pathogenic organism.

# External morphology of Triatominae bugs

#### Adults (figure 18.1)

These bugs vary in size from about 1 - 4 cm. They are easily recognised by their elongate snout-like head which bears a pair of prominent dark coloured eyes, in front of which are a pair of laterally situated long and



Fig 18.1 An adult Rhodnius species as an example of a triatomine bug.

thin four-segmented antennae. The three-segmented proboscis, sometimes called the rostrum, is relatively thin and straight and as in bedbugs lies closely appressed to the ventral surface of the head (figure 18.2a). When the Triatominae take a blood-meal the proboscis is swung forward and downwards (figure 18.2b). Bugs in other subfamilies of the Reduviidae are predacious on small insects and the proboscis is usually



Fig 18.2 Lateral views of the head of a *Rhodnius* species showing proboscis (a) closely appressed to ventral side of the elongate head and (b) swung forward in a feeding position.

thicker and more robust than in the Triatominae and is distinctly curved. Another difference is that in plantbugs the antennae arise from the dorsal surface of the head and not from its sides as in the Triatominae.

The dorsal part of the first segment of the thorax of the Triatominae consists of a very conspicuous triangular pronotum. The meso- and metathorax are completely hidden dorsally by the folded fore wings which are called hemielytra. The basal part of each hemielytron is thickened and relatively hard, whereas the more distal part is membraneous (figure 18.3). The



Fig 18.3 The fore wing or hemielytron of a triatomine bug showing the thickened and hard basal part and the membraneous distal part.

hind wings are entirely membraneous but when the bug is not flying they remain hidden underneath the hemielytra. The thorax has three pairs of relatively long and slender legs which end in paired small claws.

The abdomen is more or less oval in shape but is mostly covered by the wings, except for the lateral margins which are bent upwards slightly and are visible dorsally. In the males, but not females, there is a slight bulge on the underside of the abdomen near its tip, but apart from this there are no very obvious morphological differences between the sexes, and both take blood-meals.

Triatominae are frequently a rather dull brown-

black colour, but some species are more colourful having contrasting yellow or red markings, usually present as bands on the pronotum, basal part of the fore wings or margins of the abdomen. The three medically most important genera may be separated as follows (figure 18.4). *Panstrongylus* (figure 18.4a) – head relatively short and the antennae are situated close to the eyes; *Triatoma* (figure 18.4c) – head also relatively short, but antennae are situated about half way along the head; *Rhodnius* (figure 18.4b) – head distinctly longer and the antennae are situated near end of the head.

#### Life-cycle

Eggs are deposited in or near the habitation of their hosts, such as in cracks and crevices in walls, floors, ceilings and furniture of houses, especially old dilapidated mud-walled and thatched-roofed houses in rural areas, or slums at the edge of towns. Adults also lay their eggs in rodent burrows and a variety of shelters used by mammalian hosts upon which the bugs feed. Some species feed on birds and deposit their eggs in bird nests and on leaves of trees. Eggs are about 1.5 -2.5 mm long, pink, yellowish or white in colour depending on the species and have a smooth shell. They are oval in shape but have a slight constriction before the operculum (figure 18.5). Some species lay eggs in small batches which may be glued to the substrate, others lay them more or less singly, either free or cemented to the substrate. The total number of eggs laid by females varies between about 50-800 depending on the species, their longevity and the number of blood-meals they take. The life-cycle is hemimetabolous.

Small pale nymphs, which resemble adults but lack wings, hatch from the eggs after about 7-15 days, but the incubation period may extend to 60 days. These newly emerged nymphs usually remain hidden in cracks and crevices for a few days before they seek out blood-meals. There are five nymphal instars, each instar requires at least one complete blood-meal before it changes into a succeeding one. Rudimentary wing pads begin to be clearly visible in the fourth and fifth nymphal stages, but only adults have fully developed functional wings. Young nymphs can ingest as much as ten times their own weight of blood and as a result their abdomens may become so greatly distended that they resemble blood-red balloons. Successive instars take relatively less blood, so that the fifth and last nymphal stage takes about three to four times its own weight of blood, and adults ingest about two to three times their weight of blood. Sometimes hungry nymphs and adult bugs pierce the swollen abdomens of freshly engorged nymphs and take a blood-meal from them without apparently causing any harm.



Fig 18.4 Dorsal and lateral views of the heads of species of (a) Panstrongylus, (b) Rhodnius and (c) Triatoma to show generic differences in the length of the head and distances from the eyes to the insertion of the antennae.



Fig 18.5 Hatched and unhatched triatomine eggs.

Nymphs and adults of both sexes feed at night on their hosts, and feeding is a lengthy process lasting 25 minutes or more. When people are covered with blankets the bugs feed on exposed parts of the body such as the nose and around the eyes and mouth, but in hot weather they will readily feed on other exposed areas of the body. Bites are usually relatively painless and do not awaken people, but some species cause considerable discomfort and there may be prolonged after effects. Many triatominae defaecate during feeding and this behaviour is very important in the transmission of Chagas' disease.

Because of the relatively long time required, even under optimum laboratory conditions, to digest their large blood-meals, the life-cycle from egg to egg takes at least about three to three and a half months. Under natural conditions it often takes about one year, but sometimes the life-cycle is two years or even longer.

There are almost 100 species and subspecies of bugs in the subfamily Triatominae inhabiting both forests and drier areas of the Americas. Many species feed on a variety of wild animals, such as armadillos, opossums, rats, mice, marsupials, ground squirrels, skunks, iguanas, bats and also birds. Adults and nymphs are usually found in the burrows or nests of these animals. In addition to these sylvatic species, certain bugs have become highly domesticated and feed on animals such as donkeys, cattle, goats, horses, pigs, cats, dogs and especially chickens which in some areas appear to be particularly important hosts, and of course man. These domestic species often live in man-made shelters including houses, especially primitive ones made of wood, mud and thatch. Some species are partially sylvatic and domestic in their feeding and resting habits. Sylvatic species may sometimes readily move into houses from the forest as it is cut down and man occupies previously uninhabited areas.

If hosts vacate their shelters or homes hungry nymphs eventually crawl out from their hiding places to seek new hosts, while adults, which are strong fliers, fly out to find new hosts and shelters. Some species are attracted into houses by lights.

## Medical importance

#### Chagas' disease

Triatominae are of medical importance because they are vectors of Trypanosoma cruzi the causative agent of Chagas' disease, sometimes referred to as American trypanosomiasis. Parasites ingested with a blood-meal from a man undergo their entire development within the gut of the bug, and after 6-15 days infective metacyclic trypomastigotes (metatrypanosomes) of T. cruzi are present in the lumen of the hind gut. Bugs may also become infected by feeding on recently engorged nymphs. Blood-feeding commonly lasts 10-20 minutes or longer and during this time, or soon afterwards, many species of bugs excrete liquid or semi-liquid faeces which may be contaminated with the metacyclic forms of T. cruzi derived from a previous blood-meal. Man becomes infected when the excreta is scratched either into abrasions in the skin or in the site of the bug's bite, or when it gets rubbed into the eyes or other mucous membranes. If the bug's bite produces local irritation causing the person to scratch this facilitates infection. Transmission is not by the bite of the insect, solely through its faeces.

It appears that all Triatominae of the Western hemisphere can transmit Chagas' disease, and more than half have already been recorded naturally infected; but some strains of T. cruzi are unable to develop in the gut of some species. In practice, however, only those species (about 36) that have adapted to living in close association with man and therefore regularly feed on him are important vectors. The efficiency of a vector will also depend on the speed of feeding and whether or not the bug defaecates on a person during feeding. Among the important vectors are Rhodnius prolixus (French Guiana, Guatemala, Guyana, Nicaragua, Panama, Surinam, Venezuela), Pangstrongylus megistus (Brazil), Triatoma dimidiata (Belize, Colombia, Costa Rica, Ecuador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Venezuela) and T. infestans (Argentina, Bolivia, Brazil, Chile, Paraguay, Peru, Uruguay). Less important vectors, although they may contribute a significant role locally in transmission, include *Triatoma maculata* (Brazil, Colombia, French Guiana, Venezuela), *T. brasiliensis* (Brazil) and *T. sordida* (Argentina, Bolivia, Brazil, Chile, Paraguay, Uruguay).

Chagas' disease is a zoonosis. *T. cruzi* is essentially a parasite of wild animals, such as opossums (especially *Didelphis* spp.), armadillos, many species of wild and urban rats and mice, squirrels, carnivores, monkeys and possibly bats, all of which may serve as reservoirs of infection for man. The bug itself is also a reservoir of infection, and in some areas man is considered to be the principal one. Apart from acquiring infections with *T. cruzi* through faeces, some animals become infected by eating the bugs, or by eating infected animals such as carnivores eating rodents infected with *T. cruzi*. Man can also become infected by eating infected meat (for example inadequately cooked opossums) or food contaminated with excrement of infected mammals.

The infection rates of Triatominae are often exceptionally high. For example, it is not uncommon to find infection rates of about 25 or even 40 per cent or more. Even higher infection rates (78 per cent) have been found in *Triatoma protracta* in California, but because this species very rarely bites man it is not considered a vector to humans.

Another trypanosome, Trypanosoma rangeli, which is apparently non-pathogenic in man, is also transmitted by the Triatominae including T. infestans and T. dimidiata, but the most important vector is Rhodnius prolixus. In the insect vector the trypanosomes undergo dual development, some of the metacyclic infective forms migrate to the hind gut but others penetrate the gut wall, pass across into the haemocoel and then migrate to the salivary glands. Man is infected by both the bug's faeces and bite, but the latter seems to be the most important method of transmission.

A few arboviruses have been recovered from the Triatominae, but they have not been incriminated as important vectors of any diseases other than Chagas'.

## Control

The usual control methods consist of applying residual insecticides to the interior surfaces of walls and roofs of houses. The most common formulation is a water dispersable powder of 1.25 per cent HCH applied at the rate of 0.5 g/m<sup>2</sup>; 2.5 per cent dieldrin at a dosage of 1 g/m<sup>2</sup> is also effective, but careless handling by spray men has resulted in cases of poisoning. DDT has mainly proved ineffective, because of the development of resistance. Alternative insecticides that have shown promise include propoxur (Arprocarb) and malathion. House-spraying will only destroy bugs resting in

houses, it will not kill those resting in natural outdoor shelters and, depending on the area and habits of the species of Triatominae biting man, these outdoor populations may be important in the transmission of Chagas' disease. The spraying of houses with residual insecticides in malaria control campaigns has frequently reduced populations of Triatominae and the incidence of Chagas' disease. Replacement of mud and thatch dilapidated houses with those built of brick or cement blocks and corrugated metal roofs should significantly contribute towards the elimination of domestic bugs. However, because of the high cost of such measures, rehousing has not been carried out on a sufficiently large scale for it to produce any significant impact on transmission, except on a local basis.

## 19 Cockroaches (Order Dictyoptera)

#### Species

There are almost 4000 species of cockroaches. About 50 species have become domestic pests, and the most important medically are Blatella germanica (the German cockroach), Blatta orientalis (the oriental cockroach), Periplaneta americana (the American cockroach) P. australasiae (the Australian cockroach), Supella longipalpa (= S. supellectilium) (the brown-banded cockroach) and Leucophaea maderae (the Madeira cockroach). Cockroaches are sometimes called roaches or steam-bugs.

#### Distribution

World-wide, but especially in the tropics and sub-tropics.

## **Medical** importance

Cockroaches almost certainly aid in the transmission and harbourage of various pathogenic viruses, bacteria, protozoa and helminths. They can be intermediate hosts of certain nematodes, and an acanthocephalid that rarely parasitises man.

## External morphology of cockroaches

#### Adults (figures 19.1)

The following general description refers to the more common household pest species. They are usually chestnut brown or black in colour, about 1.0-5.0 cm long, flattened dorsoventrally and have a smooth, shiny and tough integument. Viewed from above the head appears small, and it is sometimes almost hidden by the large rounded pronotum. A pair of long and prominent filiform antennae arise from the front of the head between the eyes. The cockroach mouthparts are developed for chewing, gnawing and scraping, they are not composed of piercing stylets and therefore cockroaches cannot suck blood. In adults of both sexes there are two pairs of wings (figure 19.1a, b) but in certain household species those of the female may be shorter than those of the male, and in female *Blatta orientalis* they are very small and non-functional. The fore wings are rather leathery and are called tegima; they are not used in flight but serve as protective covers for the hind wings which are membraneous and can be used for flying, but when not in use are folded shut fanlike over the body. Although cockroaches possess wings they rarely fly in temperate climates. There are three pairs of legs which are well developed and covered with prominent small spines and bristles; they terminate in a pair of claws.

The segmented abdomen is more or less oval in shape but is either completely or partly hidden from view, depending on the species, by the folded overlapping wings. In both sexes, a pair of prominent segmented pilose cerci arise from the last abdominal segment, but they are hidden from view in some species by the wings (figure 19.1b). In the males a pair of styles which are unsegmented and thinner than the cerci project from the end of the abdomen between the cerci (figure 19.1a).

Cockroaches are most readily distinguished from beetles (order *Coleoptera*) by having the fore wings placed over the abdomen in a scissor-like manner, in beetles the fore wings (elytra) are not crossed over but meet dorsally to form a distinct line down the centre of the abdomen. Also the elytra of beetles are generally stouter than the tegima of cockroaches.

## Life-cycle

Cockroaches like warmth and during the day they hide away behind radiators, hot-water pipes, and in warm countries where these may be absent, in almost any dark places, such as cesspits, septic tanks, sewers,



Fig 19.1 Adult cockroaches: (a) male Blatta orientalis and (b) Periplaneta americana.

rubbish dumps, refuse tips, dustbins, cupboards, drawers, underneath chairs, tables, sinks, baths and beds, behind refrigerators, cooking stoves, dishes in kitchens, in fact in almost any dark place. They are usually common in kitchen especially if remains of food are left out overnight. They abound in restaurants, hotels, bakeries, breweries, laundries and on ships. Cockroaches are nocturnal in habits and are rarely seen during the day unless they are disturbed from their hiding places, but they become very active at night, crawling over floors, tables and other furniture to seek food. They move very quickly and can be both seen and heard scuttling along when lights are suddenly switched on.

They are omnivorous and voracious feeders, any type of man's food is eaten. They also consume paper, clothes, particularly starched ones, books, hair, shoes, wallpaper, dried blood, sputum, excreta, dead insects, almost any animal or vegetable matter. They have even been recorded gnawing at the fingers and toes of sleeping or comatose people and on vagabonds even infesting the hair. They habitually disgorge partially digested food and deposit their excreta on almost anything, including food. Cockroaches may live for five to ten weeks without water and for many months without food, but in practice this is not an important limiting factor because they very rarely occur in areas where food of some kind is not available. Nymphs may die within about seven to ten days in the absence of food.

Eggs are laid encased in a brown bean-shaped case or capsule called an ootheca, which is composed of two parallel rows of chambers containing the eggs (figure 19.2b). The shape of the ootheca and the number of eggs it contains (12-48) varies according to the species of cockroach. Cockroaches are often seen running around with an ootheca partly protruding from the tip of the abdomen. In some species such as *Blattella* germanica the ootheca remains projecting from the



Fig 19.2 (a) Ootheca protruding from the abdomen of Blattella germanica and (b) a typical cockroach ootheca.

abdomen until just before the nymphs hatch, while in other species it is retained for considerably less time (figure 19.2a). The oothecae are deposited in cracks and crevices in dark and secluded places. In some species they are cemented to surfaces such as the undersides of tables, chairs and beds. Oothecae are often cemented to packing crates and other packaging materials and this can lead to new infestations when such boxes and crates are transported to new premises.

Adult cockroaches live for many months to a year or more, and during this time the female will lay about four to 90 oothecae, the number varying considerably according to species. Cockroaches have a hemimetabolous life-cycle. Nymphs hatch from the eggs after about one to three months, the time depending on both temperature and species. Young nymphs are very pale and delicate versions of the adults, while the older ones are progressively darker and resemble more the adults. The nymphs are wingless, but wings gradually develop with ensuing nymphal instars, but only adults may have fully developed wings. There are usually six nymphal instars, but in Periplaneta americana there may be as many as 12 nymphal instars. The duration of the nymphal stage varies according to temperature, abundance of food and species. For example, the nymphal stage may occupy only about 10-22 weeks in Blattella germanica, 12-18 months in Blatta orientalis, and up to two and a half to three years in Periplaneta americana.

Cockroaches spread very rapidly from infested houses to adjoining ones. They often gain entry by climbing up water pipes and waste pipes. They are also spread as oothecae, nymphs and adults in furniture and other belongings.

## Medical importance

The presence of cockroaches in houses and hotels etc. has for a long time been regarded as highly undesirable because of their dirty habits of feeding indiscriminately on both excreta and foods, and their practice of excreting and regurgitating their partially digested meals over food. Because of these insanitary habits they have been suspected as aiding the transmission of various illnesses. For example, they are known to carry pathogenic viruses such as poliomyelitis, protozoa such as Entamoeba histolytica, Trichomonas hominis, Giardia intestinalis and Balantidium coli, and bacteria such as Escherichia coli, Staphylococcus aureus, Shigella dysenteriae and Salmonella species including S. typhi and S. typhimurium. They are also known to be intermediate hosts of the acanthocephalid Moniliformis moniliformis which is common in rodents and can also infect cats and dogs and very rarely man, and nematodes such as Gongylonema pulchrum, a common parasite of herbivores and occasionally man, and *Enterobius* (=Oxyuris) vermicularis which is an extremely common worm in man. They have been found naturally infected with *Toxoplasma gondi* and suspected of transmitting this parasite to cats and possibly to man by feeding on cat's faeces.

Although there is little doubt that cockroaches contribute to the spread of a number of, mainly intestinal, diseases it is difficult to assess their real importance as vectors because many of the pathogens that cockroaches can transmit are spread in many other different ways.

Some people are allergic to cockroaches. It appears that sensitised people can react to cockroach allergens by eating cockroach contaminated food, or by inhaling their dried faecal pellets.

## Control

Ensuring that neither food nor dirty kitchen utensils are left out overnight will help reduce the number of cockroaches, but if they are present in adjoining or nearby houses, good hygiene in itself will not prevent cockroaches from entering houses.

Insecticidal spraying or dusting of selected sites such as cupboards, wardrobes, kitchen furniture and fixtures, underneath sinks, stoves, refrigerators, and nearby dustbins, is recommended. Unfortunately Blattella germanica is almost universally resistant to most organochlorines and therefore for general purposes it is best to use 0.5 per cent sprays or I - 2 per cent dusts of organophosphate insecticides, such as fenthion (Baytex), malathion, diazinon, chlorpyrifos (Dursban) or dichlorvos (DDVP), or carbamates such as propoxur (Arprocarb) or carbaryl (Sevin). Sprays based on kerosene (paraffin) may leave unsightly stains on walls, and are of course dangerous near naked flames of cookers. The residual efficiency of sprays greatly depends on the surfaces on which they are applied, for example on most painted and shiny surfaces residual activity often lasts only about one to four weeks. The residual action of insecticidal dusts is less affected by the nature of the surface, but dusting is unsightly and therefore objected to by many householders. The addition of pyrethrum or synthetic pyrethroids to insecticidal sprays or aerosols is useful because it irritates cockroaches and flushes them out from their hiding places. Pyrethrum formulations, however, especially dusts, may cause allergic reactions in some people. Synthetic pyrethroids such as resmethrin or bioresmethrin applied as a spray, or more effectively as an aerosol, can produce spectacular results in flushing out and killing cockroaches. The newer pyrethroids, such as permethrin, have the advantage of also being residual insecticides, but as yet they have been little used in cockroach control.

The best control is often obtained by the applications of both sprays and dusts of the same, or different, insecticides. Boric acid powder (borax) still remains a very safe and useful chemical, acting both as a contact insecticide and a stomach poison. Sodium fluoride, although highly poisonous, is sometimes still used in poison baits for cockroach control.

Most organophosphate and carbamate insecticides can be added (I per cent) to baits which are eaten by cockroaches and so cause their death. The addition of glycerol to poison baits provides an additional feeding stimulus.

# 20 Soft ticks (Order Metastigmata: Family Argasidae)

#### Species

There are about 150 species of soft ticks (Argasidae), contained in four genera - Argas, Ornithodoros, Otobius and Antricola, but only the first three contain species that commonly attack man. Species of Antricola occur mainly on bats in the Americas, while species of Argas are principally parasitic on domestic and wild birds and also bats, but one species, Argas persicus, often attacks man. Otobius parasitise a wide variety of domestic and wild animals, a common species being Otobius megnini which sometimes bites man. The medically important soft ticks belong to the genus Ornithodoros and the most important vector species include the Ornithodoros moubata complex, O. erraticus, O. tholozani, O. hermsi, O. talaje, O. turicata, O. rudis (= venezuelensis) and O. verrucosus. Soft ticks are sometimes called tampan or non-scutate ticks.

#### Distribution

Soft ticks have a more or less world-wide distribution, but are especially common in dry areas.

#### Medical importance

Vectors of tick-borne (endemic) relapsing fever caused by Borrelia (= Treponema) duttoni.

### Ornithodoros ticks

## External morphology

#### Adults (figure 20.1)

Adults are flattened dorsoventrally and oval in outline, but the shape varies according to the species. The integument is tough and leathery, wrinkled and usually has fine tubercles, granulations or radially arranged discs or polygonal areas. Depending on the species simple eyes may, or may not, be present along the lateral margins of the body. There is no scutum or dorsal shield as is found in ixodid (hard) ticks. The capitulum or 'false head' is situated ventrally (figure 20. Ib) and so is not visible dorsally, a character which



Fig 20.1 Adult Ornithodoros moubata, (a) dorsal view and (b) ventral view.

separates adult and nymphal soft ticks from hard ticks (Ixodidae) which have the capitulum projecting forward and clearly visible dorsally. The mouthparts (figures 2.17 and 20.2) collectively known as the capitulum, consist of a pair of four-segmented leg-like palps (pedipalps) and a central toothed hypostome on either side of which are the chelicerae which have smooth not denticulate sheaths. The powerful cutting chelicerae have strong teeth at their tips and together with the hypostome penetrate the host during feeding. The palps are sensory and do not enter the skin of the host.



Fig 20.2 Capitulum of an adult Ornithodoros species showing leglike palps and non-denticulate cheliceral sheaths.

The four pairs of well developed legs are each composed of six segments (coxa, trochanter, femur, genu, tibia and tarsus) and terminate in a pair of claws. The anus opens ventrally about one third of the distance from the posterior margin of the body, while the genital opening is found just behind the base of the capitulum. The spiracles (stigmata) are situated in front of the coxae of the hind legs. The coxal organs (glands) open between the bases of the coxae of the first and second pairs of legs (figure 20.1b).

Males and females are very similar in external appearance but the shape of the genital opening differs, and in the male is more conspicuous than that of the female. However, as both sexes feed on blood and can consequently be disease vectors, it is not so important to be able to distinguish between them.

#### Internal anatomy

A brief account of the internal anatomy of a tick is necessary in order to understand the mechanisms of disease transmission.

During feeding, blood from the host passes up the

space formed by the apposition of the ventral hypostome and the dorsal chelicerae into the buccal cavity of the capitulum. The blood-meal then passes into the muscular pharynx, through the narrow oeso-phagus and into the stomach or mid gut which is provided with numerous branching diverticula (figure 20.3). The ramifications of the diverticula enable the adult tick to ingest large volumes of blood (about six to eight times its own weight), resulting in great distension of the tick's body. The hind gut may be absent or be represented by a very thin and rudimentary cord-like structure. A single pair of large and convoluted Malpighian tubules open into a relatively large sacculate rectum (bladder) which leads to the ventrally situated anus.



Fig 20.3 Diagrammatic representation of the internal anatomy of a soft (argasid) tick; adapted and modified from various authors.

During feeding saliva, which often contains powerful anticoagulants, is secreted by a pair of large grapelike salivary glands and flows down the salivary ducts to the pharynx and capitulum. Argasid ticks have a pair of coxal organs, which although sometimes called coxal glands, are not actually glandular but filter off excess fluid and salts from ingested blood-meals. This fluid is passed out through a small opening located between the bases of the coxae of the first two pairs of legs. When a soft tick is infected with tick-borne relapsing fever (*Borrelia duttoni*) many of the spirochaetes in the haemolymph enter the coxal organs and are then passed out through their openings.

Coxal organs are present only in soft ticks, not in hard ticks.

In females of both soft and hard ticks a peculiar

structure termed Gene's organ is located in front of the mid gut and during oviposition is extruded from a small opening above the capitulum. It secretes waxy waterproofing substances that coat the eggs during oviposition and thus enables them to withstand desiccation, immersion in water and other adverse environmental conditions.

## Life-cycle

A blood-meal is essential for maturation of the ovaries and egg laying. After each blood-meal female argasid ticks lay several (often four to six) small egg batches, each of about 15-100 spherical eggs; occasionally fewer but larger egg batches comprising as many as 200-300 eggs are laid. Adult ticks can live for many years, so a female may lay thousands of eggs during her life-time. Eggs are deposited in or near the resting places of the adult ticks, such as in cracks and crevices in the walls, floors and furniture of houses, or in mud, dust and debris, in rodent holes or in the more exposed resting or sleeping places of wild animals and birds.

Eggs hatch usually within one to four weeks, but because they have been coated during oviposition with a protective waxy secretion from Gene's organ (see above) they can remain viable for many months under adverse climatic conditions.

Both argasid and ixodid ticks have a hemimetabolous life-cycle, that is eggs hatch to produce sixlegged larvae which superficially resemble the adults and which moult to produce eight-legged nymphs



Fig 20.4 Larva of an Ornithodoros tick showing the small capitulum projecting anteriorly beyond the body outline.

which resemble even more closely the adults. In argasid ticks the six-legged larva (figure 20.4) is usually very active and searches for a host from which to take a blood-meal. The capitulum projects from the body and is visible from above. Blood-feeding on the host may take several minutes to several hours, but longer in slow feeding Argas larvae. When the larva is fully engorged it drops to the ground and after a few days moults to produce an eight-legged nymph, which seeks out a host and feeds for about 5-30 minutes before it falls to the ground. Argasid ticks have several distinct nymphal instars (figure 20.5) (often four to five but in some species up to eight), each of which requires a blood-meal before it can proceed to the next nymphal instar. Blood-feeding usually occurs in the evenings or during the night.

Larvae of the Ornithodoros moubata complex differ from most other argasid larval ticks in that they do not take blood-meals but remain within their egg shells after hatching, moulting to produce first-instar nymphs which crawl from the egg shells to seek their blood-meals.

The duration of the life-cycle, from egg hatching to adult, depends on the species of tick, temperature and the availability of blood-meals, but is often about 6– 12 months. Adult soft ticks can live for several years, up to 14 years in the laboratory, and they can remain alive for five to seven years after a single blood-meal. In the absence of suitable hosts argasid ticks can also survive long periods of starvation.

The distribution of the larvae, nymphs and adults of argasid ticks is usually patchy and restricted to the homes, nests and resting places of their hosts, but in these places there can be quite large populations of ticks. Species that commonly feed on man, such as some members of the Ornithodoros moubata complex in Africa, are found around human settlements especially in village huts. However, in many parts of Africa these ticks are becoming uncommon. This may be due to changes in life-style, in particular to the increased numbers of people sleeping on beds raised from the floor, which makes it more difficult for the ticks to feed on man.

Because the larvae, all the nymphal instars and adults, take blood but nevertheless remain attached for only relatively short periods, many hosts, comprising both different species and individuals, are fed upon during their life-cycle. Argasid ticks are consequently referred to as 'many-host' or 'multi-host' ticks.

#### O. moubata complex

This complex is found only in Africa and was originally thought to consist of only a single species O. moubata, but there are at least four closely related



Fig 20.5 Diagrammatic representation of the life-cycle of Ornithodoros moubata showing larvae retained in egg shells and four nymphal instars.

species in the complex, differing in their host preferences. O. moubata sensu stricto feeds on man, domestic animals and sometimes wild animals and is mainly found in arid and semi-arid areas; it is the principal vector of tick-borne relapsing fever in Africa. Two species, O. compactus and O. apertus feed on various wild animals and very rarely bite man. The fourth species of the moubata complex is O. porcinus which is divided into two subspecies, O. porcinus porcinus, which feeds mainly on warthogs, and O. porcinus domesticus. This latter subspecies has now been even further subdivided into three races or physiological forms which differ in their host preferences and environmental requirements; two of these races feed on man in villages in East and south-east Africa.

# Identification of medically important genera of argasid ticks

#### Ornithodoros

This genus occurs in North, Central and South America, Asia, Africa, Europe, having an almost world-wide distribution, but appears to be more common in warm countries rather than in temperate ones.

It may or may not have eyes, there is no sutural line separating the ventral and dorsal surfaces as found in *Argas* species, the integumental pattern consists of small tubercles or mammillae which are continuous ventrally and dorsally, and the hypostome is well developed.

#### Argas

This genus has a more or less world-wide distribution. Eyes are absent, and the ticks are distinctly flattened with the edges of the body remaining thin and compressed even when they are fully engorged; the integument is wrinkled and creased. Many species feed on domestic and wild birds. Species of the Argas persicus complex are commonly known as fowl ticks. Another common species attacking pigeons is A. reflexus. Both species occasionally bite man, especially if he is living in close association with chicken pens or sleeping in abandoned chicken houses.

#### Otobius

Otobius species are found in North and South America, India and Africa. Eyes are absent and the hypostome is very small in the adults. Only two species are recognised, the most common being Otobius megnini, the spinose ear tick. This species is covered with small spines and both larvae and nymphs occur in the ears of cattle, horses, sheep, cats, dogs and a variety of wild mammals. Nymphs usually remain attached to the ears of their hosts for about 7–14 days but attachment for up to 121 days has been recorded. This is unusual behaviour for an argasid tick, being more like that of an ixodid tick. Otobius adults do not take blood-meals and are non-parasitic.

The second Otobius species, O. lagophilus, is primarily a parasite of rabbits and hares.

#### Medical importance

#### Tick-borne relapsing fever

Soft ticks can inflict painful bites but they do not cause severe allergies such as tick paralysis as do some hard ticks (see p. 164-5). Tick-borne relapsing fever is the only important disease transmitted to man by soft ticks. This infection occurs throughout most of the tropics, sub-tropics and in many areas of the temperate regions such as North America and Europe, but it is absent from Australia and New Zealand.

The causative agent is Borrelia (= Treponema) duttoni. Some authorities recognise this as a complex of different antigenic forms and have named different spirochaete species as causing relapsing fever in different parts of the world; for example, Borrelia persica spread by Ornithodoros tholozani in Middle Eastern countries and Russia, B. turicatae transmitted in the U.S.A. and Mexico by O. turicata, and B. hermsii transmitted by O. hermsi in North America. In the New World the different forms of duttoni are more or less host specific in the ticks. In the present book, however, all populations and forms of the disease are assigned to a single species *Borrelia duttoni*.

Spirochaetes ingested with a blood-meal multiply in the gut and congregate along the wall of the tick's mid gut and then pass across into the haemocoel where they can be found after 24 hours. In the haemocoel the spirochaetes multiply enormously and invade nearly all tissues and organs of the tick's body; within three days they begin to arrive in the salivary glands, the coxal organs and the central ganglion. In the O. moubata complex the salivary glands in the nymphs appear to be more heavily infected than do those of the adults in which the infection tends to diminish and die out. In contrast the coxal organs of the nymphs are usually only lightly infected whereas those of the adults become heavily infected. When either the immature stages or adults of O. moubata complex feed on man, or some other host, saliva is injected intermittently into the bite and spirochaetes can be introduced by this route, especially by nymphal ticks. During feeding excess body fluids are filtered from the haemocoel by the coxal organs and in infected ticks, especially adults, the coxal fluids contain spirochaetes ingested with a previous blood-meal. These can enter the host through the puncture of the tick's bite or through the intact skin. Man can therefore become infected with B. duttoni by either or both the bite of O. moubata complex and the coxal fluids.

In other Ornithodoros species changes in the level of spirochaete infection with age in the salivary glands and coxal organs does not necessarily follow the same pattern. For example, in all stages of O. tholozani and O. turicata the salivary glands tend to remain heavily infected, whereas spirochaetes are often absent from the coxal organs. Moreover, these two species tend to excrete excess fluids only when they have left their host, and hence transmission by these species is mainly by the bites of the ticks. In no species of Ornithodoros is infection spread by the faeces.

Various wild animals, especially rodents, can be reservoirs of tick-borne relapsing fever but the tick itself is usually regarded as the most important reservoir, especially as there is hereditary (transovarial) transmission. The ovaries of adult female ticks become infected with spirochaetes which are then passed on to the eggs so that the newly hatched larvae, and all nymphal instars and adults of both sexes, are infected. Thus, although the larvae, nymphs and adults may not have fed on a host infected with *B. duttoni* they can nevertheless be infected and transmit the disease to other hosts. This phenomenon is called transovarial transmission and can be continued for some three to four generations.

Another rather similar and associated method of transmission is transstadial transmission. This involves one of the immature stages of a tick becoming infected by biting a host and then passing the infection on to one or more later stages, for example a larva might become infected by feeding on an infected host and pass the spirochaetes to the nymphs and adults, or the infection might start with a nymph and be passed to subsequent nymphal instars and the adults. In all cases transovarial transmission can follow.

The most important vectors of *B. duttoni* include the O. moubata complex (especially O. moubata and O. porcinus domesticus) which is found in much of Africa, O. tholozani which occurs in Asia, O. erraticus in the Mediterranean area and North Africa, O. talaje and O. rudis ( = venezuelensis) in Central and South America and O. hermsi, O. turicata and O. parkeri in North America.

## Q-fever

This is a rickettsial disease caused by *Coxiella burneti*. It has a more or less world-wide distribution and is primarily an infection of rodents and other small mammals and domestic livestock. It can be readily transmitted to people who consume contaminated milk and other foods, and also by the bites of argasid and possibly ixodid ticks. Transovarial transmission occurs.

## Control

Ticks can be removed by pulling them from their host, but this is not always easy, especially with ixodids. It is sometimes recommended that the capitulum is compressed and pushed deeper into the skin to loosen the grip of the teeth of the hypostome, and thus prevent the body being torn off whilst the capitulum remains embedded in the host's tissues. If this happens considerable irritation and secondary infections may result. The glowing end of a cigarette often stimulates a tick to withdraw its mouthparts. Alternatively, ticks can be coated with castor oil or medicinal paraffin, vaseline or nail varnish which prevents respiration through the spiracles (stigmata) and causes them to slowly release their hold so that they can be removed or drop off some hours later. Often the best method for removing ticks is to dab them with chloroform, ether, ethyl acetate, benzene or some other anaesthetic before carefully pulling them off.

Suitable repellents such as dimethyl phthalate, dibutyl phthalate, dimethyl carbate, butyl mesityloxide oxalate (indalone), diethyltoluamide and benzyl benzoate can be used on the skin, or alternatively clothing can be impregnated with these chemicals or ethyl hexanediol, to help prevent tick infestations.

Houses infested with argasid ticks such as Ornithodoros species can be sprayed with oil solutions or emulsions of insecticides such as 5 per cent DDT, 3 per cent malathion, 5 per cent carbaryl (Sevin), 0.5 per cent naled (Dibrom), 0.5 per cent diazinon or 1 per cent propoxur (Arprocarb). Special care should be taken to spray the floors and cracks and crevices in walls and furniture and any other sites where ticks may be resting. In areas where houses have been sprayed with residual insecticides for malaria campaigns there has often been a corresponding reduction in the numbers of Ornithodoros species.

## 21 Hard ticks (Order Metastigmata: Family Ixodidae)

#### Species

There are about 650 species of hard ticks belonging to 11 genera. From the medical point of view the more important genera and species are Ixodes (I. ricinus, I. persulcatus), Dermacentor (D. andersoni, D. variabilis, D. pictus), Amblyomma (A. hebraeum, A. americanum, A. cajennense, A. variegatum), Haemaphysalis (H. concinna, H. spinigera) and Hyalomma (H. marginatum). Ticks of veterinary importance include Boophilus annulatus, B. microplus, Rhipicephalus appendiculatus, R. sanguineus, Haemaphysalis leporispalustris and H. leachi, although these do not usually bite man they may be important reservoirs of various zoonotic diseases.

#### Distribution

Ixodid ticks have a world-wide distribution, but they occur more frequently in temperate regions than soft ticks (Argasidae).

#### Medical importance

Ticks are vectors of Rocky Mountain spotted fever, also called tick-borne typhus (*Rickettsia rickettsii*), Boutonneuse fever (*R. conori*), Q-fever (*Coxiella burneti*), and many arboviruses – including Russian spring-summer encephalitis, tick-borne (Central European) encephalitis, Omsk haemorrhagic fever, Kyasanur Forest disease, Colorado tick fever, and Crimean-Congo haemorrhagic fever. They may also transmit tularaemia (*Pasteurella* (= *Francisella*) tularensis) and causes tick paralysis.

## **Ixodid ticks**

#### **External morphology**

#### Adults (figure 21.1)

Adult hard ticks are flattened dorsoventrally and are

oval in shape, measuring from about 3-23 mm in length depending on species and whether they are unfed or fully engorged with blood. The females are nearly always larger than the males, and because they take larger blood-meals they enlarge much more than males during feeding.

The capitulum or 'false head' projects forwards beyond the body outline and is visible from above (figure 21.1) thus distinguishing adult hard (ixodid) ticks from soft (argasid) ticks (figure 20.1a, b). There are also differences in the shape and structure of the capitulum which serve to separate the two families. For example, in hard ticks the palps (pedipalps) are swollen and club-shaped rather than leg-shaped as in the soft ticks, and the palps of most species of hard ticks are composed of only three apparent segments, the basal one being much smaller than the other segments (figure 21.2). Closer examination of the ventral surface of the palps, however, will reveal a small fourth segment lying in a pit-like depression on the third segment. In the larval and nymphal stages the fourth palpal segment is larger and easier to see. The toothed hypostome is located between a pair of chelicerae, which unlike those of soft ticks have their cheliceral sheaths covered with very small denticles, giving them what is sometimes termed a 'shagreened' appearance. As in argasid ticks both the hypostome and chelicerae penetrate the host during feeding.

The posterior margin of the body in species of the genera *Dermacentor*, *Rhipicephalus*, *Haemaphysalis* has a number of rectangular indentations called 'festoons'. However, in fully engorged females these indentations may be difficult to see due to the body's distension with blood.

All hard ticks have a dorsal plate called a shield or scutum, which is absent in soft ticks. In the males the scutum is large and covers almost the entire dorsal surface of the body (figure 21.1), whereas in the females it is much smaller and is restricted to the anterior part of the body just behind the capitulum (figure 21.1). In engorged females the scutum may be



Fig 21.1 Adults of a male and a female Dermacentor species, showing eyes on margin of scutum, body ornamentation and a small scutum in the female and a large one in the male. Festoons are also present.



Fig 21.2 Capitulum of an adult ixodid tick showing club-shaped palps with very small fourth segment and denticulate cheliceral sheaths.

difficult to see because it appears small in relation to the enlarged body and becomes pushed forwards so that it is almost vertical in position (figure 2.16). The scutum provides a method of immediately recognising hard ticks and also of differentiating between the sexes, although this may not be very important as both sexes bite animals and are therefore potential disease vectors. In the larval and nymphal stages the scutum is small in both sexes. Adult female ixodid ticks can be distinguished from the nymphal stages by having a genital opening near the base of the second pair of legs and a pair of depressions (porose areas) on the dorsal surface at the base of the capitulum. In ixodid ticks eyes may be either absent or one pair present along the edges of the scutum, but they are never near the edges of the body as in argasid ticks.

The body has four pairs of legs which are sixsegmented (coxa, trochanter, femur, genu, tibia and tarsus) and terminate in a pair of claws. Although hard ticks are generally dark or light brown some species have coloured markings on the scutum and body, and sometimes there are shiny patches of colour on the legs; such coloured ticks are referred to as ornate species.

As in soft ticks the anus is located ventrally on the posteror third of the body, while the genital aperture is at about the level of the base of the second or third pair of legs. There are no coxal organs in ixodid ticks. Unlike soft ticks males of some species of hard ticks may have sclerotised plates ventrally, and their number, arrangement and colour may be of taxonomic importance. The spiracular plate in ixodid ticks is well developed and often approximately commashaped.

#### Life-cycle

Both the Ixodidae and Argasidae have hemimetabolous life-cycles, that is there is incomplete metamorphosis involving a larval and nymphal stage. There are, however, important differences between the lifecycles and ecology of ticks in these two families.

Adults of both sexes and also the larval and nymphal stages are haematophagous, and a blood-meal is essential for egg maturation and oviposition. Adult ixodid ticks remain attached to their hosts for long periods as blood-feeding often lasts for one to four weeks. When feeding has finished the enormously engorged tick drops from the host to the ground and seeks shelter under leaves, stones, detritus, amongst surface roots of grasses and shrubs, or buries itself in the surface soil. The time taken for females to digest their blood-meal and commence laying eggs varies according to species and environmental conditions, especially temperature. Sometimes oviposition begins three to six days after the female drops from the host, but egg laying may not start until several weeks, or occasionally months, after the end of feeding. Thousands (often 1000 - 8000) of small spherical eggs are laid in a gelatinous mass which is formed in front and on top of the scutum of the tick (figure 21.3). Some species lay as many as 20000 eggs and the egg mass may become larger than the ovipositing female. Oviposition may last for ten days or extend over about five weeks. As in argasid ticks the eggs are coated with a waxy secretion produced by Gene's organ, which in the case of ixodid ticks also helps to transfer the eggs from the genital opening to the scutum. The ixodid female lays only one batch of eggs, after which she dies.

After two to three weeks to several months six-

legged larvae hatch from the eggs. The larvae are minute, being about 0.5-1.5 mm long, and are sometimes referred to as 'seed ticks'. On cursory examination they superficially resemble larval mites, but the presence of a *toothed* hypostome immediately identifies them as ticks. After emergence the larvae remain inactive for a few days after which they become very active and swarm over the ground and climb up vegetation and cluster at the tips of grasses and leaves. When suitable hosts pass through a tick-infested habitat the larvae respond to stimuli such as host odours, warmth, shadows, vibrations and movements by waving their front legs in the air. This host-seeking behaviour, exhibited by the larvae, and also the nymphs and adults, is called 'questing'. Larvae climb on to their hosts and crawl to their favoured sites for attachment, commonly in the ears or on the eyelids, but the selected site depends on the species of tick and host. The chelicerae and hypostome are inserted deep into the skin of the host and the larvae commence blood-feeding. The larvae remain attached to their hosts for about three to seven days, and then drop to the ground and seek shelter amongst vegetation or under stones. Larvae normally take two to seven days to digest their blood-meals but in cooler weather digestion may extend over several weeks. After all blood has been digested the larvae remain inactive for a few days before they moult to become nymphs.



Fig 21.3 Diagrammatic representation of the life-cycle of a hard (ixodid) tick, showing only one nymphal stage and a female with a very large egg mass.

The newly formed eight-legged nymphs crawl over the ground and climb vegetation and behave similarly to the larvae (that is questing) in seeking out a suitable passing host. They attach themselves to selected sites on the body and begin to feed, and about five to ten days later the fully engorged nymphs drop to the ground and again seek shelter under stones or amongst vegetation. They remain quiescent for about three to four weeks, during which time the blood-meal is digested, and afterwards the nymphs moult to produce male or female adult ixodid ticks. There is only one nymphal stage in the life-cycle of ixodid ticks (figure 21.3), whereas argasid ticks have several nymphal stages.

The newly formed adults remain more or less inactive for about seven days, after which they climb vegetation and start questing for passing hosts. Adult female ixodid ticks take very large blood-meals and remain feeding on their hosts for some one to four weeks. Male ixodid ticks take smaller blood-meals, but in some species they remain on their hosts for considerable periods in order to seek out and copulate with females while these are injesting blood. In other species male ticks remain on their hosts for only short periods, falling to the ground as soon as they have engorged. When female ticks are fully engorged they may increase several times their original size. On the ground they find shelter under stones, surface vegetation and amongst roots of plants.

## Behaviour and habits

Certain species of ixodid tick are more or less host specific, for example Boophilus species feed mainly on cattle, but other species, including many of those of medical importance, are less host specific and feed on a wide variety of mammals. Diversity of host species often increases the likelihood of ticks transmitting diseases amongst their hosts, including man. Larvae and nymphs of many, but not all, ticks seem to have a predilection for small animals such as rodents, cats and dogs, and ground-inhabiting birds, whereas adults seem to prefer to feed on cattle, horses and a variety of large and wild mammals. Humans are parasitised by all life-stages of ixodid ticks, but often less so by adults than by the younger stages. The tick's life-cycle may be prolonged by months or even years by lack of suitable hosts. Some species, for example Ixodes ricinus, have a life-cycle that even under favourable conditions lasts about three years. In temperate regions development may also be prolonged or cease temporarily during winter months, but in warm countries development and breeding may continue throughout the year, but there may be seasonal fluctuations. Adults of some ixodid species may live up to seven years.

Although ticks can tolerate considerable variations in temperature and humidity, most species are absent from both very dry and very wet areas, but certain *Hyalomma* species occur in arid areas and deserts. Humidity at soil level can be an important factor in tick survival, and this may be very different from humidities measured at greater heights. Microclimatic conditions at soil level are greatly influenced by the amount and type of ground vegetation; so that the distribution of various species of ticks can often be closely associated with particular types of vegetation.

Both immature and adult ixodid ticks remain on their hosts much longer than do argasid ticks and may be carried many kilometres by their hosts or even across continents by migrating birds before they drop off. They are therefore not restricted to their host's homes or resting places as are most argasid ticks, but are more widely dispersed.

#### **Three-host ticks**

The life-cycle previously described refers to a threehost tick; that is a different individual host, which may be the same or different species, is parasitised by the larva, nymph and adult, and moulting occurs on the ground. Most ixodid ticks have this type of life-cycle and medically important species of three-host ticks are found in the genera *Ixodes*, *Dermacentor*, *Rhipicephalus* and *Haemaphysalis*. Ticks which feed on three hosts are more likely to become infected with pathogens and be potential vectors of disease than species that feed on one or two hosts.

#### **Two-host ticks**

Some species of ticks, in particular many of those in the genera *Hyalomma* and *Rhipicephalus*, are two-host ticks. After the larva has completed feeding it remains on the host and moults to produce a nymph which then feeds on the same host. The engorged nymph drops off, moults and the resultant adult feeds on a different host.

#### **One-host ticks**

In a few ticks, such as *Boophilus* species, the larva, nymph and adult all feed on the same host and moulting also takes place on that host. The only stage that leaves the host is the blood-engorged female tick which drops to the ground to lay eggs. One-host ticks are less likely to acquire infections with pathogens than ticks which feed on several hosts, and clearly the only method by which infection can be spread from one host to another by these ticks is by transovarial transmission. One-host ticks are of little or no medical importance, but certain species of *Boophilus* are important vectors of several animal diseases including babesiosis such as Texas cattle fever (*Babesia bigemina*) and bovine anaplasmosis (*Anaplasma marginale*).

# Identification of medically important ixodid genera

The following brief descriptive notes provide a means of recognising the principal genera of medically important ticks, and also the genus *Boophilus* which contains ticks of mainly veterinary importance.

#### Ixodes

World-wide distribution.

Eyes absent, anal groove surrounding anus in *front*, festoons absent, palps are conical with second papal segment at least as wide as long, usually small species, scutum not ornate.

#### Hyalomma

Occurs in southern Europe, Middle East, Arabia to India and Bangladesh, and Africa.

Eyes present and submarginal on the scutum, hypostome and chelicerae (mouthparts) much longer than length of basal part of capitulum which is called the *basis capituli*, second palpal segment considerably longer than wide but less than twice as long as segment three, scutum not ornate, if any ornamentation is present then confined to pale bands on legs, festoons absent or if present more or less coalesced, usually large ticks.

#### Amblyomma

Occurs in North, Central and South America, Africa, Europe and Asia.

Eyes present at margins of scutum, hypostome and chelicerae longer than *basis capituli*, second palpal segment much longer than wide and more than twice as long as segment three, scutum usually ornate having coloured pattern, festoons present and not coalesced.

#### Dermacentor

Occurs in North and Central America, Asia, Europe and Africa.

Eyes present, hypostome and chelicerae about as long as *basis capituli*, second palpal segment as long as wide, 11 festoons present, scutum usually ornate, ventral plates absent in both sexes.

#### Rhipicephalus

Species of this genus are widely distributed in the Old World, *R. sanguineus* is more or less cosmopolitan and the only species found in the Americas.

Eyes present, hypostome and chelicerae about as long as *basis capituli*, second palpal segment about as wide as long, festoons present. These ticks are rather similar to those of *Dermacentor* but the scutum is not ornate and a ventral plate is present in the male, although it is absent in the female.

#### Haemaphysalis

This genus is widely distributed in both the Old and New Worlds.

Eyes absent, anal groove not extending anteriorly around anus, palps short and conical, second palpal segment at least as wide as long and projects laterally beyond *basis capituli*. Scutum is not ornate, and festoons are present.

#### Boophilus (mainly of veterinary importance)

Occurs in North, Central and South America, Asia, Africa and Australasia.

Eyes present, hypostome and chelicerae about as long as *basis capituli*, second palpal segment very short, being about as wide as long, festoons always absent, segments of hind leg not greatly swollen or bead-like, no ornamentation.

## Medical importance

Ixodid ticks are vectors of numerous arboviruses to man, other mammals and birds, various rickettsiae, a few bacteria such as those causing tularaemia, and various piroplasmas of veterinary importance. They also cause a condition known as tick paralysis.

#### Tick paralysis

Certain hard ticks, especially Dermacentor andersoni and various species of Ixodes, Hyalomma, Rhipicephalus and Haemaphysalis, can cause a condition in man, farm animals, pets and wild animals called tick paralysis. The symptoms in man are an acute ascending paralysis affecting the legs with the result that the person cannot walk or stand, and has difficulty in speaking, swallowing and breathing due to paralysis of the motor nerves. The symptoms are painless and there is very rarely any rise in the patient's temperature. Tick paralysis can be confused with paralysis due to poliomyelitis and certain other paralytic infections. Young children especially up to the age of two years are most severely affected. Death in animals and in rare cases also in man, can result due to respiratory failure. After ticks have been removed the patient usually makes a full recovery within a few days or weeks.

Tick paralysis is not caused by any pathogens but by various toxins contained in the female tick's saliva which is continually pumped into the host during the long period the tick is feeding on the host. Different species of ticks and also different populations of the same species may vary markedly in their ability to produce tick paralysis in man and animals.

#### Arboviruses

All arboviruses are transmitted by the tick's bite, and transovarial transmission can occur in all tick vectors.

Russian spring-summer encephalitis (RSSE) This is caused by one of several closely related viruses of the RSSE complex which vary in their pathogenicity to man. It is associated with the taiga forests of Russia, Siberia, northern Asia and China. The main vector is Ixodes persulcatus, but in certain areas Haemaphysalis concinna seems to be an important vector. After multiplication in the tick the virus accumulates in the salivary glands, and infection is through the tick's bite. Various small mammals and birds in addition to ticks serve as reservoirs.

Omsk haemorrhagic fever (OHF) This virus produces a disease with symptoms rather similar to those produced by KFD virus; it occurs in south-western Siberia and can cause a serious disease and often death in muskrat handlers. The principal vectors appear to be Dermacentor reticulatus, Ixodes persulcatus and I. apronophorus. These ticks, and also small rodents, appear to serve as the reservoirs of infection. The ecology and epidemiology of this disease remain unclear.

Kyasanur forest disease (KFD) The disease occurs in tropical forests of southern India and is spread by Haemaphysalis species, in particular H. spinigera. Small rodents and other mammals including monkeys, bats and birds may serve as reservoir hosts.

Crimean-Congo haemorrhagic fever (CCHF) Two viruses are included in this group, the most important is Congo virus which occurs in Bulgaria, areas of Russia especially the Crimea, West Pakistan and certain areas of West, Central and East Africa. It is transmitted by ticks of the Hyalomma marginatum complex, other species of Hyalomma and also by Amblyomma, Rhipicephalus and Boophilus species. These ticks occur on a variety of animals, including birds that fly from Russia to Africa, Asia and Western Europe and thus may aid in spreading the disease. The other virus in the group is Hazara which is also spread by ixodid ticks and which occurs in Asia.

Colorado tick fever (CTF) This is a virus disease which occurs in the U.S.A. and Canada and is transmitted to man by Dermacentor andersoni and D. occidentalis, but spread amongst rabbits, hares and squirrels, and other rodents by other species of Dermacentor, and also by Haemaphysalis and the argasid tick Otobius lagophilus. Both rodents and ticks form the reservoirs of the virus.

Tick-borne (Central European) encephalitis (TBE) This virus produces a disease with symptoms very similar to that of RSSE. It occurs in central Europe from Scandinavia to the Balkans. The principal vectors appear to be *Ixodes ricinus* and *Dermacentor marginatus*; various small mammals appear to serve as reservoirs. TBE virus accumulates in the mammary glands of goats and cows, and people usually become infected not by tick bites but by drinking infected milk.

Miscellaneous arboviruses Ixodid ticks spread many other arboviruses, including Powassan encephalitis (POW) virus in North America, Langat (LGT) virus in Malaysia, Kemerovo fever (KEM) virus in Siberia, Quaranfil fever (QRF) in Egypt, and Louping ill (LI) an important virus disease of sheep in Britain which is spread by *Ixodid ricinus* and is occasionally acquired by humans. West Nile (WN) is spread mainly by mosquitoes but both ixodid and argasid ticks may also be responsible for some transmission.

In addition hard ticks may play a more or less incidental role in the transmission of other viruses such as Sindbis (SIN) virus, Japanese (JE), St Louis (SLE), Eastern Equine (EEE) and Western Equine encephalitis (WEE) viruses which are normally transmitted by mosquitoes (chapter 6).

#### Rickettsiae

Rocky Mountain spotted fever (RMSF) This disease is also known as Mexican spotted fever, São Paulo spotted fever, American tick-borne typhus and several other local names. Different strains of the causative organism, Rickettsia rickettsii (a form of the spotted fever group), vary considerably in their virulence. Rocky Mountain spotted fever occurs in North, Central and South America, where it can cause death in man. The principal vectors in North America are Dermacentor andersoni and D. variabilis. Dogs, rabbits and small rodents also become infected and the disease is spread amongst them by various species of ticks belonging to the genera Dermacentor, Haemaphysalis, Amblyomma and Rhipicephalus. Various animals may act as reservoirs of the disease but as they remain infectious for relatively short periods, the main reservoir is the tick, in which the rickettsiae can survive during the winter. In South America Amblyomma species are the main vectors.

There is an incubation period of about 9-12 days before an infected tick becomes infective, and transmission is normally through the bite of any stage in the life-cycle of the tick. An infective tick, however, must remain feeding on a host for at least two hours before infection is passed from it to the host, thus early removal of ticks may help prevent transmission. Transmission can also be through tick faeces and by crushing ticks in the fingers and accidentally rubbing the rickettsiae into the eyes or abrasions. There is transovarial and transstadial transmission.

Siberian tick typhus (STT) This disease is similar to Rocky Mountain spotted fever, and the causative organism, Rickettsia siberica, is antigenically close to R rickettsii. It occurs in Russia, Pacific areas and on the Japanese islands. Vectors include species of Dermacentor, Haemaphysalis, Rhipicephalus and Hyalomma. Infection is by bites and both transstadial and transovarial transmission occur. Ticks appear to be the main reservoir of infection. Certain mammals may also serve as reservoirs, but since infection in these animals is short-lived they are probably not important in maintaining the reservoir of infection.

Boutonneuse fever Also known as fievre boutonneuse, Marseilles fever, South African tick typhus, Kenyan tick typhus, Indian tick typhus, Crimean tick typhus etc. This disease is caused by *R. conori* and the symptoms in man are similar to those caused by Rocky Mountain spotted fever (*R. rickettsii*). It occurs in the Mediterranean region, the Middle East, Crimea, most of India, Southern Asia and Africa. One of the principal vectors is *Rhipicephalus sanguineus*, but it appears that ticks of most ixodid genera can transmit the disease to man. Transmission is by the tick's bite and both transstadial and transovarial transmission occur. Infection can also occur if infected ticks are crushed and the rickettsiae rubbed into abrasions or in the eyes. Both ticks and rodents serve as reservoirs.

Miscellaneous rickettsiae A disease known as Queensland tick typhus (R. australis) is transmitted by Ixodes ticks and occurs in the Queensland area of Australia.

Rickettsia prowazeki, responsible for louse-borne (epidemic) typhus, has been found in ticks (Hyalomma and Amblyomma species) and large domestic animals in Ethiopia. It is possible that under certain conditions ixodid ticks may be involved in the transmission of R. prowazeki to man.

Another rickettsial disease is Q-fever (*Coxiella bur*neti) which has a world-wide distribution and is mainly a disease of rodents and other mammals. It is mainly spread by consuming contaminated milk and meat from cattle and the inhalation of dried infected faeces by those working with cattle. Hard ticks may be able to help maintain an enzootic cycle, and possibly even transmit the disease to man. Transovarial transmission occurs.

A few other non-pathogenic or apparently unimportant rickettsiae have been isolated from ticks. In addition to causing human diseases ixodid ticks are also vectors of several rickettsiae of veterinary importance to animals such as cattle, sheep and dogs.

#### Tularaemia

A bacterial disease caused by Pasteurella (= Francisella) tularensis which occurs in North America, Europe, Japan and Asia and infects mainly rabbits, but other rodents and even birds can be infected. The disease is spread by a variety of direct contact methods such as handling infected live animals, carcasses, drinking contaminated water, eating raw or uncooked meats and also by the bite of various hard ticks. Chrysops discalis is also a vector (p. 93).

## Control

Methods for the removal of argasid ticks described on page 159 are also applicable to the removal of ixodid ticks, which if not removed may remain attached for several days or even weeks. The types of insect repellents described on page 159 can also be used to prevent attachment of ixodid ticks.

Various insecticidal formulations can be applied to domestic pets, such as dogs, to rid them of their ticks. Recommended treatments include solutions of 0.5 per cent malathion, 0.1 per cent dichlorvos (DDVP), 1 per cent carbaryl (Sevin), 0.1 per cent dioxathion, 0.2 per cent naled (Dibrom) and 1.0 per cent coumaphos. Alternatively, dusts of 5 per cent carbaryl or 0.5 per cent coumaphos, 3-5 per cent malathion or 1 per cent trichlorphon can be applied to the coats of pets. Floors of houses, porches, verandahs and other sites where infected pets sleep should be sprayed with oil solutions or emulsions of organochlorine or organophosphate insecticides, such as I per cent propoxur (Arprocarb), 0.5 per cent diazinon, 2 per cent malathion, 5 per cent carbaryl (Sevin), 0.5 per cent chlorpyrifos (Dursban), to kill ticks still attached to hosts as well as those that have dropped off. Ultra-low-volume (ULV) spraying with propoxur at the rate of about 0.5-2 kg/ha may give good tick control for about six weeks.

The dipping of sheep and cattle, and sometimes other domestic livestock, in acaricidal baths, or spraying them with insecticides, is often crucial if ticks and tick-borne diseases of man as well as of livestock are to be effectively controlled.

# 22 Scabies mites (Order Astigmata: Family Sarcoptidae)

#### Species

The scabies or itch mite which occurs on man belongs to the species Sarcoptes scabiei. Similar mites that cannot be reliably separated from S. scabiei are found on numerous wild and domesticated animals and have in the past been given separate names for example Sarcoptes canis, S. equi, S. ovis and S. leonis. They have also been regarded as named varieties; for example the mite on man was called S. scabiei var. hominis and that on dogs S. scabiei var. canis. They are now regarded as different biological forms of S. scabiei which are morphologically indistinguishable, but which nevertheless are physiologically different and host specific. Thus scabies mites on dogs, horses and other animals very rarely infect man.

The family Sarcoptidae also contains mites of two other genera, Notoedres and Knemidokoptes (= Cnemidocoptes). Mites of the former genus can cause mange in cats and also dogs and rodents, while some Knemidokoptes species are parasitic on the legs, or the skin at the base of the feathers, of domestic poultry, and other species cause mange in budgerigars.

Mites belonging to the genus *Psoroptes* (family Psoroptidae) superficially resemble scabies mites, but their tarsal 'suckers' are borne on distinctly segmented pedicels; in addition the legs are longer. There are numerous species of *Psoroptes* infecting a wide range of domestic and wild animals, and they often become established in laboratory animals, especially rabbits.

#### Distribution

S. scabiei has a more or less world-wide distribution.

#### Medical importance

Scabies mites are not vectors of any disease but cause the conditions known in man as scabies, acariasis, Norwegian itch, crusted scabies or seven-year itch: in non-human mammals the condition is known as sarcoptic mange and in birds as scaly leg.

## Sarcoptes scabiei

## External morphology

#### Adults (figures 22.1 and 22.3)

The female mite is just about visible without the aid of a hand lens (0.30-0.45 mm). It is whitish and discshaped, distinctly convex dorsally but more or less flattened ventrally (figure 22.1). Dorsally the mite is covered with numerous small peg-like protuberances



*Fig* 22.1 Dorsal view of an adult mature female of the scabies mite, *Sarcoptes scabiei*.

and a few bristles, and both dorsally and ventrally there are series of lines across the body giving the mite a striated appearance. Adults have four pairs of short and cylindrical legs divided into five apparent ring-like segments. The first two pairs of legs end in short stalks called pedicels which terminate in thin-walled roundish structures often termed 'suckers'. In the females the posterior two pairs of legs do not have 'suckers' but end in long and very conspicuous bristles.

There is no real distinct head, but the short and fat palps and pincer-like chelicerae of the mouthparts protrude anteriorly from the body. The small triangular untoothed hypostome is best seen in ventral view (figure 22.2).



Fig 22.2 The mouthparts of Sarcoptes scabiei.

Adult male scabies mites are only about 0.20-0.25 mm long, and apart from their smaller size may also be distinguished from females by the presence of 'suckers' on the last pairs of legs (figure 22.3).

## Life-cycle

The female scabies mite selects places on the body where the skin is thin and wrinkled, such as between the fingers, wrists, elbows, feet, penis, scrotum, buttocks and axillae. The majority (63 per cent) of mites are found on the hands and wrists and about 11 per cent occur on the elbows. Using the cutting edges on the tibiae of the front pair of legs and the sharp chelicerae of the mouthparts the mite digs and eats her way into the surface layers of the skin - the stratum corneum. She takes about an hour to bury herself. In women mites may often be found burrowing beneath and around the breasts and nipples. In young children whose skin is soft and tender they may be found burrowing on the face and other parts of the body, and often the greatest number of mites on children up to a year old are found on the feet. When the females have burrowed into the superficial layers of the skin they



Fig 22.3 Ventral view of an adult male Sarcoptes scabiei showing 'suckers' on the last pair of legs.

excavate winding tunnels at the rate of about 2-3 mm per day, which are seen on the skin as very thin twisting lines a few mm to several cm long. The mite feeds on liquids oozing from dermal cells she has chewed. During the mite's progress along the tunnel she lays about four to six large eggs (100–150  $\mu$ m) and defaecates daily.

The eggs hatch within three to five days and small six-legged larvae emerge which look like miniature adults. These larvae crawl out of the tunnels on to the surface of the skin where a large number die, but a few succeed in either burrowing into the stratum corneum or enter a hair follicle to produce not a tunnel but a small pocket called a 'moulting pocket'. The larva feeds on fluids seeping from damaged skin cells and grows, but does not extend the pocket into a tunnel, and after two to three days moults in the pocket to produce an eight-legged nymph. It was originally thought that if this nymph was destined to become an adult female mite it moulted again to produce a second-stage nymph, which then moulted to become an adult female. This interpretation of the life-cycle is given in most text books but appears to be erroneous. It seems that a nymph destined to become a female mite

moults to produce not another nymph but a sexually immature female which remains more or less quiescent in the moulting pocket until she is fertilised by a male, after which she enlarges in size to become a mature (ovigerous) female. Mating may be accomplished by the male either burrowing through the surface of the skin into the moulting pocket containing the female, or on the surface of the skin. Only after fertilisation does the female commence to burrow through the skin, and after about four to five days she starts to lay eggs in the tunnel. Female mites very rarely leave their burrows.

The life-cycle from egg-egg takes about 14-31 days. Female scabies mites may live about one to two months on man, away from man they may survive for about seven to ten days under ideal conditions but they usually live only two to four days.

In the life-cycle of the male mite the six-legged larva moults to become a nymph which stays in the moulting pocket until it changes into an adult male, this stage being reached four to six days after the eggs have hatched. Adult males, which are about half the size of mature female mites, can be found in either very short burrows (usually less than 1 mm) or in small pockets in the skin, but they probably spend most of their life wandering around on the surface of the skin seeking females awaiting to be fertilised.

The principal methods by which scabies mites are transferred from one individual to another are not properly understood. Some authorities believe that it is mainly the larvae that spread the infection to a new host while others consider it is principally either the immature or ovigerous females that spread from person to person. Scabies is a contagious complaint which is transmitted only by close contact; it is therefore a family disease spreading amongst those living in close association, especially when they sleep together in the same bed. It can be spread amongst courting couples who are habitually holding hands. It appears that the actual transfer of mites from person to person takes about 15-20 minutes of close contact. The incidence of scabies often increases during wars and disasters, such as earthquakes, floods and famines when people are sleeping and living in very overcrowded situations. It is also possible to get infected by sleeping in a bed formerly occupied by an infected person, but experimental work has indicated that this very rarely happens. It is therefore not usually worthwhile fumigating or sterilising clothing or bedclothes to prevent scabies spreading, but in epidemics or cases of Norwegian or crusted scabies resulting from the use of immunosuppressive drugs such measures may be needed. Ten minutes at 50°C will kill the mites, alternatively clothing and bedding can be kept unused for about four days which usually results in their death. Laundering will also kill any mites on clothing.

#### Other forms of scabies

Many wild and domesticated animals have scabies mites and are said to be suffering from mange or sarcoptic mange. The mites are morphologically indistinguishable from those on man but are distinct physiological forms that are adapted to their own particular hosts. Whereas few scabies mites are found on man (an average of 11 mature female mites) hundreds or even thousands of Sarcoptes may occur on domestic animals. Because of this, people working in close contact with animals, such as veterinary scientists, may become infected with a few Sarcoptes originating from domestic animals. As another example people constantly riding horses sometimes become infected with mites normally found on these animals, and this has led to a condition known as 'cavalryman's itch'. Scarcoptes mites from animals seem poorly adapted to life on man; they do not usually make burrows in the skin, and an infection often lasts only a few days or weeks before it dies out.

# Detection and identification of scabies infections

Scabies in man can be diagnosed by detection of the female mite's thin twisting tunnels, which are easier to see on fair-skinned than dark-skinned people. The faeces deposited in the tunnels may be visible through the skin and appear as pepper-like spots. However, for those with less experience in scabies detection a more reliable procedure is to remove and identify the mites. The best procedure is to wear a watchmaker's eye glass (that is a magnifying glass) which leaves both hands free to search the skin, especially between the fingers and wrists, for tunnels having a slight dilatation at the end which indicates the presence of the female mite. The surface layers of the skin at the end of the tunnel should be gently scratched away with sharp dissecting needles and the mites, which usually readily adhere to the points of the needles, removed. They can be transferred alive to a dry microscope slide or placed in a water-immiscible mountant and examined under a × 50 magnification. The average number of adult female scabies mites found on a person is about 11; most patients have one to 15 mites, only about 3 per cent have more than 50 mites.

#### The scabies rash

This is a follicular papular eruption that occurs mainly on areas of the body not infected with burrowing mites, such as the buttocks and around the waist and the shoulders, but the rash can also occur on other parts
of the body such as the arms, calves and ankles. It does not appear on the head, centre of the chest or back, nor on the palms of the hands or soles of the feet. The rash is produced in response to the patient being sensitised, that is the rash is an allergic reaction produced by the mites. Frequently a patient is unaware he is harbouring mites until a rash appears.

When a person is infected for the first time with itch mites the rash does not appear until about four to six weeks later, but in individuals who have previously been infected a rash may develop within a few days after reinfection. The rash may persist for several weeks after all scabies mites have been destroyed. The severe puritis which soon develops results in vigorous and constant scratching, especially at night, and this frequently leads to the development of secondary bacterial infections. These may be quite severe leading to boils, pustules, ecthyma, eczema and impetigo contagiosa. These complications tend to mask the nature of the complaint and as a consequence correct diagnosis of scabies may not be made. The seriousness of the symptoms is not always directly related to the number of mites, and severe reactions may be found on people harbouring few mites.

The condition in Europe known as Norwegian or crusted scabies is rare but highly contagious due to the vast numbers of mites in the exfoliating scales. It is characterised by the formation of thick keralatic crusts over the hands and feet, scaling eruptions on other parts of the body, and usually large numbers of mites, but a much less pronounced degree of itching. At one time it was erroneously thought that this form of scabies was due to a distinct variety of mite called S. scabiei crustosea. It is not clear why the condition develops, but it may be due to a loss of immunity in man which allows the establishment of enormous numbers of mites. This hypothesis is supported by the finding that the development of Norwegian scabies has sometimes been associated with the extensive use of corticosteroids. This reduces irritation and consequently reduces the patient's scratching, an act which helps in the removal and destruction of some mites.

#### **Treatment of scabies**

All cases of scabies can be cured, there are no resistant infections. Methods aimed at killing the mites will do little to immediately alleviate the nuisance and irritation caused by the rash, although this will eventually disappear. Separate medical treatment, however, may be necessary especially if secondary infections have become established. In the past a common procedure was to give the patient a hot bath and vigorous scrubbing with a brush until he bled, but this is not very effective at either removing or killing the mites. However, as many, but certainly not all, patients with scabies are dirty, an ordinary bath before treatment may be advisable for general hygienic reasons. However, if large numbers of patients suffering from scabies are to be treated, such as in epidemic situations, bathing may not be practical.

Numerous organic and inorganic compounds including benzyl benzoate and sulphur preparations such as 'Mitigal' and 'Tetmosol' have been used to treat scabies.

A 20-25 per cent benzyl benzoate emulsion can be painted on a patient from the neck downwards and after allowing some 5-10 minutes for this application to dry the patient can redress. A single efficient treatment should in most cases result in a complete eradication of the mites but a repeat treatment on the third day may be advisable. Only in rare cases does dermatitis result from the use of benzyl benzoate and this is more likely to happen in young children.

Mitigal is a yellowish oily liquid sulphur preparation (2, 7-dimethylthianthrene) which is painted undiluted over the body from the neck downwards. A single treatment should be 100 per cent effective. A mild form of dermatitis may be produced in some people. Tetmosol (tetraethylthiuram monosulphide) is another sulphur compound sometimes used to treat scabies. It is slow in its action and usually about three treatments 24 hours apart are recommended for a complete cure, and is therefore of limited use in mass treatments. It has been combined with soap and sold as Tetmosol soap and in this form when regularly used in washing and bathing it has a slow curative effect and also acts as a prophylactic.

Although these two sulphur preparations and benzyl benzoate are still used, a better treatment for scabies consists of applying a I per cent HCH cream or lotion to the body, but this is not always available in sufficient quantities to treat large numbers of people. Although, as with benzyl benzoate and Mitigal, a single treatment has a high success rate a second application two to seven days later, if this is possible, ensures a complete cure.

With a highly contagious condition like scabies it is important to treat all members of a family, or community living in close association, not just the individual with a particularly bad infestation of mites, otherwise reinfestation will soon occur.

Scabies is often commoner in communities with inadequate water supply and poor standards of hygiene than in those having piped water and thus plenty available for washing.

### 23 Scrub typhus mites (Order Prostigmata: Family Trombiculidae)

#### Species

There are several hundred species of trombiculid mites belonging to several genera (Eutrombicula, Neotrombicula, Ascoschoengastia etc.), but from the medical point of view the most important are species of the Leptotrombidium deliense group, the closely related L. akamushi and L. fletcheri (all formerly placed in the genus Trombicula). Larvae of trombiculid mites are often called red bugs or chiggers. This latter word, however, may lead to some confusion, especially in pronunciation, as it can be confused with jiggers a name often given to fleas of the species Tunga penetrans which invade the feet of man (chapter 15). In Europe larvae of Neotrombiculum autumnalis, although not disease vectors, often attack man and are frequently known as harvest mites.

#### Distribution

The Trombiculidae have a more or less world-wide distribution in temperate and tropical regions, but the medically important species, such as the L. deliense group, L. akamushi and L. fletcheri are found only in Asia. L. akamushi occurs only in certain areas of Japan; references to this species outside Japan such as in China, Taiwan, much of Indonesia to New Guinea and the Philippines refer to L. fletcheri. There has been considerable confusion over the identity of these two species and in many previous papers and books the two have incorrectly been regarded as synonymous. Both species are particularly common in cultivated land inundated by floods in the spring and summer. The L. deliense group has a wider distribution and is more associated with the forests of Pakistan, India, Burma, Indonesia, Malaysia, Philippines, New Guinea and northern Australia.

#### Medical importance

Certain species are vectors of scrub typhus (Rickettsia

tsutsugamushi) in parts of Asiatic Russia, India, Sri Lanka to south-east Asia, Burma, China, Korea, Japan, Taiwan, New Guinea and northern Australia etc. Other trombiculid mites in many parts of the world cause itching and a form of dermatitis in man, known as scrub-itch or trombidiosis.

#### Leptotrombidium species

#### External morphology

#### Adults and nymphs (figure 23.1)

Adults are small mites (1.0-2.0 mm), usually reddish and covered dorsally and ventrally with numerous feathered hairs giving them a velvety appearance. There are four pairs of seven-segmented legs ending in paired claws. The body is distinctly constricted between the third and fourth pairs of legs giving it an outline resembling a figure eight. The palps and mouthparts project in front of the body and are clearly visible from above.

The nymph resembles the adult but is smaller (0.5–1.0 mm) and the body is less densely covered with feathered hairs.

Neither the adults nor nymphs are of direct medical importance; they do not bite man or animals but feed on small arthropods and their eggs. It is only the larvae that are parasitic and hence responsible for the spread of diseases.

#### Larvae (figure 23.2)

Larvae are very small (0.15-0.3 mm), but after engorging they may increase six-fold in size, they are usually reddish or orange but may be pale yellow or straw coloured. There are three pairs of six or sevensegmented legs which terminate in a pair of relatively large claws, and both legs and body are covered with



Fig 23.1 Dorsal view of an adult trombiculid mite of the genus Leptotrombidium.

fine feathered hairs. The five-segmented palps and blade-like chelicerae are large and conspicuous, giving the larvae the appearance of having a false head. Dorsally and on the anterior part of the body there is a rectangular or pentagonal shaped scutum bearing three to six setae, but because the scutum is weakly sclerotised it is often difficult to see under the microscope, unless the light is correctly aligned. More easily detected are a pair of eyes on either side of the scutum.



Fig 23.2 Dorsal view of a larval trombiculid mite of the genus Leptotrombidium.

In medically important species there are five feathered setae on the scutum and in addition a pair of specialised feathered and flagelliform hairs known as sensillae which arise from distinct bases (pseudostigmatic organs). The combination of a body covered with feathered hairs, seven-segmented legs, five scutal hairs, a pair of flagelliform sensillae and large pigmented eyes distinguishes larvae of *Leptotrombidium* from larvae of other mite genera.

#### Life-cycle

Adult trombiculid mites are not parasitic but live in the soil feeding on a variety of small soil inhabiting arthropods and their eggs. A female mite lays one to five spherical eggs (0.2 mm diameter) each day on the surface of damp soil or under leaves. About 30–100 eggs a month are deposited. In hot climates egg laying continues unmterrupted for a year or more but in cooler areas of south–east Asia, including Japan, oviposition apparently ceases during the cooler months of the year, and adults enter into partial or complete hibernation.

After about five to seven days the egg shell splits; the six-legged larva does not emerge but remains within the egg shell and is called the deutovum. After about five to seven days the larva emerges from the egg shell and usually becomes very active and swarms over the ground and climbs up grasses and other low-lying vegetation. Larvae attach themselves to birds or mammals, including man, walking through infested vegetation. When the larval mites have climbed on to a suitable host they congregate where the skin is soft and moist, such as the ears, genitalia and around the anus. On man larvae seek out areas where clothing is tight against the skin, such as around the waist or the ankles.

Larvae pierce the host's skin with their powerful chelicerae and inject saliva into the wound which causes disintegration of the cells. Larvae do not normally suck up blood but lymph and other fluid and semi-digested materials. The repeated injection of saliva into the wound produces a skin reaction in the host and the formation of a peculiar tube-like structure which extends vertically downwards in the host's skin, and is known as the stylostome, hypostome or histiosiphon. Some trombiculid mites remain attached to their hosts for as long as about one month, but the *Leptotrombidium* vectors of scrub typhus remain on man for only about two to ten days. The engorged larva drops to the ground and buries itself just below the surface of the soil or underneath debris.

The larva having concealed itself becomes quiescent

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and this stage is known as the protonymph, nymphochrysalis or nymphophane stage. After seven to ten days the nymphochrysalis moults to produce an eightlegged nymph, which is reddish, about 0.5-1.0 mm long and covered with feathered hairs. The nymphs are not parasitic, but feed on soil-inhabiting arthropods and their eggs. After a period of some days to about two weeks the nymph ceases feeding and becomes inactive, and is called a preadult, imagochrysalis or teleiophane stage, which after about another 14 days moults to give rise to an adult. The adults, described on page 171, resemble the nymphs but are larger, and like them are free-living and feed on small soil animals.

The life-cycle usually takes about two to three months but may be as long as eight to ten months. The stages in the life-cycle can be summarised as follows: - egg - deutovum - larva - nympho-chrysalis - nymph - imagochrysalis - adult.

#### Ecology

The free-living nymphs and adults of Leptotrombidium have specialised ecological requirements, for example habitats must contain sufficient numbers of suitable arthropod fauna to serve as food for the nymphs and adults. The habitat must also be one in which host animals such as rodents regularly traverse so that larvae will have opportunities of attaching themselves to their hosts. Wild rats of the genus Rattus, subgenus Rattus, are very important hosts of Leptotrombidium larvae. In Japan, and possibly elsewhere, small rodents such as species of Apodemus and Microtus and other subgenera of Rattus are also important hosts. Domestic rats play little or no part in the ecology or epidemiology of scrub typhus. In addition to hosts such as these which maintain the mite population in an area, other more or less incidental hosts may be important in aiding the dispersal of larvae to other areas.

Relatively small changes in moisture content of the soil, temperature and humidity can be crucial, as they may cause adults to bury deeper into the soil and cease egg-laying. Habitats favouring the survival and development of the nymphs and adults are therefore in a very delicate ecological balance, and frequently only very small areas of ground, often just a few square metres, prove to be suitable habitats. This can result in a very patchy distribution of *Leptotrombidium* mites over small areas, but in some situations habitats may comprise several square kilometres. Areas suitable for mite survival and development are often called 'mite islands', a term which emphasises their frequent isolation from other ecologically suitable areas.

#### Medical importance

#### Nuisance

Several species of Trombiculidae attack man in temperate and tropical regions of the world and although not responsible for transmitting any disease, they can nevertheless cause intense itching and irritation, commonly referred to as 'harvest-bug itch', 'autumnal itch' or 'scrub itch'. Irritation is due to the host's sensitisation with the mite's saliva, and is usually most intense 12-24 hours after attachment of the mites. In a previously sensitised individual, however, irritation becomes established more quickly; in some individuals a high degree of immunity develops. Larval mites commonly attack the legs. If they are forcibly removed, their mouthparts frequently remain imbedded in the skin and this may promote further irritation or inflammation and sepsis. People usually become infested with these mites after walking through long grass or scrub vegetation. In Africa, however, there appear to be few instances of man being attacked by larval trombiculids.

#### Scrub typhus

The causative organism is Rickettsia tsutsugamushi ( = orientalis) and the disease is commonly known as scrub typhus, mite-borne typhus, rural typhus, Japanese river fever or tsutsugamushi disease. The disease is restricted to Asia and occurs over a large area, extending from the Primorye regions of Russia, India, Sri Lanka, Burma, Korea, Malaysia, south-east Asia, China, Taiwan, Japan, Philippines and New Guinea to northern Australia and neighbouring south-west Pacific islands south to about the tropic of Capricorn. Although most cases are reported from low-lying areas, infections occur up to a height of 1000 m in many areas, and even up to about 3200 m in Taiwan and 3500 m in the Himalayas. In India, Leptotrombidium mites have been found at elevations of 2700 m. During the Second World War (1939-1945) the incidence of scrub typhus in troops in the Asiatic-Pacific areas was second only to malaria.

Scrub typhus has often been regarded as a zoonosis, but although *R. tsutsugamushi* occurs in forest and rural rodents and *Tupaia* (tree-shrews) it appears that these animals have a very minor role, if any, in the maintenance of scrub typhus. Experimentally it has been shown that it is very difficult to infect trombiculid larvae by feeding them on infected rodents, and even if they do become infected the rickettsiae are not transovarially transmitted to their progeny. It appears that larvae infected by feeding on man can pass the infection to their own progeny by transovarial transmission, and subsequently to man and rodents, but it is very rare for an infection to be acquired from a rodent and then passed to other rodents or man. Leptotrombidium mites themselves are the main reservoirs of infection.

Man becomes infected following the bite of infected larval trombiculid mites, especially by Leptotrombidium akamushi and the L. deliense group of species, but also by bites from larvae of L. fletcheri, L. arenicola, L. pallidum, L. scutellare and L. pavlovskyi. People usually get bitten when they visit or work in areas having socalled mite islands, that is patches of vegetation harbouring large numbers of host-seeking larvae. These mite islands may be at the edge of the forest or bush or on cleared and cultivated land that harbours rodents and is also suitable for the survival and development of the mites. The disease is often associated with 'fringe habitats', in other words habitats separating two major vegetation zones such as forests and plantations, because these areas are often heavily populated with rodent hosts. The risk of scrub typhus transmission is often related to the number of areas of different types of vegetation, that is habitat diversity.

One of the more important ecological factors in the spread of scrub typhus is the 'slash and burn' agriculture or 'shifting cultivation' that is widely practised in many parts of India and south—east Asia. This consists of cutting down forests and burning vegetation to provide land for cultivation. After a few harvests the cleared land is usually abandoned and soon becomes covered with shrubs, grasses and young tree saplings, and this vegetative cover combined with a few remaining crops provide ideal habitats for the proliferation of rats of the subgenus *Rattus*, which in turn facilitate increased populations of trombiculid mites.

All known foci of scrub typhus are characterised by natural or man-made changes in environmental conditions. The very close association between (I) *Leptotrombidium* mites, (2) wild rodents such as *Rattus* (*Rattus*), (3) transitional secondary vegetation, for example, grasses, shrubs and saplings, and (4) *R. tsutsugamushi* has been described as a 'zoonotic tetrad of chigger-borne rickettsiosis'.

Because larval mites attach themselves to only a single host during their life-cycle the disease cannot be spread by larvae feeding on an infected host (for example man) then another. The infection acquired by mites feeding on hosts with rickettsiae is passed on to the free-living nymphal stages and then to the freeliving adults. When the female lays her eggs they are infected with rickettsiae and this infection is passed on to the emerging larvae. So, although they have not previously fed on man they are already infected and consequently transmit the disease to their hosts (man or rodents) when they feed for the first and only time. This inherited type of transmission is called transovarial transmission and can be maintained for several mite generations before the rickettsiae are reduced in numbers and finally disappear. It is possible that on rare occasions larval mites may become detached before they are fully engorged and consequently feed on another host, thus conceivably in some instances scrub typhus could be transmitted in this way.

#### Control

The application to the body of suitable insect repellents such as dimethyl phthalate, diethyltoluamide, dibutyl phthalate, ethyl hexanediol and benzyl benzoate may be of help in reducing the likelihood of people getting infected with mites. Clothing can also be impregnated with suitable repellents.

If mite islands can be identified then it may be possible to remove the scrub vegetation mechanically or by herbicides and so ensure that the habitat is no longer suitable for the survival of the mites. This, however, frequently is not possible, especially if mites are inhabiting cultivated land where ground vegetation consists mainly of crops. Spraying areas known or suspected of harbouring mites with residual insecticides such as DDT, HCH, dieldrin, fenthion (Baytex), malathion, propoxur (Arprocarb), toxaphene (camphechlor), diazinon or other insecticides, preferably as fogs or emulsions, can do much to reduce the mite population. Insecticidal sprays can be applied from knapsack sprayers, or from equipment mounted on vehicles or aircraft which generates insecticidal aerosols or fogs. Ultra-low-volume spraying has also been used in some areas.

24 Miscellaneous mites of minor medical importance (Order Prostigmata: Families Demodicidae and Pyemotidae: Order Astigmata: Families Acaridae, and Pyroglyphidae: Order Mesostigmata: Family Dermanyssidae)

#### Family Demodicidae

#### Demodex folliculorum (figure 24.1)

There are several aberrant species of mites within the genus *Demodex* some of which cause severe forms of mange in animals, but only one species, *D. folliculorum*, the hair follicle mite, infects man. The mite is extremely small (0.3-0.4 mm), has a striated abdomen and is remarkably non-mite like. It looks rather like a segmented worm, but the thorax has four pairs of very short and fat five-segmented legs. It cannot be confused with any other arthropod infecting man.

Demodex are found in the hair follicles and sebaceous glands of man where they feed on subcutaneous secretions, especially sebum. They are particularly common on the nose, eyelids and cheeks adjacent to the nose. They have also been found in ear wax and in the extruded contents of comedones ('black-heads'). Females lay eggs within the hair follicles and these hatch to produce six-legged larvae which moult to give rise to nymphs and finally adults. All the developmental stages, which extend over 13-15 days, occur within the hair follicles or sebaceous glands. Little is known about their biology but apparently a high proportion of adults, especially women, unknowingly have these mites. They are seldom found on children or adolescents.

Normally they do not appear to produce any adverse effects, although possibly they may sometimes cause dermatitis, such as acne, rosacea, impetigo contagiosa or demodes blepharites which affects the eyelids. Daily washing with soap and water can reduce infections. In severe infections resulting in dermatitis 'Danish ointment', which contains a compound polysulphide can be used but this should not be applied to



Fig 24.1 Ventral view of Demodex folliculorum, the hair follicle mite, showing the four small and stumpy legs.

the eyelids otherwise irritation may occur. Alternative treatment consists of applying 0.5 per cent selenium sulphide cream, 10 per cent sulphur or 5 per cent balsam of Peru, which contains benzyl benzoate.

#### Family Pyemotidae

#### Pyemotes ( = Pediculoides) ventricosus (figure 24.2)

This mite, commonly known as the grain itch or straw itch mite, can cause dermatitis and is the only species in the family of any real medical importance.

The adult mite is about 0.2-0.3 mm in length, white or yellowish and readily identified by the following combination of characters:- absence of any prominent projecting mouthparts, a transverse line dividing the body into two main parts and the last two pairs of legs separated by a considerable distance from the first two pairs of legs. In the female the presence of a pair of club-shaped setae inserted dorsally near the edge of the body between the first and second pair of legs serves to identify *P. ventricosus* (figure 24.2a). Males are shorter (0.16 mm) than non-gravid females but they have broader bodies and do not have club-shaped thoracic setae.

An unusual feature of the life-cycle is that the female is viviparous. The posterior part of the opisthosoma (abdomen) of a gravid female becomes enormously enlarged and sac-like resulting in the female becoming  $1-2 \text{ mm} \log 200-300 \text{ eggs}$  are produced and hatched within the female and the progeny are retained in the abdomen until they become sexually mature



Fig 24.2 Pyemotes ventricosus, (a) an adult female and (b) a female that has given birth to sexually mature males which are congregating around the genital orifice to fertilise the females as soon as they are born.

adults. Males emerge first (fig. 24.2b) and congregate around the genital orifice of the female and fertilise the young females as soon as they emerge from the parent. The life-cycle from the production of eggs to the emergence of adults takes about 7–10 days.

These mites are not normally parasitic on vertebrates but parasitise the larvae of a variety of insects many of which infest grain, straw, hay, cotton etc. People continually handling these infested materials may develop allergic dermatitis, sometimes known as straw, hay or grain itch, due to the presence of mites on the larval insects and /or their bites. The symptoms in man can be quite severe and include intense itching and irritation, wheals, headache, fever and even vomiting. Prolonged scratching can give rise to secondary infections. Those most likely to be affected with this type of dermatitis are workers unloading cargoes of grain and other materials, farm workers, and also people sleeping on straw mattresses. Preventive treatment consists of applying 5 per cent betanaphthol ointment, 5 per cent carbolic acid or proprietary sulphur ointments to parts of the body likely to become infected.

#### Family Acaridae ( = Tyroglyphidae)

#### Tyrophagus and Acarus species etc. (figure 24.3)

Mites belonging to several genera (*Tyrophagus, Acarus, Glycyphagus, Carpoglyphus* etc.) are often collectively known as grocer's itch mites. They may cause dermatitis and bronchial asthma in man in both temperate and tropical regions of the world. The mites are oval in outline, about 0.4-0.5 mm long, whitish or pale yellow in colour and have the body divided into two parts by a transverse suture. In this latter respect they resemble *Pyemotes* mites, but differ in that the chelicerae are large and prominent and the setae on the body are considerably longer and more conspicuous (figure 24.3) and the females do not have club-shaped thoracic setae as do female *Pyemotes* mites.

There is a complicated life history, often involving the moulting of the nymph to a hypopus, a stage in the life-cycle which lacks any mouthparts, but which with its stumpy legs attaches itself to insects for dispersal. In some species the hypopus encysts to withstand desiccation. It is not considered necessary to describe further details of the life-cycle of these mites, as it has little relevance to their medical importance.

Acarid mites are non-parasitic and the different species feed on a variety of materials including flour, rice, cheese, roots, bulbs, dried fruits, copra and vanilla pods. Persons habitually handling these materials may develop an allergic dermatitis if they are infested with these mites. The resultant dermatitis is called miller's,



Fig 24.3 A species of Tyrophagus as an example of an acarid mite.

baker's, grocer's or copra itch or vanilla worker's rash etc. depending on the material being handled. It is possible that some people exhibiting classical symptoms of hay fever such as sneezing, wheezing, rhinitis, inflamed and running eyes, may be reacting against the presence of *Glycyphagus* mites in hay.

#### Family Pyroglyphidae

# Dermatophagoides pteronyssinus and other species (figure 24.4)

Some species of Dermatophagoides (= Mealia) are found wandering on or burrowing in the skin of birds and mammals including man, and there may be persistent (seven years or more) infestations on the scalp. Other species are more common in fish meal and animal foods, in the nests of birds, in resting places of animals, amongst bed clothes and mattresses, carpets and in general house dusts, thus giving rise to their popular name of house-dust mites. They are exceedingly small mites (0.3 mm) and are very rarely seen although the allergic symptoms they and their faeces produce may be quite common. Relatively little is known about their biology, except that they feed on



Fig 24.4 Ventral view of Dermatophagoides pteronyssinus, one of the house-dust mites.

skin detritus, scurf and other organic debris and have five life-stages, – egg, larva, protonymph, tritonymph and adult, spanning about 20–30 days. Medically important species such as *D. pteronyssinus* and *D. farinae* have been reported from Europe, the U.S.A., south– east Asia, Japan etc. They can produce allergic reactions in man such as asthma and rhinitis, but until comparatively recently their importance in causing such allergies has been largely overlooked.

#### Family Dermanyssidae

### Ornithonyssus and Liponyssoides species (figure 24.5)

The genus Ornithonyssus was previously placed in the family Laelaptidae but is now regarded as belonging to the Dermanyssidae; it contains mites that can cause dermatitis in man in both temperate and tropical areas. The principal species of Dermanyssidae that give rise to dermatitis in man include Ornithonyssus (= Liponyssus) bacoti (tropical rat mite), O. bursa (tropical fowl mite), O. sylviarum (northern fowl mite), Dermanyssus gallinae (red poultry or chicken mite) and Liponyssoides (= Allodermanyssus) sanguineus (house-mouse mite). In addition a few Ornithonyssus species are vectors of certain rickettsiae, including flea-borne endemic typhus (Rickettsia mooseri), amongst domestic rodents. Liponyssoides sanguineus is suspected of aiding the transmission of rickettsial pox (R. akari).

A description of a tropical dermanyssid mite of medical importance such as O. bacoti is as follows. Unfed female adults are about 0.8-1 mm long, oval in outline and pale yellow or straw coloured, but bloodengorged individuals are 1.2-1.4 mm in length and are bright to dark red depending on the stage of blood digestion. Males are about 0.6-0.8 mm long. The nine-segmented legs and body are covered dorsally and ventrally with numerous, fine, short and simple hairs. Dorsally the body has a weakly sclerotised dorsal shield (scutum) which bears numerous setae (figure 24.5b). Ventrally there are several sclerotised small shields, their number and the number of setae they have differs according to the species (figure 24.5a). Careful examination shows that there is a spiracle near the lateral margin of each side of the body between the coxae of the third and fourth pairs of legs. Each spiracle opens into a narrow delicate tube-like structure, termed the peritreme, which extends forwards along the lateral margin of the body as far as the base of the coxae of the first pair of legs. In Ornithonyssus mites the mouthparts project conspicuously forwards in front of the body and consist of a pair of narrow five-segmented palps and a pair of slender chelicerae which are used to pierce the skin of their host.

Female Ornithonyssus lay minute oval white eggs which are most commonly found amongst debris and litter of the nests or resting places of their hosts. They hatch within one to two days to produce six-legged larvae which do not feed but moult within one to two days to give rise to the first-stage nymph (protonymph) which seeks out a suitable host and takes a blood-meal and then drops to the ground. After blood digestion has been completed the protonymph moults to produce the second-stage nymph (deutonymph), which does not take a blood-meal but moults after one to two days to become an adult male or female mite. Both sexes take blood-meals. The life-cycle from eggadult takes about 8-16 days, the duration depending on both species and temperature. Adult mites can live for about two to three months and can withstand considerable periods of starvation.

People working with poultry or in close association with animals infected with dermanyssid mites are the most likely to become infected. Bites from the mites can cause considerable irritation and sometimes dermatitis.

Ornithonyssus bacoti occurs throughout the tropics but in temperate areas it is mainly restricted to sea ports. Although it has not been incriminated as transmitting any disease to man it is a minor vector in the maintenance of endemic flea-borne typhus (*R. mooseri*) in rat populations.

Liponyssoides ( = Allodermanyssus) sanguineus occurs in North America, Europe, Africa and Asia. It is



Fig 24.5 Ornithonyssus bacoti, (a) ventral view showing three sclerotised plates, spiracles and peritremes, and (b) dorsal view weakly sclerotised single dorsal plate (scutum).

superficially like Ornithonyssus bacoti and has a similar life-cycle but has two (not one) sclerotised dorsal plates. It also differs in that the deutonymph as well as the protonymph takes a blood-meal. The life-cycle occupies 15-20 days. It normally feeds on the housemouse but will readily attack rats, other rodents and man. There is circumstantial evidence that *L. sanguineus* can transmit *Rickettsia akari* (rickettsial pox) to man, but this is probably a relatively rare occurrence. The mite is also a possible vector of Q-fever (*Coxiella burneti*), but as there are so many other routes of transmission for this organism, many of which do not involve arthropod vectors, it is unlikely that this is an important method of transmission. Transmission of both R. akari and C. burneti is by the bites of the nymphs or adults; transovarial transmission also occurs.

#### Other mites

There are numerous other mites that either suck the blood or other body fluids, or burrow into the skin, of wild and domesticated mammals and birds, and others that infest a variety of stored products or are pests of fruit trees and other crops. Several of these mites occasionally occur on man causing either dermatitis or irritations due to their bites.

### 25 Miscellaneous poisonous, venomous and allergic arthropods

The principal arthropods transmitting diseases to man have been described in the preceding chapters, but there remains a miscellaneous assortment of stinging, biting and venomous arthropods that may cause discomfort or affect man's health. Although these arthropods are of relative minor medical importance a brief account of them is presented here.

#### **Biting arthropods**

Blood-sucking arthropods may produce severe local inflammation and irritation. It might be expected that bites from insects with large and clumsy mouthparts such as Tabanidae would produce much more severe reactions in their hosts than insects with small and delicate mouthparts such as Culicoides and phlebotomine sandflies, but this is not always so. In many people, for example, bites from phlebotomine sandflies produce an intense burning sensation, like a redhot needle piercing the skin, a reaction out of proportion to the size of the insects. Simulium bites can also be especially troublesome because of the presence of toxic chemicals in their saliva. Toxic substances in the saliva of various ticks, especially species of Ixodes in Africa and Australia and Amblyomma and Dermacentor in North America, are responsible for the condition known as tick paralysis (chapter 21).

Some people are more attractive to biting insects than others and consequently receive more bites, but they may possess a high degree of immunity so that they produce little reaction, and are therefore largely unaware they have been bitten. In contrast other people may receive relatively few bites, but because of their intense allergic reactions they are more aware of being bitten. Complaints from people being bitten are therefore usually related not only to the number of bites they have received but also to the severity of the reactions.

There are often two types of host reaction, an

immediate one which results in weals being produced within minutes at the site of the bite but which usually disappear within an hour, and a delayed reaction producing a papular eruption which occurs some hours or days later. A person not previously bitten by a particular insect may show little immediate or delayed reactions after the first few bites, but thereafter due to sensitisation bites may produce firstly a delayed and then an immediate reaction. If the person is continually exposed to bites he may develop immunity and show very little delayed reaction and finally little or no immediate reaction. Immunity developed following exposure to the bites of one species of insect sometimes confers immunity to bites from other insects, but in other instances immunity is more or less species specific.

In addition to bites from blood-sucking insects, people are sometimes bitten by other types of insects. For example, various predacious reduviid bugs and aquatic insects (for example Belastomatidae and Notonectidae) of the order Hemiptera which normally prey on invertebrates and small vertebrates, and certain phytophagous Hemiptera which suck plant juices, may very occasionally bite man and cause irritation. Very small insects, commonly called thrips, belonging to the order Thysanoptera and which are plant-feeders and pests of cereal crops occasionally bite man, especially if he is working with crops and cereals heavily infested with them.

#### Stinging insects (Hymenoptera)

Stinging insects, which comprise the bees, wasps and ants of the order Hymenoptera, usually sting man only as a defensive reaction. The stinging apparatus is a modified ovipositor. The sting of most bees has barbs and after it is inserted it takes some time before the bee can extract it, but before this can be accomplished the victim usually brushes the insect aside which result in the stinging apparatus being torn from the bee and remaining in the body. Wasps and ants, however, have unbarbed stings which enables them to withdraw their apparatus quickly after stinging and escape. The severity of the reaction and pain resulting from bee and wasp stings depends in part on the size of the insect, the numbers of insects stinging, the species or even subspecies, the individual's sensitivity, and the site of stinging. Stings on the skin are usually followed by oedema, redness, local irritation, pain and in very rare cases necrosis, but stings on the tongue, fauces, and inside the mouth may cause much more severe symptoms including respiratory obstruction and asphyxia. Bee stings can cause neurotoxic, haemorrhagic and haemolytic damage. A few people are extremely sensitive to bee stings and may become very ill, and on very rare occasions die, after a single sting. In a normal healthy person some 400-500 stings are probably needed to cause death, although this is considerably fewer for younger children and babies. Man rarely gets stung by such large numbers of bees or wasps, although, at least in honey bees (Apis mellifera), there are 'alarm odours' which are produced by a bee when it stings and which incite other bees in the colony, or nearby, to sting. In Europe and the U.S.A. there are more deaths from anaphylaxis following bee and wasp stings than from all other venomous bites and stings combined. Tropical African honey bees are particularly aggressive, and following their introduction to South America to boost the honey industry they have hybridised with local honey bees and in some areas have produced an even more vicious bee. In Brazil, however, the hybrids appear to be more docile.

The small stingless bees of the genus *Trigona* are greatly attracted to sweat and can be annoying in African savanna areas by alighting in considerable numbers on the face and getting into the eyes and ears. (They are sometimes mistaken for *Simulium* flies (chapter 7.)

Not all ants possess stings but many do, and large numbers may attack man simultaneously giving rise to considerable discomfort. Some ants do not sting but cause pain and irritation by their bites, while others spray formic acid from the tips of their abdomens, which causes inflammation and irritation especially if it comes in contact with skin abrasions or punctures left by the ant's bite.

# Insects with urticating hairs (Lepidoptera)

Caterpillars (larvae) of several species of moths and butterflies (Order Lepidoptera) possess urticating hairs. These hairs are often hollow and in many species contain toxins secreted by poison gland cells situated at their bases. When they come into contact with the skin they cause irritation due to their toxins stimulating the release of histamine. Irritation may also be produced by a physical effect, rather similar to that which can be caused by fine glass fibres. If urticating hairs get into the eyes irritation and inflammation can be severe and blindness has occasionally resulted. Inhalation may also cause asthma and other respiratory conditions.

Caterpillars of several species of moths live gregariously amongst vegetation and when such vegetation is being cleared clouds of urticating hairs from their cast off skins are released into the air and cause irritation, conjunctivitis and respiratory problems.

A few species of moths frequent the eyes of mammals, including very occasionally man, and feed upon eye discharges. Although they do not penetrate the skin they cause irritation to the eyes, and may act as mechanical vectors of conjunctivitis. At least one species of moth which occurs in Asia, however, has been found to penetrate the skin of man and other mammals.

#### Blister or vesicant beetles (Coleoptera)

Several species of beetles (Coleoptera) contain chemical compounds such as cantharidin or pederin which are vesicating agents. Consequently when either dead or live beetles come into contact with the skin they produce intense local irritation which results in fluidfilled blisters on the skin (that is vesication). Fluid from these blisters may produce secondary blisters. If the beetles or the fluids from their crushed bodies come into contact with the eyes serious conjunctivitis and inflammation results. There are several different species of vesicating beetle but the most notorious is Lytta vesicatoria, often called Spanish-fly, a misnomer because it is a beetle not a fly. Small black and orange coloured beetles of the genus Paederus can also be troublesome, especially if they get into the eyes. Vesicating beetles are often attracted to light and consequently have a tendency to land on people who are sitting under lights in houses or on verandahs.

#### Venomous spiders (Araneae)

Spiders kill their prey, usually insects but occasionally small vertebrates, by biting and introducing venom through their chelicerae, very few spiders possess a sufficiently poisonous venom to harm people. Large hairy spiders (15-19 cm across the spread of the legs)belonging to several families and genera but commonly called 'tarantulas' are found in certain areas of Europe, North America and most tropical countries, and although they may look dangerous very few produce severe poisonous symptoms in man. The few that can inflict painful bites usually cause only localised swelling and reactions, not general poisoning.

The much smaller black or brown widow spiders of the genus Latrodectus can be more dangerous to man than the larger and more alarming looking tarantulas. Latrodectus species are found throughout most of the tropics, subtropics and in some areas of the temperate regions; there are about six closely related species which are venomous to man. The name black widow spider is commonly used to describe any spider in the genus Latrodectus, but in the U.S.A. it is usually referable to L. mactans, which in the females has a red hour-glass shaped marking ventrally on the abdomen. The actual bite of the black widow spider is not normally very painful and may pass unnoticed, but afterwards three red spots and some local swelling and oedema may occur. About 15 minutes to about three hours after the neurotoxic poison has been injected severe and generalised symptoms of poisoning become apparent. Clinical symptoms include intense muscular pains, especially over the abdomen, sweating, nausea, tightness across the chest and difficulty in breathing and sometimes also in speaking. These symptoms usually disappear within one to two days, but may take up to a week. When death occurs this is due to asphyxia caused by respiratory paralysis.

Spiders are not aggressive but bite as a defensive mechanism, and people usually get bitten when they accidently disturb places where spiders are resting or have spun their webs. Black widow spiders frequently shelter amongst vegetation, under logs, on fences, in farm buildings, in temporary discarded shoes and clothing and on the underside of lavatory seats. In fact a high proportion of those bitten by black widows are bitten on the buttocks and in the genital regions.

In North, Central and South America bites from spiders belonging to the genus *Loxosceles*, produce a condition known as necrotic arachnidism, which involves gangrenous shedding of the skin around the site of the bite and localised necrosis; death may occur. Other dangerous spiders belong to the genera *Phoneutria*, *Atrax* and *Mastophera*.

#### **Scorpions (Scorpiones)**

There are some 350 different species of scorpions occurring throughout most of the subtropics and tropics, and in some areas they are clinically more important than snakes. The venom is contained in two glands situated in a bulbous caudal vesicle near the tip of the tail which terminates in a curved spine-like 'stinger'. If given the opportunity scorpions sting their prey or victims several times. There is no simple relationship between the size of scorpions and their venomousness. Some, but not all, big scorpions are relatively harmless, their sting producing only localised swelling and a burning pain, whereas many of the smaller species (for example 2-4 cm long) belonging to the family Buthidae are more dangerous. Scorpion venom contains neurotoxins and produces generalised poison symptoms, including rapid breathing, profuse sweating, excessive salivation, choking sensations, nausea, vomiting and convulsions. Death is usually caused by cardiac failure or cessation of breathing due to respiratory paralysis. Drop for drop the poison of Androctonus australis of North Africa is more toxic than cobra venom. The most dangerous scorpions occur in North Africa (Buthus, Androctonus, Buthacus, Leiurus spp.), South Africa (Parabuthus spp.), Middle East (Buthus, Leiurus, Androctonus spp.), Trinidad (Tityus spp.), Brazil (Tityus spp.), Mexico (Centruroides spp.) and Arizona in the U.S.A. (Centruroides spp.).

Fatality rates from dangerous scorpions depend on the scorpion species, age of the individual that has been stung and whether there have been repeated stings or only a single sting.

#### **Centipedes** (Chilopoda)

Centipedes have long segmented bodies, are more or less flattened dorsoventrally, and have one pair of legs per segment, which may amount to 15 to more than 100 pairs of legs. They are predacious and kill their small prey by a pair of poison claws (maxillipeds) which are situated ventrally to the mouth and are connected to large poison glands. A few species are capable of biting man and causing local swelling and pain, the severity of which is similar to that caused by bee or wasp stings. Bites from centipedes are not serious and apparently the only authenticated death from a centipede bite involved a seven-year old child in the Philippines.

#### Millipedes (Diplopoda)

Millipedes are long, cylindrical, segmented animals with all but the first four pairs of apparent segments possessing two pairs of legs. They are not predacious but are vegetarian and do not possess any sting or poison fangs. One group (*Chilognatha*), however, has repugnatorial glands from which oozes an irritating fluid. In some species this can be squirted from pores placed laterally along the body with considerable force and up to 82 cm. On contact with the skin this fluid causes localised irritation and swelling, and if it reaches the eyes or mouth severe inflammation can result. Children often suffer due to them either sucking their fingers or rubbing their eyes after playing with millipedes. Usually, however, millipedes are of very minor medical importance.

#### Entomophobia

Phobias exist to most things including insects. Many

people dislike insects and spiders but in a few individuals there is an almost pathological abhorrence to them. Moreover, this phobia often involves not real insects but imaginary ones thought to be infesting houses, beds or crawling over the body. Those with such phobias often continually scratch and will try ingenious methods to kill and get rid of these imaginary, but to them very real, tormenting pests. Such conditions can lead to nervous disorders and hallucinations and may require medical and psychiatric treatment.

# 26 Methods for dissecting arthropod vectors to determine infection rates

In entomological surveys after man-biting insects have been collected and identified it is often important to determine whether they are disease vectors; for example, whether anopheline mosquitoes caught feeding on man are vectors of malaria or filariasis, and if so the infective proportion.

Most practical procedures are best learnt by demonstrations and practice consequently the following descriptions of methods for dissecting vectors can only serve as a guide. Frequently a number of different techniques have been devised to dissect medically important arthropods, the following methods are those that have been found most convenient by the author or his colleagues.

Useful dissecting instruments include one or two pairs of finely pointed forceps (for example watchmaker's tweezers), small scapel blades, a pair of fine spring-type scissors such as used by eye surgeons, dissecting needles consisting of entomological pins or micropins screwed or stuck into suitable holders, and if possible a pair of stainless steel, spear-shaped Shute's needles (figure 26.2). Most dissections can be perform-



Fig 26.1 A simple arrangement of a lens, mirror and glass stage which is suitable for the dissection of insects for the removal of the guts and salivary glands.

ed under a very simple dissecting microscope consisting of a  $\times 10$  or  $\times 15$  lens, a glass stage and a mirror (figure 26.1), although it may be easier, especially for beginners, to use a more sophisticated dissecting microscope. A compound microscope having eye pieces (oculars) of about  $\times 10$  and objectives of  $\times 10$  $\times 40$  is needed, an oil immersion objective of about  $\times 100$  is useful but not essential. Normal physiological saline (8.5 g sodium chlorine/litre) is required and Giemsa or Leishman stains are useful for some dissections.

#### **Dissecting techniques**

#### Malarial infections in anopheline mosquitoes

The female mosquito is killed with chloroform or some other anaesthetic and the legs and wings pulled or cut from the body which is placed on a clean microscope slide.

#### Stomach dissections for oocysts (figure 26.2)

A small drop of normal saline is placed on the slide around the abdomen of the mosquito, and under a  $\times$  10 or  $\times$  15 lens the integument of the abdomen is cut with dissecting needles or Shute's needles on either side of about the penultimate segment. One needle is held flat across the thorax to hold the mosquito in place while the other needle, or a pair of forceps, is used to gently but firmly pull away the partially severed tip of the abdomen. In so doing the hind gut and mid gut, and sometimes the fore gut, are withdrawn on to the slide (figure 26.2). If the intestine breaks before the mid gut is fully extracted fine needles can be used to cut along the abdominal integument and the mid gut exposed and withdrawn. When the gut has been dissected out a cut is made immediately in front of the



Fig 26.2 Female anopheline mosquito with legs and wings removed and with the abdomen placed in a drop of normal saline on a microscope slide. Spear-shaped Shute's needles are used to withdraw the gut from the mosquito.

Malpighian tubules and the hind gut discarded, a similar cut is made to separate the fore gut. Other parts of the mosquito are removed from the slide and a cover glass is placed on the mid gut which is then examined under a  $\times$  10 objective. If the mosquito is infected with oocysts they are seen on the stomach wall as circular granulated cysts (50–60  $\mu$ m), confirmation can be made under a  $\times$  40 objective.

## Salivary gland dissection for sporozoites (figure 26.3)

The mosquito with wings and legs removed is placed on a slide with a small drop of saline placed around the head which is cleanly cut off near the thorax. The dissection is done under a  $\times 10$  or  $\times 15$  lens and it is essential that illumination by transmitted light (daylight will suffice) is adjusted so that the glands show up clearly against the background.

A needle, preferably a Shute's needle, is held in the left hand and placed flat across the thorax. Very gentle pressure is exerted which causes the two trilobed salivary glands to pop out from the end of the neck, accompanied by fat globules, some muscle fibre and other tissues (figure 26.3). The needle which is held flat across the thorax should not pierce it or be pressed down too hard otherwise much tissue will be squeezed out and it will then be difficult to locate the glands. When the glands have been located they are freed from the debris and pulled to the edge of the drop of saline and the corner of a small (about  $8 \times 8$  or  $5 \times 5$  mm) square cover glass is placed over them. The glands are best located under the  $\times$  10 objective of a compound microscope, and after ensuring there is adequate saline on the slide they are ruptured by pressing down on the cover slip with a dissecting needle. The ruptured glands are then examined under a  $\times$  40 objective and if the mosquito is infective the small slightly sickle-shaped motile sporozoites (about 15 µm long) are easily seen.

It is not essential to remove both salivary glands, nor all lobes of each gland, but removal of both glands and all lobes increases the likelihood of detecting sporozoites, especially if the glands are only slightly infected.

Stomach and salivary gland dissections can be made on the same female *Anopheles* mosquito, and it is usually more convenient to dissect out the glands before the stomach is removed. Separate slides should be used for each.

#### Dissection of mosquitoes for filarial parasites

After the legs and wings have been removed the female mosquito is placed in a drop of normal saline on a microscope slide, and under a  $\times 10$  or  $\times 15$  lens clean cuts are made to separate the head, thorax and abdomen. The severed head is transferred to a separate drop of saline and held in position with a dissecting needle while another fine needle is used to separate the gutter-shaped labium from the other parts of the proboscis. If infective filarial worms (about 2 mm long) are present they will emerge from the labium into the saline.



Fig 26.3 Method for cutting off the head from a female anopheline mosquito and gently pressing out the salivary glands into a drop of normal saline on a microscope slide.

The remainder of the head, and also the thorax and abdomen, are transferred to fresh drops of saline and carefully teased apart under the high power of a dissecting microscope and examined for immature filarial worms.

Careful examination and staining (Giemsa) of the filarial worms may be necessary to determine whether they are those of *Wuchereria bancrofti*, *Brugia malayi* or *B. timori*, or filarial worms of animals and therefore not of medical importance.

#### Dissection of Simulium, Chrysops and Culicoides for filarial parasites

Female Simulium species are dissected for the presence of filarial worms of Onchocerca volvulus, Chrysops species for filarial worms of Loa loa and Culicoides species for worms of Dipetalonema perstans and D. streptocerca.

The dissecting techniques are similar to those described above for the detection of filarial worms in mosquitoes, but the location of infective worms may differ. For example, most infective filarial worms in Simulium and Culicoides species are found in the head and proboscis, whereas infective stages of *Loa loa* are commonly found in the abdomen and thorax as well as in the head and proboscis, thus all parts of the body should be examined.

The presence of filarial worms in any of these vectors does not necessarily indicate that the fly is infected with nematodes infective to man because they may be parasites of non-human hosts.

### Dissection of phlebotomine sandflies for leishmanial parasites

Dissections are often made in normal saline but this may interfere with subsequent staining, and some workers prefer to dissect sandflies in 5 per cent glucose (or serum). The freshly killed insect is placed in a drop of saline or glucose on a microscope slide and examined under a  $\times 15$  lens. If the abdomen contains mature eggs the terminal segments are cut off and the abdomen gently stroked towards the tip with a dissecting needle to squeeze out the eggs which are discarded. A fine dissecting needle is placed across the thorax and the head cut off, then the gut is extracted through the posterior end of the abdomen. If the dissection has been done in saline this should be removed from the slide with filter paper before the gut is ruptured and stained with Giemsa or Leishman and examined under a ×40 objective for leptomonad forms of leishmanial parasites. Unfortunately the presence of leptomonads does not necessarily indicate that a sandfly is infected with leishmaniasis, as many non-pathogenic *Leishmania*-like protozoa may be present in sandflies. Because of this difficulty it is usual to triturate the sandfly gut and inoculate this into susceptible animals such as hamsters to see whether they develop leishmanial parasites.

In addition to dissecting out the gut the head of the sandfly should also be dissected for *Leishmania* parasites in the pharynx.

## Dissection of tsetse flies for trypanosomes (figure 26.4)

After removal of the wings and legs the tsetse fly is placed dorsal side uppermost on a microscope slide in a relatively large drop of normal saline, but if trypanosomes found in the glands are to be stained it is better to use 5 per cent glucose solution. Salivary gland dissections can be performed without a lens or under a magnification of  $\times$  5 or  $\times$  10. The thorax is gently gripped with a pair of forceps and another pair firmly grasp the head which is pulled forwards and results in breaking the neck of the tsetse. The head is then carefully pulled further away and the fine thread-like salivary glands are withdrawn from the thorax and abdomen (figure 26.4). If they break during their extraction they can often be gripped with fine entomological forceps and pulled from the tsetse's body. Sometimes, however, when they break they spring back into the body and can only be obtained by dissecting the abdomen under a  $\times$  10 lens. Cuts are

made with a sharp scapel blade near both lateral margins of the anterior abdominal segments and the dorsal tergites removed with forceps to expose the mid gut. Gentle probing on either side of the gut should locate the salivary glands which can be withdrawn through the opening made in the abdomen.

The extracted salivary glands are placed in saline and examined under a  $\times$  40 objective. If they are infected it is often possible to observe trypanosomes emerging from their cut anterior ends, but if they are not seen the glands should be crushed under a cover glass and re-examined.

The presence of trypanosomes in the salivary glands means that the fly contains infective forms of either Trypanosoma brucei brucei (of veterinary but not medical importance), or T. brucei gambiense or T. brucei rhodesiense the causative organisms of human sleeping sickness. It is impossible to differentiate between infective forms of these three trypanosomes in the salivary glands. It may, however, be possible to differentiate between brucei brucei and brucei rhodesiense infections by a Blood-Incubation-Infectivity-Test (BIIT). This involves inoculating salivary gland trypanosomes into a rat and then after allowing time for multiplication bleeding the rat and incubating the trypanosomes in vitro in human blood. Finally, this culture is inoculated into another rat and if the infection develops in the rat it suggests that parasites are brucei rhodesiense.

The absence of trypanosomes in the salivary glands shows that the tsetse fly is not infective to man, at least at the time of examination, but it may be carrying infective forms of animal trypanosomes such as *T. vivax*, *T.* uniforme, *T.* congolense or *T.* simiae. These are located in other parts of the body, and to detect infections with these trypanosomes the gut and proboscis have to be dissected and examined.



Fig 26.4 Technique of using two pairs of entomological forceps for pulling the head from a tsetse fly to withdraw the thin thread-like salivary glands.

#### Dissections of reduviid bugs for trypanosomes

After the wings and legs have been removed the insect is placed on a slide and the tip of the abdomen surrounded by a large drop of saline, or 5 per cent glucose if trypanosomes are to be stained. Dissections can be performed without magnification or under a  $\times$  5 lens. The lateral margins of the penultimate segment of the abdomen are cut and the thorax gripped with forceps while with another pair the tip of the abdomen is pulled away to withdraw the intestines. The hind gut is isolated and transferred to a fresh drop of saline and cut into several pieces, covered with a cover glass and examined for trypanosomes under a × 40 objective. The presence of trypanosomes is indicative of either Trypanosoma cruzi, the causative agent of South American trypanosomiasis, or T. rangeli, a non-pathogenic parasite of man. The metacyclic infective forms of T. rangeli will also be present in the salivary glands of the triatomine bugs, whereas T. cruzi is restricted to the hind gut.

A much simpler method that is often effective is to place the bug on a microscope slide and roll a pencil over the ventral surface of the abdomen to squeeze out the gut contents on to the slide. The extruded fluids are then examined for the presence of trypanosomes. Care should be taken that handling of the insects or their tissues does not result in the infection of the person undertaking the dissection.

### Detection of pathogenic bacteria, rickettisae and spirochaetes

Theoretically it is possible to dissect arthropod vectors such as lice, fleas and ticks and make stained smears of appropriate organs and tissues and examine them for bacteria, rickettsiae and spirochaetes, but in practice this procedure is generally unreliable and of limited application. Moreover, it is very difficult to identify some pathogens by examination of arthropod tissues, for example Yersinia (= Pasteurella) pestis in the alimentary canal of fleas is difficult to diagnose with certainty. Identification of these types of pathogens in arthropods can be made by macerating potential vectors (for example body lice, fleas, ticks) under sterile conditions in normal saline and inoculating them into susceptible laboratory animals. The development of the pathogens in experimental animals indicates that the arthropods concerned were infected. The final proof, however, that they are vectors is obtained by allowing the arthropods to feed on clean hosts and determining whether they become infected.

#### Detection of arboviruses in arthropods

It is impossible to detect arboviruses in mosquitoes, ticks and other arthropods by direct examination. Arbovirus detection and isolation is achieved by inoculating susceptible animals, such as baby mice, young chickens or monkeys with macerated extracts of the vectors and later bleeding these animals to detect the presence of arbovisuses. Isolations of arboviruses are not performed in entomological laboratories but in specially equipped virology centres.

Arthropods collected for subsequent arbovirus isolations should not be anaesthetised with ether because many arboviruses are ether sensitive and will be killed. Potential vectors can be killed by placing them in a deep freeze (about  $-20^{\circ}$ C), after which they should be sorted and counted while still frozen. It is usual to group the arthropods together in pools (batches) of 25, 50 or more, and if possible each pool should consist of a single species, but this may not always be practical. After pooling the arthropods should be stored in liquid nitrogen (about  $-196^{\circ}$ C) until they can be macerated for virus isolation studies.

#### Dissections made for identification of bloodmeals

Serological tests, such as the precipitin test, can be made on the blood contents of arthropod's stomachs to determine upon which animal species they have fed. This is a valuable technique as it is important in epidemiological studies to determine not only what species feed on man but also what proportion of the population do so, which may vary according to locality, habitat, season and availability of man and alternative hosts.

Blood-engorged arthropods required for bloodmeal analyses can be collected from houses, animal shelters, rodent burrows and from a variety of outdoor resting sites. The abdomen of blood-fed mosquitoes, simuliids and smaller insects can be squashed on to as small an area as possible of filter paper (no. I grade if possible). With larger insects such as tsetse flies and triatomine bugs it is better to dissect out the mid gut and to squash this rather than the entire abdomen. Blood-meals from 8-16 vectors can be smeared separately along the periphery of 9 cm diameter filter paper. Each smear should be uniquely numbered and the number entered on to a record sheet with relevant data such as arthropod species, date of collection, locality and habitat from which it was collected.

Filter papers smeared with the blood contents of vectors should be interleafed with non-absorbent paper, such as flimsy typing paper, and stored in a desiccator over phosphorus pentoxide or silica gel until they can be tested; if this is not possible they should be stored in a refrigerator or deep freeze. In hot climates it may be advisable to combine both procedures. Batches of filter paper together with all relevant data can be posted air mail to appropriate laboratories specialising in the identification of blood-meals from vectors.

# 27 Methods for collecting and preserving medically important arthropods

Many different methods have been devised for collecting medically important insects, some of which are very simple while others are more complicated and make use of ingenious traps. Certain methods can be used to collect a wide variety of insects while others are designed specifically to collect insects of a particular kind. It is not the intention in this chapter to describe in detail the numerous collecting methods used by specialists, but to provide simple guidelines so that the non-specialist can collect medically important arthropods and forward them to medical entomologists and other specialists for identification.

#### General considerations

#### Killing agents

Numerous chemicals are used to kill arthropods, including potassium or sodium cyanide but these are dangerous and are not recommended. Chloroform, ether, ethyl acetate, ethylene tetrachloride and carbon tetrachloride etc. are useful killing agents. They should not be allowed to wet insects because this may damage certain taxonomic characters needed for identification.

#### **Preserving fluids**

Eggs and larvae of many arthropods and some adults can be both killed and kept by placing them in preserving fluids. The most easily obtained all-purpose preserving fluid is 70-80 per cent ethyl alcohol (methyl alcohol may be used or even industrial methylated spirits). About 3 per cent glycerol (glycerine) can be added to the alcohol to prevent the insects drying out if the alcohol evaporates, but in some insects this may obscure certain taxonomic characters. An alternative preservative is 7-10 per cent formalin (formaldehyde), but this tends to make insects hard and brittle and thus easily damaged. If these preservatives are not available specimens can be temporarily preserved in undiluted spirits such as whisky, gin or brandy (alcohol content being about 40 per cent) or in petrol (gasoline) or kerosene (paraffin), and transferred to 70 per cent alcohol or 10 per cent formalin as soon as it is available.

Sometimes individuals belonging to species complexes such as Anopheles gambiae, An. farauti and Simulium damnosum, can only be reliably identified to species by examination of the banding patterns on the giant polytene chromosomes of their larval salivary glands, or sometimes of the ovaries of half-gravid females. Specimens requiring cytotaxonomic identifications must be placed in a modified Carnoy's solution which consists of equal parts 95 per cent ethyl alcohol and 45 per cent acetic acid. Best results are obtained if the Carnoy's solution is freshly made before use. Preserved material should be kept in a refrigerator, but not a deep freeze.

After specimens have been placed with preserving fluid in small glass or plastic tubes (for example  $40 \times 10$  mm,  $50 \times 12.5$  mm), a tight fitting plug of cotton wool should be pushed down to confine them to the bottom of the specimen tube and prevent damage by shaking. Specimen tubes fitted with plastic or metal screw-on or snap-on tops are the best. If, however, corks, rubber bungs or cotton wool plugs are used as stoppers, the tubes should be placed in a large suitable container such as a 'Mason' or 'Kilner' jar, or a screw-cap food container which is filled with 70 or 80 per cent alcohol or 10 per cent formalin to prevent the specimens drying out due to poorly fitting stoppers (figure 27.1).

#### Pinning adult insects (figures 27.2 and 27.3)

After having been killed with chloroform or some other suitable anaesthetic adults of most large or medium sized flies, triatomine bugs, beetles, wasps, bees etc. can have an entomological pin pushed down



Fig 27.1 Tubes of fluid-preserved specimens placed in a large glass container filled with alcohol.

through the dorsal surface of the thorax just lateral to the mid-line (figure 27.2a, b). Entomological pins are either white and made of stainless steel or coated with a black lacquer. They are available in different lengths and thicknesses, the thinnest pin possible should be used to minimise damage to the insect. Ordinary household



Fig 27.2 Pinned adult housefly showing (a) position of an entomological pin through thorax and (b) position of insec and data labels on the pin.

or dressmaker's pins are usually too thick for many insects, and moreover the body fluids in the insects cause them to corrode and finally break. They can, however, be used if nothing else is available.

Even the smallest entomological pins are too big for small insects such as mosquitoes, simuliids and ceratopogonids. These insects are mounted on pins in two stages. An extremely thin headless pin made of stainless steel and sometimes called a steel point, micropin or minuten (about 0.15-0.25 mm in diameter and 10-15 mm long), is pushed through a small (10-15 mm long) strip of very soft material, such as polyporus. (This is sold by entomological dealers and scientific suppliers and consists of thin strips cut from a bracket fungus.) If polyporus strips are not available, the micropins can be carefully pushed through thin strips of cork, thin celluloid-type plastic, stiff cardboard or certain types of soft polythene foam such as plastazoate. The strip of polyporus with the micropin sticking through it is held by fine forceps and the small insect impaled with the micropin either ventrally between the legs or through one side of the thorax (figure 27.3a). Pinning should be done on a microscope slide to prevent the micropin emerging from the other side of the thorax. An entomological or household pin is passed through the other end of the polyporus strip and the pinned insect placed in an entomological storage box lined with cork, or in some other air tight container (figure 27.3b).



Fig 27.3 Pinned mosquitoes: (a) a mosquito pinned to a thin strip of polyporus by a headless micropin (minuten) and an entomological pin pushed through the polyporus strip and data labels, and (b) a pinned mosquito inserted into the cork of a  $75 \times 25$  mm specimen tube.

Dry pinned insects are readily eaten by cockroaches, ants, mites and other scavengers and may also become attacked by fungi. Pinned insects should consequently be stored in airtight containers. If they are kept for long periods in boxes these should be liberally painted with the following mixture – 6 parts naphthalene (mothballs) or paradichlorobenzene\*, 1 part chloroform, 1 part Beechwood creosote and 4 parts benzene or petrol (gasoline) – this mixture affords protection against both insect scavengers and fungi. Alternatively storage boxes can be painted with 10% chlorocresol in 95% ethanol.

Individual insects can be pinned on to cork stoppers which are then firmly placed in the openings of  $75 \times 25$ mm glass specimen tubes (figure 27.3b).

#### Labelling specimens (figures 27.2 and 27.3)

All specimens, whether pinned, preserved in fluid or mounted on microscope slides, must be labelled with the data written in pencil or indian ink. One to three data labels, cut from white cards (for example index or filing cards) or from typing papers, should be placed

\* Care is needed as this substance is carcinogenic.

underneath the insect on the pin (figures 27.2b and 27.3a, b). With specimens preserved in fluids the data can be written in pencil and the label placed inside the tube, in addition a label may be glued on the outside of the tube.

All labels should have (1) the locality from where the specimen was caught, (2) how it was caught, such as light trap, biting man, from skin of rodents, from soil, tree holes or rotting vegetation or whether it was reared from the immature stages, (3) the date, (4) the name and initials of the collector, (5) any other relevant information and (6) a serial number. More detailed notes can be given in a record book against the serial number.

#### Postage of specimens (figure 27.3)

It is often necessary to post specimens to specialists for identification. Dead specimens can usually be sent within, and between countries without difficulty with custom's clearance if packets are marked 'Preserved biological material, no commercial value'. It is essential to ensure that tubes containing fluid-preserved material have tight fitting stoppers. Tubes can be placed in either self-sealing plastic envelopes or in small plastic bags which have the openings tied tightly to prevent the package becoming wet if a tube breaks. Specimen tubes placed in plastic bags or envelopes should be packed surrounded by cotton wool, tissue paper, paper towelling or polystyrene chips and placed in a strong container.

Pinned adult insects can be posted in small entomological storage boxes, but if they are unavailable specimens can be pinned to sheets of cork or polystyrene which are placed firmly in a suitable rigid plastic or metal container such as sweet, tobacco or biscuit tins, plastic freezer boxes or sandwich boxes. The join between the lid and the container must be sellotaped to exclude ants and mites. Alternatively, individual pinned specimens can be firmly inserted into cork stoppers which are placed in  $75 \times 25$  mm glass specimen tubes (figure 27.3b). These are then either placed in small rectangular wooden containers made specially for the tubes or packed in some other protective container.

It is important that the specimens despatched to specialists should be correctly labelled and accompanied by a letter explaining why identification is needed, and whether or not the specimens should be returned to the sender.

#### Collecting methods for specific vectors

#### Lice

Body hairs or fibres from clothing to which eggs (nits),

nymphs or adult lice are attached can be cut and placed in small tubes containing alcohol. Alternatively, specimens can be removed with very fine forceps.

#### Fleas

These may be relatively difficult to catch. Fleas on man and animals may be collected in small aspirators (figure 27.4a) or by coating a matchstick with glue and



Fig 27.4 Aspirators (pooters), (a) a reservoir type for collecting small insects such as midges and sandflies and (b) a larger type suitable for the collection of mosquitoes or tsetse flies.

touching them with this; pieces of 'fly paper' may also be used. Fleas from rodents can be collected by killing the rodent and placing it in a plastic bag with a piece of cotton wool soaked in an anaesthetic and tying up the open end of the bag. After about 30 minutes the rodent is removed on to a sheet of white paper and combed with a very fine metal comb to remove fleas and other ectoparasites. The plastic bag is shaken over another sheet of paper and any fleas are collected with fine forceps or a matchstick smeared with glycerine or castor oil, and then transferred to alcohol. Alternatively small rodents can be placed alive in a strong plastic bag and killed together with their fleas by chloroform. Dogs and cats can be placed in plastic bags which are closed at the neck of the animal thus leaving the head outside, carbon dioxide or some other anaesthetic can be introduced through a length of rubber tubing inserted into the other end of the bag.

Gloves should be worn to protect the handler from bites and scratches of wild rodents and other animals.

Fleas can sometimes be collected from infested buildings by wrapping 'fly paper', with the sticky side out, around the ankles and lower legs of collectors, fleas are trapped on the fly paper as they jump on to the collector's legs.

#### Scabies mites

These can be carefully removed from their tunnels in the skin with fine forceps and placed in alcohol (p. 169).

#### Leptotrombidium mites

Larval mites on man can be collected by firstly applying a small piece of cotton wool soaked in chloroform to anaesthetise or kill them. They can then be removed by carefully dissecting out, with fine sterile needles, their embedded mouthparts from the skin. The mites can be preserved in alcohol.

#### Ticks

Ticks attached to their hosts can occasionally be removed by grasping them around the capitulum with a pair of fine forceps and gently pulling. This procedure may, however, leave the mouthparts embedded in the hosts and result in the wound becoming septic. A better method is to apply a small piece of cotton wool soaked in chloroform, or some other anaesthetic, to the tick which often causes it to withdraw its mouthparts. Less satisfactory agents such as petrol, kerosene (paraffin), benzyl benzoate, various insect repellents or even the glowing end of a cigarette can be applied to stimulate the tick to withdraw its mouthparts. Alternatively ticks can be smeared with castor oil, medical paraffin, vaseline or nail varnish to block their spiracles and prevent respiration which should eventually cause the ticks to dislodge (see also p. 159).

Ticks can also be collected from small mammals by placing them in a plastic bag and anaesthetising them, a procedure which is described in more detail for the collection of fleas.

Some argasid ticks, such as certain Ornithodoros species, shelter, when not feeding, in cracks and crevices in houses from which they can be collected.

Ticks can sometimes be collected from vegetation by wrapping a towel or piece of flannelette round a rubber or plastic hot water bottle which is filled with warm water and slowly dragged over the ground. Adult and immature ticks respond to both the warmth of the bottle and its motion and attach themselves to the covered bottle. A simpler technique consists of just dragging towelling, muslin, flannelette or a blanket over the ground. Ticks should be preserved in alcohol or formalin.

It may be advisable to wear gloves when collecting ticks to prevent accidental infection with tick-borne pathogens.

#### Bedbugs

Bedbugs can be collected either from their daytime

resting places, such as cracks and crevices in walls, floors and furniture and amongst bedding, or when they are feeding on house occupants during the night. They can be picked up with forceps but, if this proves difficult, small pieces of cotton wool soaked in chloroform can be applied to anaesthetise them and facilitate their collection. Bedbugs can be preserved in alcohol.

#### **Triatomine bugs**

These can be caught either with forceps or in small glass tubes when they are biting man or other hosts, or collected from rodent burrows, nests or cracks and crevices in houses and furniture. Adults can be preserved in alcohol or pinned. Before preservation a note should be made of their colour pattern because in many species this fades after death.

#### Myiasis-producing larvae

These can be picked from wounds and sores, or in the case of the tumbu fly (*Cordylobia anthropophaga*) the small boil-like holes they produce in the body can be covered with liquid paraffin or castor oil and the larvae gently squeezed out. Larvae are best killed by dropping them into boiling water as this results in the extension of the body segments and facilitates identification, but if this is not practical they can be killed, and preserved, in alcohol.

#### Non-biting flies

Houseflies and related flies and the metallic and nonmetallic calliphorid flies etc. can be collected with small hand nets, in glass tubes or assorted containers from houses, kitchens, latrines and sometimes at their breeding sites. After killing with an anaesthetic they should be pinned. If pinning is not possible they can be preserved dry by carefully wrapping them in cotton wool, soft tissue or toilet paper and placing them in specimen tubes. They can also be preserved in alcohol, but this is generally not so satisfactory because fluid preserved specimens may be difficult to identify to species.

#### **Blood-sucking flies**

Mosquitoes The simplest and most direct method of catching mosquitoes is by a human-bait catch. This consists of one or two people sitting down and allowing mosquitoes to alight on them, but before they have inserted their mouthparts a test tube (about 12 mm diameter 12.5 cm long) is carefully placed over the mosquito which reacts by flying up into it. The open end is plugged with a small piece of dry cotton wool. A single mosquito, or several separated by cotton wool plugs, can be collected in each test tube. There are usually differences between the mosquito species biting man at different times of the day and night, and consequently bait catches are often made at different times. At night torches are used during collecting. Catches can be made in houses, on verandahs, in village compounds, in the centre of towns, amongst farms, on cleared lands, in forests etc. Both time and location of catches depend upon the mosquito populations being sampled. Catches can last for less than an hour to several hours.

With the aid of torches mosquitoes resting on the walls, ceilings and roofs of houses, and also in dark

collecting indoor resting mosquitoes from village houses and huts consists of placing white calico sheets over the floor and, after closing all doors and windows, space-spraying the house with 0.1 per cent pyrethrum in kerosene synergised with piperonyl butoxide. The sprayman then leaves the house but returns after about 10-15 minutes to collect the dead and dying mosquitoes that have fallen on to the sheets.

Other methods used to collect mosquitoes include animal-baited traps which have entrances of slits or cones which allow mosquitoes to enter but prevent, or at least hinder, their escape. Large traps can be baited with animals such as cattle, donkeys and goats (figure 27.5), smaller ones with rabbits, mice, rats and other



Fig 27.5 A wooden stable-type of trap with the door open, suitable for containing bait animals such as goats, calves, pigs etc. A slit-like baffle is placed just below the mosquito gauze section on all sides to allow unfed mosquitoes to enter but to prevent their escape.

places such as under beds and behind cupboards, can be collected in test tubes. Alternatively, they can be sucked up into simple aspirators (figure 27.4b) and blown into suitable collecting containers such as cardboard or plastic cups which have the openings covered with netting. Another common method of



Fig 27.6 A cylindrical metal trap with removable cone funnels at each end, suitable for containing bait animals such as rodents or birds.

rodents, or birds (figure 27.6). Animal-baited traps can be used during the day or night.

Certain species of mosquitoes are attracted to carbon dioxide and light. Consequently traps baited with carbon dioxide, obtained either from gas cylinders or more simply by the sublimation of solid carbon dioxide ('dry ice'), have been used to trap mosquitoes. Carbon dioxide traps can be used during the day or night.

There is voluminous literature on the use of light traps to catch mosquitoes. Basically these traps have a light source such as a 40 to 100-watt tungsten light bulb, a mercury vapour discharge lamp, a small fluorescent tube or torch bulb to attract mosquitoes. Light intensity and spectral emission greatly influence both the numbers and species of mosquitoes caught. For example, some mosquitoes are caught in greatest numbers in traps employing ultraviolet light, whereas other species are more attracted to small incandescent lights. After attraction to the light trap mosquitoes are sucked down by a small fan into a collecting bag or some other type of container. Some light traps are about 30 cm or more in diameter, contain a large fan and operate from 110 or 230-volt mains supply of electricity (figure 27.7a). More useful traps are the miniature ones employing torch bulbs or small fluorescent tubes which operate from 6-volt dry cell batteries or 12-volt car batteries, and are consequently independent of electricity and can therefore be used in remote areas.



Fig 27.7 Light traps: (a) diagram of a New Jersey-type of light trap employing a tungsten bulb and a 25-cm diameter fan to draw the catch down into a collecting bottle, this trap operates from a mains supply (110 or 230 V) of electricity, and (b) a miniature Monks Wood-type of light trap employing a 23-cm fluorescent light tube and a miniature motor and fan to draw the catch down into a cloth and netting bag, this trap operates from a 12 V battery.

A typical miniature battery-operated trap is the Monks Wood light trap which has a 6-watt 23-cm fluorescent light tube and a small 12-volt d.c. fan which sucks the catch down into a cloth collecting bag (figure 27.7b). The trap operates from a 12-volt car battery. Light traps are used only at night.

Mosquitoes collected by any methods should be pinned as soon as possible after they have been killed. Great care is needed to avoid rubbing the scales from the wings, legs and body as this will make them difficult, if not impossible, to identify. If pinning facilities are not available adults can be placed between layers of thin tissue or soft toilet paper, or cotton wool, and carefully packed in specimen tubes, sweet or tobacco tins etc. If mosquitoes are preserved in alcohol they may prove impossible to identify to species.

Biting midges (Ceratopogonidae) These are usually col-

lected by human-bait catches performed out of doors in the evenings or at night. They can be collected in test tubes or sucked into reservoir-type aspirators (pooters) (figure 27.4). Light traps, especially those using ultraviolet light such as the Monks Wood trap, are also useful in collecting Ceratopogonidae. After killing, adults can be pinned with micropins or preserved in alcohol.

Phlebotomine sandflies Sandflies attracted to man can be caught in reservoir-type aspirators (figure 27.4a); they appear to survive better in the glass vial of the aspirator if the bottom and sides are covered with plaster of Paris which is slightly dampened before collections are made. Aspirators can also be used to collect sandflies from their daytime resting sites, such as from forest vegetation, buttress roots of trees, rodent burrows, holes formed at the base of termite mounds and from houses.

They can also be caught by placing sheets of paper (for example typing paper) soaked in castor oil near rodent holes, buttress roots of trees, under the overhanging eaves of houses etc., or in any other site where sandflies are thought to be sheltering. The entrapped sandflies can be removed from the oiled papers with an artist's small paint brush or forceps. It is best to soak them in saline containing a few drops of detergent before they are preserved in alcohol as this helps remove oil sticking to their bodies. Sticky papers are more useful in dry than wet environments where rain tends to wash the castor oil and insects from the papers.

Some species of sandflies are caught in light traps and animal-baited traps.

Sandflies collected by any method should not be pinned but be placed in alcohol.

Blackflies Blackflies are mainly day-biters and can be collected in test tubes or reservoir-type aspirators (figure 27.4a) during human-bait catches performed out of doors. Many species that bite man also feed on cattle, birds and other domestic and wild animals, and sometimes these can be collected with aspirators or in small hand nets. Light traps are not usually very useful for collecting simuliids.

Adult blackflies should be pinned on micropins or carefully placed amongst soft tissue paper or cotton wool. They should not usually be preserved in alcohol as this often renders them difficult or impossible to identify.

Tsetse flies Host seeking adults (both sexes) bite during the day and are attracted to moving hosts in preference to stationary ones. They are usually collected by people acting as both baits and catchers slowly walking through an infested area. Tsetse flies are also commonly attracted to vehicles and can often be collected from the bodies or tyres of cars or lorries parked in the bush or on the road. Tsetse flies are not caught in light traps.

Adults should be pinned or carefully wrapped in soft toilet paper or cotton wool, or as a last resort preserved in alcohol.

Horseflies The collecting methods are much the same as for tsetse flies, but in addition certain species can be collected in light traps. They should be pinned or carefully wrapped in tissue paper or cotton wool; it may prove difficult or impossible to identify adults preserved in alcohol.

Stableflies The collection and preservation methods are similar to those for tsetse flies.

#### Dipterous larvae

Dipterous larvae, and sometimes pupae or puparia, can

be collected from their breeding places. Specimens should be preserved in alcohol or formalin, but if they are placed live in these fluids they may turn brown. This discolouration can be avoided if larvae are killed by dropping them in boiling water before preserving them in alcohol. Live pupae or puparia can be placed in small containers and if adult flies emerge they can be killed and preserved in alcohol or pinned. The empty pupal cases can be preserved in alcohol with the adults, or stuck on to small pieces of cardboard and placed on the same pin as the adult insect.

#### Miscellaneous arthropods

Bees, wasps, and beetles should be pinned or wrapped in tissue paper or cotton wool, but they can be placed in alcohol if pinning facilities are not available. Ants are best preserved in alcohol.

The eggs, larvae and pupae of most insects, and also arthropods like spiders, millipedes and centipedes can be placed in alcohol or formalin. Scorpions can be kept in a dry state or preserved in alcohol.

### 28 Insecticides and insecticide resistance

#### Introduction

There are many books on insecticides describing their use for the control of arthropods of medical, veterinary and agricultural importance, their classification, insecticidal properties, toxicities etc. which can be consulted. The present account is therefore confined to a brief summary of insecticides and their uses in medical entomology.

#### **Pollution controversy**

Despite concern expressed by some environmentalists, conservationists, biologists, politicians and others, insecticides remain the principal, and sometimes the only practical, method of effectively killing insect vectors and thus reducing disease transmission. There can be little serious objection to the use of toxicologically safe insecticides and other chemicals to kill obnoxious pests such as bedbugs, head, body and pubic lice, scabies mites and flea infestations in houses, as this leads to no contamination of the environment. In areas of tropical Africa having 100 inhabitats/km<sup>2</sup> the application of residual sprays of 2g DDT/m<sup>2</sup> to the interior surfaces of houses in malaria campaigns results in depositing only about 10g/ha of insecticide, twice a year, on the soil. This concentration is very small compared with the often normal practice of spraying cotton and other crops with 2 kg/ha of DDT many times a year. There can be little doubt that the enormous benefits arising from the use of DDT in malaria control far outweigh the very small detrimental effects.

It is usually the more widespread uses of insecticide outside the home, such as spraying large areas of vegetation to kill tsetse flies, spraying rivers to kill blackfly larvae and spraying aquatic habitats to kill mosquito larvae that arouses controversy. Such criticism and concern, although occasionally justified, is often emotional and irrational.

There is no doubt, however, that repeated spraying of mosquito breeding places with residual insecticides, especially the very persistent organochlorines, may result in indiscriminately killing large numbers and species of invertebrates, and sometimes also vertebrates such as fish. Because aquatic habitats can be ecologically damaged by insecticidal applications the World Health Organization urges that wherever possible less persistent and more biodegradable insecticides such as the organophosphates and carbamates be used as mosquito larvicides. Although these insecticides may kill a broad spectrum of invertebrates, their deposits in the environment are rapidly broken down.

Another example of the concern over the use of insecticides, is the insecticidal spraying of vegetation to control tsetse flies which has sometimes resulted in the destruction of non-target invertebrates, including beneficial ones such as bees, and also some vertebrates. However, it is not generally appreciated that this is usually a reversible change, because when areas have been cleared of tsetse flies and spraying ceases these areas will be gradually recolonised by neighbouring unaffected faunas. In marked contrast after areas have been freed of tsetse flies the vegetation is often removed and the land farmed, and this leads to habitat loss and more permanent ecological damage than perpetuated by insecticides. As a final example, spraying rivers with organochlorine or organophosphate insecticides to kill larvae of the blackfly vectors of onchocerciasis may be detrimental to the ecology of river systems, but the benefits gained in freeing people from the threat of onchocerciasis and blindness have to be carefully balanced against the polluting side-effects arising from insecticidal spraying.

There has been increasing interest in the development of biological control methods to replace insecticides for vector control. It is, however, regrettably true that at present there is no practical biological method that can be recommended for efficient control of any of the major vectors of disease. Moreover, biological control may prove more difficult and costly to implement and maintain than insecticidal measures and, although it may be more specific than chemical methods, this may confer disadvantages as well as advantages. For example, certain insect pathogens and genetic control measures are likely only effective against a single vector species whereas there may be more than one in the area requiring control. Although at present insecticides remain the most powerful method of killing insect vectors there should nevertheless be continued efforts to integrate their use with other control measures, with the hope that eventually their usage will be minimised.

#### **Classification of insecticides**

#### Botanical insecticides and pyrethroids

This group includes pyrethrum, rotenone (Derris root) and the synthetic pyrethroids such as allethrin, bioallethrin, resmethrin, bioresmethrin, permethrin and decamethrin. They all contain only carbon, hydrogen and oxygen and some also certain halogens. Pyrethrum is obtained from the flower heads (mainly the seeds) of Chrysanthemum cinerariaefolium, and the insecticidal activity is due to six constituents: - pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin I and jasmolin II. Pyrethrum is a very useful knock-down insecticide, that is one that rapidly kills insects causing them to fall to the ground; it has no residual effect. A synergist, such as piperonyl butoxide, is usually added to enhance the activity of pyrethrum. Rotenone is commonly used in commercial preparations to kill fleas infesting domestic animals and pets. Both natural pyrethrum and many of the synthetic pyrethrum-type insecticides have very low mammalian toxicities and are therefore very safe to use.

#### **Mineral insecticides**

Paris green, which is a copper acetoarsenite and kills as a stomach poison, was formerly applied as a fine dust floating on the water to kill surface feeding *Anopheles* larvae, but it became more or less obsolete with the arrival of the persistent organochlorine insecticides. However, because of the development of insecticide resistance in many important pest mosquito species, there has been renewed interest in Paris green for mosquito control. In addition to being prepared as a fine dust, it can be formulated as granules or pellets which can either float on the water surface or sink. Mosquito larvae have not developed resistance to Paris green.

Petroleum oils, such as kerosene (paraffin), diesel oil

and fuel oils have for many years been sprayed on breeding places to kill mosquito larvae. They release into the water toxic hydrocarbons; one fraction is highly volatile and penetrates the tracheae of mosquito larvae and kills them by its toxic or anaesthetic effects, another less volatile fraction tends to physically interfere with the mechanics of respiration at the water surface. Crude oils are applied at the rate of 300-500litre/ha, the addition of castor oil reduces the application to 50-100 litre/ha. The spreading power of oils is measured in dynes/cm. The spreading power of crude oils (for example kerosene) is low, being only about 8-13 dynes/cm, that is they are relatively thick and high application rates are needed.

Oils specially formulated for mosquito control, such as Flit Mosquito Larvicidal Oil (Flit MLO) and Malariol HS, have been produced. These usually incorporate spreading agents such as 0.5 per cent Triton X-100, which increases their spreading powers to about 18-36 dynes/cm so that only 10-20 litre/ha are needed.

Although mosquitoes have not become resistant to oils, they have been decreasingly used as larvicides since the introduction of residual insecticides such as DDT.

#### Organochlorine insecticides

These man-made insecticides originally called chlorinated hydrocarbons but now usually termed organochlorines contain hydrogen, carbon and chlorine, and a few also oxygen and sulphur. They kill arthropods by acting as powerful poisons of the central nervous system, causing successive symptomatic stages of excitation, convulsive movements, paralysis and finally death. They are slow in action and the insects may not die until several hours after contact with the insecticide.

DDT was the first organochlorine to be manufactured and used as an insecticide. Other organochlorines used to kill medically important insects include methoxychlor, HCH (often called BHC, benzene hexachloride or lindane), chlordane, endosulfan and dieldrin. Most are long lasting and may remain effective in killing insects for many weeks or months. For example, a deposit of  $2 g/m^2$  of DDT powder sprayed on the interior surface of houses effectively reduces house-resting (endophilic) mosquitoes for about six months. The persistence of HCH and dieldrin is not as long, and with a deposit of  $0.4-0.6 \text{ g/m}^2$ control is achieved for about three months. Although their extreme persistence and stable nature has proved very useful in killing insects of medical, veterinary and agricultural importance, it has also sometimes resulted in their contamination of the environment. Most organochlorine insecticides are not broken down in the soil, plant or animal tissues but are passed on to animals that live in the soil, or that eat contaminated vegetation

or invertebrate or vertebrate animals containing these insecticides.

Organochlorine insecticides can be divided into two main groups – the DDT group and the lindanechlordane group. The former group contains DDT and methoxychlor which is related to DDT in structure but is less toxic. It is one of the few organochlorine insecticides that is biodegradable, and hence does not persist in the environment. The lindane-chlordane group is divided into two subgroups, the first one contains HCH and the other subgroup includes chlordane, dieldrin, endosulfan and heptachlor which are sometimes called the cyclodiene insecticides.

Many insecticides, including medically important ones, have developed resistance to organochlorine insecticides, but different selection mechanisms are involved in resistance to the two main groups. Thus, insects resistant to the DDT group remain susceptible to the lindane-chlordane group and vice versa. But within these groups there is cross resistance, so that insects resistant to HCH are also resistant to dieldrin, but not to DDT. However, insects may independently develop resistance to both these groups, so that none of the organochlorine insecticides can be used, and other insecticides such as organophosphates and carbamates have to be selected. Organochlorine insecticides, in particular DDT, dieldrin and HCH, have been widely used to control a great variety of medically important arthropods.

#### Organophosphate insecticides

These insecticides contain carbon, hydrogen, oxygen and phosphorus and some contain other elements such as chlorine, bromine and sulphur. They kill insects by inhibiting the enzyme cholinesterase and blocking transmission of nerve impulses.

Because of the increase of organochlorine resistance in medically important insects organophosphate insecticides have been increasingly used. These are biodegradable insecticides which do not accumulate and persist in the environment and consequently have much less effect on non-target organisms. However, like all insecticides they are not specific, so they kill a large variety of insects not all of which are pests. Examples of organophosphate insecticides used to kill medically important insects are malathion, dichlorvos (DDVP), naled (Dibrom), trichlorphon (Dipterex), fenthion (Baytex), temephos (Abate), fenitrothion (Sumithion), diazinon and chlorpyrifos (Dursban).

Malathion has a low toxicity to mammals and is regarded as a safe insecticide and is probably the most useful organophosphate insecticide; in many situations it has replaced DDT for killing insects such as mosquitoes, bedbugs and lice.

Dichlorvos is principally a fumigant insecticide. It is

frequently impregnated into polyvinyl plastic strips that are commonly suspended in houses, kitchens, restaurants and other rooms, where the insecticidal vapour emitted from the strips kills house-frequenting pests, such as houseflies and mosquitoes, for about eight to ten weeks. It is also used as a flea collar around the necks of cats and dogs to reduce flea infestations.

The parathion group of insecticides contains some very toxic and dangerous insecticides such as ethyl and methyl parathion and EPN which are readily absorbed through the skin and require very strict safety precautions and are consequently not recommended for controlling medically important insects. In contrast temephos (Abate) is regarded as one of the safest insecticides used in public health and it has extremely low toxicity to fish, birds and mammals; the acute oral toxicity (LD 50) to female rats is 8600 - 1300 mg/kg body weight compared with a value of 113 mg /kg for DDT and 1400 - 2800 mg /kg for malathion. Its low vertebrate toxicity, combined with its high toxicity to mosquito and *Simulium* larvae, makes it an especially useful chemical for killing larvae of these vectors.

#### Carbamate insecticides

These insecticides contain carbon, hydrogen, oxygen and nitrogen but not chlorine or phosphorus. Many carbamates act as nerve poisons and lower the cholinesterase levels; they are usually slow acting, but a few such as carbaryl (Sevin) and propoxur (Arprocarb) produce a quick knock-down of insects similar to that produced by pyrethrum compounds.

Carbaryl is widely used in public health, veterinary and agricultural programmes to kill a variety of insect pests. It can be formulated as sprays to kill adult mosquitoes and because of its low mammalian toxicity dust formulations have been used on animals to kill fleas and lice, it can also be incorporated into lotions for head lice control. Propoxur has been used as a residual insecticide to spray houses to kill adult mosquitoes, houseflies, ticks etc., it has also been used as a stomach poison and incorporated in sugar baits to kill cockroaches, and applied as a dust to animals to kill ectoparasites.

### Insect growth regulators (juvenile hormones and chitin inhibitors)

The problems of insecticide resistance, persistence of pesticides in the environment and the fact that insecticidal applications aimed at medically important arthropods often kill many non-target organisms that may be harmless or even beneficial to man, have stimulated research into alternative chemicals for insect control. This has resulted in several novel approaches including the use of insect hormones, their analogues

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and mimics, which interfere with the growth and metamorphosis of the immature stages of arthropods. Examples of synthetic juvenile hormones which maintain insects in the immature stages are farnesol and methoprene (Altosid). Other chemicals such as difluron (diflubenzuron, Dimilin) inhibit chitin formation between the pre-adult stages or delay or prevent pupal formation. All these compounds, collectively known as Insect Growth Regulators (IGRs), are more or less specific to insects and are readily decomposed in the environment and therefore do not contribute to environmental pollution. They have mainly been evaluated against mosquitoes: for example, methoprene applied at a rate of 0.056 kg/ha has given good control of mosquito larvae while not affecting other aquatic invertebrates.

Control of mosquitoes and other medically important insects by these types of chemicals is still mainly at the experimental stage.

#### Repellents

Although not classified as insecticides it is convenient to refer to repellents in this chapter. Most modern repellents are synthetic compounds that are applied to the skin or clothing to prevent attacks by obnoxious insects, ticks and mites. They can be useful in situations where control measures are either not practical or possible. Protection from bites of blood-sucking arthropods usually lasts for only about two hours, but this protection time will vary with the individual, his activity and degree of sweating, the ambient temperature and the insect pest. Repellents such as diethyltoluamide and ethyl hexanediol, however, can be impregnated into clothing and can remain effective for many weeks, especially if treated clothes are sealed in plastic bags when not in use. Repellents should not be allowed to come into contact with the eyes, nose, mouth or other sensitive areas otherwise they may cause considerable irritation. Some, but not all, repellents damage plastic materials.

Useful repellents are dimethyl phthalate (DMP or DIMP), diethyltoluamide (DET or DEET), butyl mesityloxide oxalate (indalone) and a mixture known as 6-2-2, comprising six parts dimethyl phthalate, two parts indalone and two parts ethyl hexanediol (Rutgers 612). The suitability of a repellent often depends on the arthropod pest. For example, diethyltoluamide and 6-2-2 are good all-purpose repellents effective against many pests, whereas dimethyl phthalate gives good protection against most pests but not against ticks. Similarly, benzyl benzoate is ineffective against biting flies but affords good protection against fleas and *Leptotrombidium* mites, and dibutyl phthalate (DBP) and dimethyl carbate are only really effective against *Leptotrombidium* mites. Other useful but less commonly used repellents include chlorodiethylbenzamide and butyl ethylpropanediol used to afford protection from mosquitoes, butyryl-tetrahydro quinoline against blackflies, trimethyl pentanediol against phlebotomine sandflies and biting midges (Ceratopogonidae).

#### Modes of action

Some insecticides have a single mode of action in killing insects, for example Paris green kills mosquito larvae almost entirely by being ingested and acting as a stomach poison. Dichlorvos (DDVP) usually kills insects by its toxic vapours while DDT and most other organochlorine and organophosphate insecticides normally kill by their contact with the exterior of the insect. Other insecticides, however, can kill insects by several different methods, for example chlordane can act as a stomach poison, fumigant or contact insecticide depending in part on how it is formulated and applied. Enumerated below are the principal modes of action by which medically important insects are usually killed.

#### Stomach poisons

These comprise insecticides and chemicals that are ingested during the insect's feeding. Examples are Paris green used for killing mosquito larvae, chlordecone and propoxur (Arprocarb), which when added to attractant baits kill cockroaches, and dichlorvos (DDVP) and trichlorphon (Dipterex) which can be incorporated in sugar baits for housefly control.

#### **Contact insecticides**

These insecticides are picked up through the feet or body wall of many insects or through the body wall and tracheae of aquatic insects such as mosquito larvae. Most insecticides used to control medically important insects are essentially contact insecticides. For example, DDT, HCH and malathion are used as residual sprays on the interior surfaces of houses to kill indoor resting mosquitoes, or are applied to clothing or the body to kill lice, or sprayed on vegetation to kill tsetse flies.

#### Fumigant insecticides

These are volatile chemicals whose vapours enter the body of arthropods via the spiracles and tracheae, and sometimes through the more general body surface. An example is dichlorvos, which is often impregnated into plastic strips to kill by fumigation insects resting in houses.

#### Auxiliaries

#### Solvents

A solvent dissolves an insecticide so that a solution is produced. Many insecticides have very low solubility in water, consequently oils such as xylene, kerosene (paraffin), other petroleum oils and mineral oils are used.

#### Emulsifiers

These are agents that enable a liquid to remain dispersed in another liquid, and thus produce an emulsion. (Milk for example is an emulsion consisting of minute globules of liquid buttermilk and other ingredients dispersed and suspended in water.) Insecticidal emulsions are produced by firstly dissolving an insecticide in an oil to form a concentrated solution after which the correct quantity of appropriate emulsifier (for example Triton  $\times$  100) is added to produce an emulsifiable concentrate. This is finally added to a diluent, usually water, to form an emulsion of very small oil droplets containing dissolved insecticide. A thin layer of emulsifier surrounds each droplet maintaining them in suspension and thus preventing them from coalescing and separating into two distinct layers of oil and water. Insecticide emulsions can be sprayed on to many surfaces including water, vegetation and the interior structures of houses.

#### Spreading and wetting agents

Many emulsifiers are also used as spreading or wetting agents. For example, when an emulsifier is added to a larvicidal oil it decreases the surface tension so that the oil spreads more thinly over the water surface, and less is needed.

Wetting agents are sometimes used to help the formation of a continuous film of oil or insecticide on surfaces that are water-repellent. They are also mixed with powdered insecticides to enable them to be wetted by diluents such as water, and allow wettable or water-dispersable suspensions to be formed.

#### Synergists

A synergist is a chemical which when added to another produces enhanced reaction, but which by itself has little or no effect. Piperonyl butoxide is a synergist that is added in small amounts to pyrethrum or synthetic pyrethroids to greatly increase their efficiency and activity, and thus allow less of these expensive insecticides to be used. Other synergists such as sesoxane and sulphoxide are sometimes used with insecticides.

#### Insecticidal formulations

#### Technical grade insecticide

This is the insecticide in its purest commercial form, although chemically it is rarely pure. Technical grade DDT is a white crystalline powder, whereas technical grade malathion is a clear amber coloured liquid. Technical grade insecticides are usually diluted before application, but sometimes technical grade insecticides are used undiluted for ultra-low-volume spraying. Most insecticidal dusts consist of technical grade insecticides mixed with inert carriers.

#### Dusts

Dusts usually consist of quantities of finely pulverised technical grade insecticide mixed with suitable carriers such as inert dusts. DDT dusts usually contain 5-10 per cent of the insecticide, while HCH dusts normally contain 0.5 per cent of the technical grade insecticide.

#### Granules and pellets

Insecticides may be either incorporated throughout granules or pellets or stuck only to their outsides. The size of granules (0.3-0.6 mm) and pellets (0.6-2 mm)varies considerably but they are always considerably larger than dusts. They are especially useful in penetrating vegetation covering mosquito breeding places thus ensuring that the insecticides reach the water. Some granular preparations are formulated to float, others to sink. Temephos (Abate) has been incorporated on to sand for mosquito control.

#### Wettable powders

These are sometimes termed water-dispersable powders. They consist of technical grade insecticide, an inert carrier, and a wetting agent that ensures the insecticidal powder is wetted and mixes with water to form a suspension of minute insecticidal particles. Insecticidal suspensions must be agitated or stirred before application to prevent the fine particles originally in suspension from settling to the bottom.

Suspensions of water-dispersable powders have several advantages over other insecticidal formulations, for example- the diluent, water, is cheap, nonflammable and has in itself no toxic properties. When wettable powders are sprayed on to porous materials such as concrete, plaster, unpainted wood, mud and thatch, the water is absorbed into the material leaving behind on the surface a fine deposit of insecticidal powder. Most wettable powders contain 50 or 75 per cent insecticide, but some (for example malathion) contain as little as 25 per cent.

#### Solutions

A solution consists of technical grade insecticide dissolved in a solvent, usually a mineral oil such as kerosene (paraffin), diesel oil, fuel oil, xylene or very rarely water. Some insecticidal solutions are relatively weak and are sold ready for use, while others are more concentrated and are diluted before spraying by the addition of more solvent. When solutions are sprayed on to non-absorbent surfaces the solvent evaporates leaving a crystalline deposit of insecticide, on absorbent surfaces some insecticide is left on the surface but a large amount may be absorbed into the material. They are consequently of little value for spraying mudwalled houses. An advantage of using solutions is that they have less tendency to mark surfaces than either emulsions or wettable powders, but they are usually less effective. Concentrated solutions of insecticide are sometimes used without dilution such as in ultra-lowvolume spraying.

#### Emulsions

Emulsions are made by dissolving technical grade insecticides in a solvent to which an emulsifier is added to produce an emulsion concentrate, this is then added to water and vigorously shaken to produce a milky white emulsion. Despite the addition of emulsifiers, unless they are periodically agitated, emulsions will eventually settle out into an oil layer containing the insecticide and an aqueous layer. Insecticidal emulsions are frequently used to spray mosquito larval habitats; the small emulsion droplets deposited on the water surface tend to coalesce to form a thin oil film. Because they contain relatively small amounts of insecticides spraying is best restricted to shallow water. In deeper water it is better to spray with oil solutions as they contain greater quantities of insecticide.

Insecticidal emulsions are also sometimes used to spray absorbent surfaces such as concrete, mud and thatch and non-absorbent surfaces such as painted woods. Generally their absorption and rate of crystallisation is slower than with oil solutions and they have greater persistence, but they have the disadvantage that they tend to mark surfaces more than insecticidal solutions.

#### Mists, fogs and aerosols

These are produced when insecticidal solutions are introduced from spraying machinery into a cold or hot blast of air. Mists have a volume median diameter (vmd) of about  $50-100 \mu$ m while fogs and aerosols have droplets having a vmd of about  $50 \mu$ m or less. The definition of vmd is the diameter of the droplet that divides the mist or aerosol (or spray) into two equal volumes, one half contains larger droplets and the other half contains droplets having a smaller diameter than the volume median diameter. Aerosols are commonly produced for ultra-low-volume spraying.

#### **Residual house spraying**

Spraying the interior walls, ceiling or roof of houses and other buildings with residual deposits of insecticides will kill endophilic insects, such as certain mosquitoes, sandflies, houseflies, cockroaches and triatomine bugs which rest for at least part of the day or night indoors. The usual procedure is to use a cylindrical pressurised (compression or pneumatic) sprayer that ensures that the insecticide is discharged at a constant rate (for example at 750 ml/min with a pump pressure of 2.8 kg/cm<sup>2</sup>). The nozzle of the spray lance has a T-shaped jet (0.8–1.2 mm diameter) and produces a fan-shaped spray. When the lance is held 45 cm from the surface being sprayed a 75 cm swath is produced.

Usual application rates of water-dispersable powders are as follows: -

2 g DDT/m <sup>2</sup>	200 mg organophos-
	phates/m <sup>2</sup>
0.4 g HCH/m <sup>2</sup>	200 mg carbamates/m <sup>2</sup>
0.6 g dieldrin/m <sup>2</sup>	

Further details are given under the heading Control in chapter 5, with particular reference to the control of malaria vectors.

#### Ultra-low-volume spraying

This technique is explained in more detail in connection with mosquito control in chapter 4, but can be summarised as follows. Instead of filling the insecticidal tank of a spraying machine with a small quantity of insecticide and a large bulk of diluent, for example, water for emulsions and inert talcs for insecticidal powders, the tank is filled with concentrated technical grade insecticidal solution or dust. The quantity of technical grade insecticide in the tank is consequently greater than with conventional spraying methods, and this concentrated insecticide is applied at a very low volume, hence the term ultra-low-volume (ULV) applications.

Special spraying machinery is required to produce an even dispersal of very small quantities of insecticides over relatively large areas. Normally a fog or aerosol is produced. In aerial applications the droplet size leaving the machine is usually large than that from groundbased sprayers because reduction in size due to evaporation during descent has to be allowed for. Moreover, if droplets are too small they will not settle rapidly but remain suspended in the air and get blown away from the target area. The less volatile the insecticide and the higher its specific gravity, the more suitable it is for aerial ULV spraying.

#### Insecticide toxicity

Insecticides such as parathion and dieldrin are highly toxic to man and other mammals, whereas others such as methoxychlor and temephos (Abate) are considered very safe, most insecticides have toxicities in between these two extremes.

Toxicity is usually measured in terms of milligrams of insecticide per kilogram of body weight of the test animal that produces 50 per cent mortality, that is the LD50. Oral toxicity is the toxicity following ingestion of the insecticide by mouth, whereas dermal toxicity is the toxicity following absorption of the insecticide through the skin. Some insecticides are very toxic both orally and dermally, for example ethyl parathion has an oral LD50 of 3.0 mg/kg for female rats and a dermal toxicity of 6.8 mg/kg, whereas DDT has an oral LD50 of 113 mg/kg but the LD50 for dermal toxicity is 2510 mg/kg, the dermal/oral toxicity ratio is thus about 22. Cheap supplies of insecticide may contain greater quantities of more toxic isomers and are not necessarily the best buy.

Because all insecticides are poisonous to a greater or lesser degree, the safety precautions advocated by the manufacturers or by various organizations (WHO, FAO) should be strictly enforced when people are handling, mixing, or applying insecticides.

#### Insecticide resistance

Insecticide resistance usually becomes apparent when applications of insecticide which were previously giving good control of a particular insect species are no longer effective. In other words, a change has occurred that allows many of the insects which would previously have been killed to be unaffected by the insecticide, that is the population has developed resistance. There are two main types of resistance in insects, physiological resistance, which is usually the most important, and behavioural resistance.

#### Physiological resistance

When much greater quantities of insecticides (at least two to ten times, sometimes a 100 times or more) are needed to kill insects it should be suspected that they have developed physiological resistance. The development of physiological insecticide resistance in an insect population is under genetic control and Darwinian selection and can be explained as follows. Before insecticidal spraying has started the insect population may contain a very few individuals that already have a mechanism which will allow them to survive if they are subjected to insecticides. Consequently, when the population is sprayed with insecticides these few individuals survive, but because most insects in the population are susceptible and are killed, the survival of these very few resistant individuals passes unnoticed. In fact very good control may be achieved and maintained for some time, but resistant individuals and their progeny slowly increase in number at the expense of the susceptible ones which are killed. Gradually an increasingly greater proportion of the population comprise resistant insects and it then becomes obvious that insecticides are no longer giving efficient control. In other words insecticide resistance has established itself.

The speed of development of resistance in a population depends on several factors including the proportion of resistant individuals originally present, the degree of isolation of the population from neighbouring unsprayed populations, and the reproductive rate and generation time of the insect. For example, in insects like houseflies and mosquitoes, which lay numerous eggs and have rapid life-cycles, resistance can appear quickly, whereas in tsetse flies, in which generation time is about five to seven weeks, resistance would be slow in developing and in fact there is as yet no evidence of it. Dieldrin-resistance usually arises more quickly and is more common than DDTresistance. This is because it is usually inherent in a greater proportion of the population before spraying and is dominant or semi-dominant genetically, so that individuals having only partially a genetic make-up for resistance (heterozygotes) are not killed. DDTresistance, in contrast, is governed by a recessive gene so only homozygous individuals survive. Paradoxically often the more efficient an insecticide is at killing susceptible individuals and the greater the exposure, the quicker resistance is selected. For example, if the same insecticide is applied to both mosquito larvae and adults, resistance is more likely to develop than if these two stages are exposed to a different insecticide.

Physiological resistance can arise from several different mechanisms -(1) the ability of an insect through the presence of detoxifying enzymes to convert the insecticide within its body into less harmful compounds, for example DDT to DDE, (2) the absorption and harmless storage of insecticides in body tissues such as fat, (3) the impermeability of the insect integument, which prevents the insecticides being taken up into the insect, and (4) the ability of some insects to rapidly excrete insecticides.

Insects that have become resistant to DDT usually remain susceptible to other organochlorines such as dieldrin and HCH and the carbamates and organophosphate But insects resistance to dieldrin are also cross resistant to HCH, but not DDT or other groups, and those that have developed independently resistance to DDT and dieldrin (and HCH) remain susceptible to the other groups. However, DDT resistance may confer greater tolerance to other insecticides particularly the pyrethroids. Some vectors have become resistant to many insecticides belonging to different groups, for example, in certain areas of El Salvador, the important malaria vector Anopheles albimanus is resistant to DDT, dieldrin and HCH, the organophosphates and the carbamates. In many areas houseflies are also resistant to most of the common insecticides. Recently some vectors have become resistant to the synthetic pyrethroids, but not pyrethrum, and a few insects have also developed resistance to the juvenile hormone mimics.

When resistance is suspected in vectors, samples should be collected and standardised tests detailed by the World Health Organization undertaken to determine the level of resistance. Basically, these tests involve exposing several hundred individuals to different insecticide concentrations and counting the numbers which die after a given exposure period. From these dosage-mortality experiments the LD50 for a particular insecticide is calculated and compared with the LD50 for the same species from a susceptible population. If there is a several fold (two to ten or more) difference between the LD50 values, physiological resistance is indicated.

#### Behavioural resistance

This is the ability of vectors to avoid contact with insecticides due to changes in their habitats and behaviour. For example, spraying the interior surfaces of houses with a residual insecticide may initially give efficient control of endophilic malaria vectors. Sometimes, however, an insecticide (for example DDT) has an irritant effect causing mosquitoes to settle for only short periods on sprayed surfaces and to fly out of doors to rest. Alternatively, the insecticide (for example HCH) may have a slight fumigant effect causing mosquitoes to avoid entering sprayed houses. Both types of avoidance tend to prevent mosquitoes coming into sufficient contact with insecticidal deposits to kill them.

In addition to these changes in behaviour there may be originally present în the population a proportion of exophilic mosquitoes. Initially this proportion of exophilic adults may be very small, but as the indoor resting adults are killed by insecticidal contact the exophilic components of the population may increase so that the population of a mosquito that was formerly endophilic may become predominantly exophilic. Resistance resulting from behavioural and/or ecological changes are considerably more difficult to recognise and quantify than physiological resistance, although the World Health Organization issues a standard kit to measure irritability in mosquitoes.

#### Vigour tolerance

Occasionally a population or part of a population of insects develops greater vigour and is able to better withstand adverse conditions, including insecticides. In the latter instance such insects are said to have developed vigour tolerance to insecticides, but it usually involves only a relatively small level of resistance and is usually not important.

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List of abbreviations used in bibliography.

Acta trop. (Acta tropica); Agric. Res. Serv., U.S. Dept. Agric. (Agricultural Research Service, United States Department of Agriculture); A. Rev. Ent. (Annual Review of Entomology); A. Rev. Microbiol. (Annual Review of Microbiology); Am. J. publ. Hlth (American Journal of Public Health); Ann. trop. Med. Parasit. (Annals of Tropical Medicine and Parasitology); Archs Derm. (Archives of Dermatology); Archos Zool. Est. S Paulo (Archivos de zoologia do Estado de Sao Paulo); Br. med. Bull. (British Medical Bulletin); Bull. ent. Res. (Bulletin of Entomological Research); Bull. ent. Soc. Am. (Bulletin of the Entomological Society of America); Bull. N.Y. St. Mus. (Bulletin of the New York State Museum); Bull. Wld Hlth Org. (Bulletin of the World Health Organization); Dept Hlth Educ. and Welfare (Department of Health Education and Welfare); Ent. Soc. Am. (Entomological Society of America); Ent. Tidskr. (Entomologisk tidskrift); Fla Ent. (Florida Entomologist) J. Am. med. Ass. (Journal of the American Medical Association); J. econ. Ent. (Journal of Economic Entomology); J. med. Ent. (Journal of Medical Entomology); J. Hyg., Camb. (Journal of Hygiene); Med. Arts Sci. (Medical Arts and Sciences); Med. Offr. (Medical Officer); Mem. ent. Soc. Wash. (Memoirs of the Entomological Society of Washington); Mem. Lond. Sch. Hyg. trop. Med. (Memoirs of the London School of Hygiene and Tropical Medicine); Pan. Am. Hlth Org., Sci. publ. (Pan-American Health Organization, Scientific Publication); Proc. R. Soc. Edinb. (Proceedings of the Royal Society of Edinburgh); Publs. S. Afr. Inst. med. Res. (Publication of the South African Institute of Medical Research); Smithson. misc. Collns (Smithsonian Miscellaneous Collections); Stud. Inst. med. Res. F.M.S. (Studies from the Institute for Medical Research, Federated Malay States); Symp. Br. Soc. Parasitology (Symposium British Society of Parasitology); Symp. Proc. R Soc. trop. Med. Hyg. (Symposium Proceedings, Royal Society of Tropical Medicine and Hygiene); Symp. zool. Soc. Lond. (Symposia of the Zoological Society of London); Trop. Dis. Bull. (Tropical Diseases Bulletin): Wld Hlth Org. Monograph Ser. (World Health Organization Monograph Series); Wld Hlth Org. Tech. Rep. Ser. (World Health Organization Technical Report Series); Z. tropenmed. Parasit. (Zeitschrift für Tropenmedizin und Parasitologie).

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