SHORT COMMUNICATION

Sex ratio in the aphid parasitoid *Aphidius colemani* (Hymenoptera: Braconidae) in relation to host size

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Introduction

In haplodiploid organisms such as hymenopteran parasitoids, males develop from unfertilized eggs and females from fertilized eggs. The sex of the egg is thus under the direct behavioural control of the mother, and it is relatively easy for natural selection to produce sex ratios adapted to local conditions. Sex ratios in parasitoid wasps (defined as the proportion of males in the progeny) thus show some of the clearest examples of adaptive patterns in animal behaviour (Godfray, 1994).

Mated females of some solitary parasitic Hymenoptera are able to regulate the sex ratio of their offspring in relation to the size of host they attack (reviewed by Charnov, 1982; King, 1993; Godfray, 1994). The sex ratios of progeny emerging from small hosts tend to be male-biased and those from large hosts tend to be female-biased. This behaviour may have adaptive significance as female fecundity is directly proportional to body size (King, 1987; Honěk, 1993). In nature, males are smaller than females and their fitness may be less affected by variation in body size. Hence, ovipositing male eggs in small hosts and female eggs into large hosts may allow the optimal exploitation of the available hosts (Charnov, 1979; Charnov *et al.*, 1981).

There is little doubt (see King, 1989) that the mechanism of differential allocation of male and female eggs to small and large hosts developed in solitary parasitoids that attack non-growing hosts: these hosts represent a 'fixed package' of resources available for offspring development. However, it has been suggested (Waage, 1982) that the mechanism of differential allocation of male and female eggs is unlikely to evolve in solitary parasitoids that attack growing host stages: these hosts represent 'dynamic resources' that

continue to grow during the course of offspring development. Differential allocation of male and female eggs is only likely to evolve if there is a consistent relationship between host size at oviposition and the resources available to the developing parasitoid (King, 1989).

The sex ratio determined by female choice at oviposition may be modified by differential mortality of the sexes. Therefore, mortality should be checked when interpreting parasitoid sex ratios. A biased sex ratio can result either from the female's control of fertilization at oviposition, or, alternatively, from the differential mortality of male and female offspring during immature development (Charnov *et al.*, 1981).

Quantification of the primary sex ratio and offspring mortality is difficult in endoparasitoids, in which both occur prior to the emergence of progeny. Evidence of either primary sex manipulation by females at oviposition or differential mortality of the sexes during development depends on knowing the primary sex ratio, which cannot be determined directly. Wellings *et al.* (1986) and Cloutier *et al.* (1991) proposed and tested indirect methods of distinguishing the primary sex ratio and differential mortality in solitary haplodiploid endoparasitoids. They partitioned the emergence sex ratio to give estimates of the primary sex ratio and the male and female offspring survival from oviposition to emergence.

Using these methods, Wellings *et al.* (1986) found no evidence that females of the solitary aphidiid parasitoid, *Aphidius ervi* Haliday (Hymenoptera: Braconidae), attacking a growing host, the aphid *Acyrthosiphon condoi* Shinji (Hemiptera: Aphididae), manipulated the primary sex ratio at oviposition. They observed a significantly male-biased sex ratio of *A. ervi* emerging from small aphids, but attributed this to differential progeny survival. On the other hand, Cloutier *et al.* (1991) demonstrated manipulation of the primary sex ratio by the aphidiid parasitoid *Ephedrus*

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californicus Baker (Hymenoptera: Braconidae). Differential pre-adult mortality did not modify the primary sex ratio. The results of Wellings *et al*. (1986) suggest that the uncertainty about future host quality may impose a constraint on the evolution of sex ratio regulatory mechanisms in solitary parasitoids attacking growing hosts. On the other hand, the results of Cloutier *et al*. (1991) suggest that the quality of a growing host is predictable from its initial size.

The primary sex ratio and mortality rates of male and female offspring of the aphidiid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae), attacking the aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) was studied. *Aphidius colemani* is a generalist aphid parasitoid commonly used in augmentative biological control of *M. persicae* and the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) (e.g. Hofsvang & Hågvar, 1975; Van Steenis *et al.*, 1996). This parasitoid has a similar biology and host relationship as the species used by Wellings *et al*. (1986) and Cloutier *et al*. (1991): *Aphidius colemani* males tend to emerge from smaller aphids than females (Holý, 2000), and both male and female parasitoids are large if they emerge from large rather than small aphids. Moreover, large parasitoids live on average longer and larger females lay more eggs (V. Jarošík & L. Lapchin, unpublished).

Naive females were kept with hosts of constant size so they had no experience of hosts of varying quality, in order to address the following questions:

1. Can mated females vary the primary sex ratio?
2. Are there differences in the mortality rates of male and female offspring during the pre-imaginal development?
3. Are the mortality rates dependent on host size?

By keeping females with hosts of a constant size, there was expected manipulation of the primary sex ratio if the females judge host size in absolute terms, i.e. females will produce a constant, but skewed, sex ratio in each host size independent of other host sizes they encounter. On the other hand, if females judge host size in relative terms then the sex ratio will depend on the other host sizes encountered; and if a female encounters only a single host size, she should produce the same sex ratio regardless of the host size (King, 1993).

Material and methods

The parasitoid host, the green peach aphid, *M. persicae*, originated from a laboratory colony reared at 22 ± 2°C and under a 16L:8D photoperiod. The host plant of the aphid was young pepper plants, *Capsicum sativum* L. (Solanaceae) cultivar Sonar. The parasitoid *A. colemani* originally emerged from mummies of *Aphis nerii* Boyer de Fonscolombe (Hemiptera: Aphididae) collected in southern Brazil in 1982. It was continually reared under a 16L:8D photoperiod at 23–25°C in nylon-covered 50 × 50 × 90 cm cages on plants infested with *M. persicae*.

The experiments were done at 22.5°C under a 16L:8D photoperiod, on 8-cm diameter discs cut from the leaves of pepper plants. The leaf discs were placed downside up on water just covering sand in 8-cm diameter Plexiglas Petri dishes covered with nylon fabric for ventilation.

Aphids were kept in cohorts of 150 individuals on the leaf discs. The cohorts consisted of either small first instar aphids (L1), or large third instar aphids (L3), which were

assumed to represent different host qualities for the parasitoid. There is a non-linear relationship between host quality and aphid size (Sequeira & Mackauer, 1992a). However, aphidiid parasitoids are known to prefer L3 aphids as hosts (Srivastava & Singh, 1995; Pandey & Singh, 1999).

Two sets of adult female parasitoids were established: one group was mated and the other unmated. Parasitoids were randomly allocated to these sets by removing mummies (parasitoid pupae within exoskeletons of dead aphids) from the stock culture of the parasitoid. The mummies were individually isolated in 0.4 ml gelatine capsules that were provided with a drop of honey as a food for the emerging adults. On the day of emergence the adults were sexed, and single males were introduced into one half of the capsules that contained females. Mating in the capsules was checked visually and verified by female presence in the progeny of the mated female. The females in the remaining capsules were left unmated.

The aphids on leaf discs were then exposed to virgin parasitoid females or to the mated females. The introductions were made when the parasitoids were < 24 h old. The parasitoids were naive, i.e. without any previous experience of laying eggs.

To assess the number of aphids not accepted by a female for oviposition, or those in which the immature parasitoid died before pupation, the numbers of stung aphids that survived without mummification were examined. Cohorts of L1 and a L3 aphids were exposed to either unmated or mated females. After each ovipositional attack, each stung aphid was individually caged on a small leaf disc in a 2.5 cm Petri dish. For each treatment, 60–100 stung aphids were used.

To assess the number of male and female offspring of mated and unmated *Aphidius colemani* emerging from small and large aphids, virgin parasitoid females or male-female pairs were provided either with a cohort of L1 aphids or L3 aphids for 12 h. Six unmated and 15 mated females on L1 aphids, and two unmated and four mated females on L3 aphids were used. However, the experiment did not include individual females as an independent variable, and the sample sizes used in the calculations, presented in table 1, were thus the total numbers of male and female offspring, and the total number of parasitized aphids, produced by all the females (Wellings *et al.*, 1986).

After removing the parasitoids, the aphids were individually caged on small leaf discs in 2.5 cm Petri dishes. Mummies were individually stored in 0.4 ml gelatine capsules. Each parasitoid was sexed at emergence. Mortality, mummy development and sex were recorded at 24 h intervals.

Sex-specific mortality was estimated by comparing the survival of all male broods laid by the virgin females with the survival of mixed broods laid by the females mated at emergence, following Wellings *et al*. (1986) (table 1). The technique assumes that the male progeny of virgin females suffer the same mortality as the male progeny of mated females, which appears realistic in the case of species without parental care (e.g. Godfray, 1994). The assumption that either a single egg is oviposited, or, if multiple oviposition occurs, only one survives, also appears to be met. Dissection of 150 L1 and L3 aphids revealed two eggs in only one out of the 300 aphids.

Table 1. The total number of male and female offspring of mated and unmated *Aphidius colemani* emerging from small (L1) and large (L3) *Myzus persicae* and the total number of aphids parasitized.

Aphid age at time of parasitization	No. of offspring of unmated females		No. of offspring of mated females		
	Sons	Parasitized aphids	Sons	Daughters	Parasitized aphids
L1	235	296	301	270	739
L3	64	77	55	91	204

Results

In first instar *M. persicae*, only one of the stung aphids previously exposed to unmated, and none exposed to mated *A. colemani* survived without mummification. In third instar aphids, the corresponding numbers for unmated and mated parasitoids were two and three aphids, respectively. Thus, egg and larval mortality appeared negligible, because nearly all stung aphids were mummified. The low numbers of stung aphids surviving without mummification did not differ significantly between the small and large aphids and between the mated and unmated females (2-tail probability of Fisher's exact test on contingency table = 1.0). Therefore, the possibility that the differences in the mean sex ratio of *A. colemani* were biased by differences in host acceptance, rather than a result of sex-specific offspring mortality, or female control of fertilization at oviposition, can be excluded.

The emergence sex ratio of *A. colemani* differed greatly between small and large aphids ($\chi^2 = 10.52$, df = 1, $P = 0.0012$). The estimated emergence sex ratio was 0.53 from small aphids and 0.38 from large aphids. Whether this reflected a difference in primary sex ratio is unclear.

The primary sex ratio at oviposition did not differ significantly in small and large aphids, but approached the 5% level of significance ($\chi^2 = 3.14$, df = 1, $P = 0.076$), suggesting a slight tendency to allocate male eggs to small aphids and female eggs to large aphids. The estimated primary sex ratios was 0.51 for small aphids but only 0.32 for large aphids.

Assuming that the primary sex ratio did not differ between host size and equals 0.47 (equation (11) in Wellings *et al*. (1986)), female progeny survivorship differed significantly in small and large aphids ($\chi^2 = 4.41$, df = 1, $P = 0.036$). The estimated survivorship of female progeny was higher in large (survivorship 0.75) compared to small aphids (survivorship 0.72). Survivorship of male progeny did not differ significantly in small and large aphids ($\chi^2 = 0.53$, df = 1, $P = 0.46$).

Discussion

It cannot be said that the primary sex ratio of *A. colemani* in small and large aphids differed significantly at the moment of oviposition. However, on emergence from small aphids, the sex ratio showed a significant male bias. The shift in emergence sex ratio may be attributable to a significantly higher mortality of female progeny in small than large aphids. Female offspring often have more stringent nutritional requirements than males and reach a larger size than males in hosts of equal size (Sequeira & Mackauer, 1992b, 1993). The higher female mortality in small aphids may be a consequence of resource limitation during juvenile development of females in small aphids.

The experiment constrained naive *A. colemani* females with hosts of a constant size to prevent the females from obtaining experience with hosts of varying quality. The results suggested a constant allocation of male and female eggs to different sized hosts, that were used to simulate host quality. Similar results were obtained by Wellings *et al*. (1986) when mated females were not constrained to hosts of a fixed size, but randomly presented with different sized hosts.

However, Cloutier *et al*. (1991), Srivastava & Singh (1995) and Pandey & Singh (1999) reported manipulation of the primary sex ratio by aphidiid females when females experienced different sized hosts, with male eggs allocated to small aphids and female eggs to large aphids. This suggests that manipulation of the primary sex ratio by aphidiid parasitoids can depend on a female's previous experience. The fact that manipulation of the primary sex ratio was not found by Wellings *et al*. (1986) may be because of the limited memory capabilities of the parasitoids when evaluating the availability of small and large hosts (King, 1993). For example, each day, Wellings *et al*. (1986) sequentially presented females with a maximum of five aphids, randomly selected from three aphid size classes.

Our results suggested a constant primary sex ratio in the experiment constraining females with hosts of uniform quality. By keeping females with hosts of a constant size, the manipulation of the primary sex ratio was only expected in cases where females judge host size in absolute terms. The non-significant tendency to allocate male eggs to small aphids and female eggs to large aphids can be attributed more to non-constant host quality from the parasitoid's perspective than a manipulation of the primary sex ratio. Our results do not contradict the assertion that a female's decision to fertilize an egg is affected by host size variation in growing hosts (King, 1989; Cloutier *et al*., 2000), and suggest that a female's decision can crucially depend on her information on the availability of small and large hosts (Cloutier *et al*., 1991; Srivastava & Singh 1995; Pandey & Singh, 1999). Females may pick up, store and use information about host distribution (Charnov *et al*., 1981).

Our results indicate that female *A. colemani* judge host size by comparing the availability of small and large hosts, i.e. on a relative basis. Judging host size allows a female to produce an offspring sex ratio independently of changes in the distribution of available host sizes (King, 1987). How host size is judged by the parasitoid in nature may depend

on the amount of temporal variation in host size distribution, with a shift in host abundance the most likely reason for a shift in host size utilization (Charnov *et al.*, 1981). Aphids, by virtue of short generation time and programmed anticipation of seasonal trends, are hosts with an extremely high temporal variation in population abundance (Jarošík & Dixon, 1999) and host size distribution (Dixon, 1985; Sequeira & Dixon, 1996). Therefore, the evaluation of host size quality on a relative basis can be an adaptive strategy for solitary aphid parasitoids (Honěk *et al.*, 1998).

Acknowledgements

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