The evolutionary history of testicular externalization and the origin of the scrotum

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This paper re-examines the evolution of the scrotum and testicular descent in the context of the recent phylogeny of mammals. The adaptive significance of testicular descent and scrotality is briefly discussed. We mapped four character states reflecting the position of testes and presence of scrotum onto recent mammalian phylogeny. Our results are interpreted as follows: as to the presence of testicondy in Monotremata and most of Atlantogenata, which represent the basal group of all eutherians, we argue that primary testicondy represents a plesiomorphic condition for Eutheria as well as for all mammals. This is in opposition to the previous hypothesis of Werdelin and Nilsonne that the scrotum may have evolved before the origin of mammals and then repeatedly disappeared in many groups including monotremes. We suggest that the scrotum evolved at least twice during the evolutionary history of mammals, within Marsupialia and Boreoeutheria, and has subsequently been lost by many groups; this trend is especially strong in Laurasiatheria. We suggest that the recent diversity in testicular position within mammals is the result of multiple selection pressures stemming from the need to provide conditions suitable for sperm development and storage, or to protect the male gonads from excessive physical and physiological disturbance.

1. Introduction

While the possession of a scrotum by most male mammals is considered a hallmark of mammalian evolution, surprisingly little is known about how and why this unusual organ evolved. The scrotum originated as an ultimate consequence of the process of testicular descent (*descensus testiculorum)*. During embryonic development, both testes descend from a primitive position close to the embryonic kidneys first into the inguinal region of the lower abdomen and subsequently through the inguinal canals penetrating the body wall; thus, in some mammalian groups, they actually leave the body core and become encased within a special subcutaneous diverticulum – the scrotum. Developmentally, the state wherein the testicles have descended into a scrotal pouch and are posited outside the body core is considered the most extreme manifestation of this process.

1.1 *Hypotheses describing the evolutionary origin and functioning of the scrotum*

During the past hundred years, numerous hypotheses have been put forward to explain the evolutionary origin of the scrotum; however, none has been able to provide a simple explanation of this phenomenon. One of the oldest hypotheses, and still very popular, presupposes that a temperature lower than that of the core body is necessary to avoid a disruption of spermatogenesis. The original version of this so-called 'cooling hypothesis' comes from Moore (1926) wherein the physiological requirement of lower temperature was considered as the reason for testicular descent and development of the scrotum. This classic hypothesis has been repeatedly criticized from various perspectives (Portmann 1952; Carrick and Setchell 1977; Freeman 1990; Short 1997; Bedford 2004). The cooling

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hypothesis finds a modern reinterpretation in the work of R V Short, who suggests that the reason for testicular descent and evolution of the scrotum is not because of a need to cool the testes to ensure a convenient environment for spermatogenesis, but rather because it represents a useful evolutionary mechanism for keeping the mutation rate in the male germ line at an acceptable level (Short 1997). Bedford also criticized the traditional understanding of the cooling hypothesis, arguing that the causal explanation of scrotal evolution and testicular descent is to cool the epididymides (not the testes). The epididymides are elaborations of the male (Wolffian) ducts designed for achieving final maturation of the sperm and their prolonged storage (Bedford 1978). As mentioned by Werdelin and Nilsonne (1999), this hypothesis does not explain the evolution of the scrotum explicitly because there are mammals with a subcutaneous or intra-abdominal epididymis having no scrotum. The 'training hypothesis' (Freeman 1990) provides an indirect explanation for evolution of the scrotum; wherein the scrotum serves to expose the sperm to a hostile environment in order to train it for further goals, namely fertilization. In this hypothesis, the decreased blood supply in the testes, combined with a high metabolic rate, causes oxygen-poor conditions resulting in a physiologically hostile environment; this may be only a secondary effect of testicular descent rather than its evolutionary cause. The 'display hypothesis' also considers the lower temperature in descended testes as derived secondarily, but argues that the prime mover of the descent and evolution of the scrotum was a signalling function (Portmann 1952). Accordingly, the scrotum is brightly coloured in some mammalian groups and thus signals the sexual pole of a male animal. This hypothesis is usually refuted, as it is improbable that the scrotum originated primarily in order to serve signalling (Ruibal 1957). However, as stated by Werdelin and Nilsonne (1999), it may explain why the scrotum – an easily vulnerable organ with a crucial reproductive role – was evolutionarily retained in lineages where the physiological obstacle with cooling has been solved alternatively. Finally, the 'galloping hypothesis' states that the scrotum originated in mammals which frequently gallop, leap or jump. During such a bumpy lifestyle, the process of spermatogenesis in an abdominal testis or, alternatively, the sperm stored in the epididymis, would be endangered by the fluctuations in intra-abdominal pressure; the evolutionary origin of a scrotum is a solution which accommodates these crucial reproductive organs in a relatively non-destructive surrounding (Frey 1991; Chance 1996). Recently, Bedford (2004) proposed that a kind of evolutionary trade-off might stand behind the diversity in testicular position. Freeman (1990) has already suggested that internal testes tend to be larger relative to body size compared to scrotal testes. Thus, the relative size of the testes decreases with the degree of testicular descent.

Because larger testes mean larger amounts of sperm, the degree of testicular descent reflects the existence of a trade-off between sperm production and sperm storage (Bedford 2004): 'Maximising sperm storage may "allow" both a lower sperm production and cost incurred in this, without compromising male fecundity.' This may explain the high diversity in testicular position among mammals.

1.2　*The molecular physiology of testicular descent in modern mammals*

Before assessing possible evolutionary pathways, we need to understand clearly the nature of the events recapitulated in every male embryo of a representative modern mammal, and which lead to the repositioning of the testes in an external scrotum. Most details are known for rodents, including genetically mutated mice, or for humans where the failure of the testes to descend properly is a common pathological condition (cryptorchidism). Prior to sex determination and expression of the *SRY* gene in the embryonic Sertoli cells, which occurs between the first and second trimesters of pregnancy, the indeterminate gonads (presumptive ovaries and testes), as partly mesonephric derivatives, are located close to the kidneys (metanephroi). The induction of the *SRY* gene leads to the differentiation of foetal Sertoli and Leydig cells. The latter, already at the beginning of the second trimester of pregnancy, begin producing steroid hormones, especially androgens, and the peptide hormone insulin-like factor 3 (INSL3) (Ivell and Hartung 2003). Soon after this, the Sertoli cells begin secreting anti-Mullerian hormone (AMH), which is responsible for the dissolution of the Mullerian duct (future uterus and oviduct of the female). In the male foetus, the early testis is held by two principal ligaments: dorsally, the cranial suspensory ligament (CSL) holds the gonad close to the kidney and ventrally the gubernaculum links the presumptive testis to the inguinal region. Whereas in the female the CSL strengthens to hold the future ovary in its primitive position, in the male androgens from the Leydig cells cause this ligament to involute and effectively disappear. INSL3, on the other hand, works specifically on the gubernacular ligament and participating cremaster muscle, which connects the testis ventrally to the inguinal region. Under the influence of INSL3, this ligament thickens, effectively holding the gonad in the inguinal region while the rest of the embryo grows away dorsally (Ivell and Hartung 2003; Nation *et al.* 2009). Thus, this first transabdominal phase of testicular descent is less an active downward migration of the testes than a repositioning of the gonad relative to other organs. From an evolutionary perspective, this is all that is required to achieve the so-called inguinally positioned testes.

Studies from transgenic mice overexpressing INSL3 in female animals have shown that another property of

this hormone appears to be to induce a weakening of the muscular body wall, effectively inducing a lower abdominal hernia (Koskimies *et al.* 2003). This implies that the evolutionary state with non-scrotal but subcutaneous testes might simply be the result of such INSL3 action inducing a kind of hernia. We should not be surprised at the complex involvement here of a single gene such as *INSL3*. INSL3 is what has recently been termed a 'neohormone' (Ivell and Bathgate 2006). Like the gene for the closely related peptide hormone relaxin, these genes have evolved specifically within early mammals to address the new physiology demanded by mammalian evolution (viviparity, lactation, adjustment of body fluid volume relationships, scrotal testis, post-reproductive survival).

Finally, recent studies suggest that the subsequent outgrowth of the gubernaculum into the scrotum as a special subcutaneous pouch might be due to induction of the specialized segmental genes known as *HOX* genes, which are involved in limb-bud growth (Nightingale *et al.* 2008). This second phase of testicular descent appears to be governed mostly by androgens, although the additional involvement of INSL3 and AMH cannot be excluded (Nation *et al.* 2009).

In order to understand the selection pressures that might have favoured the development of a scrotal testis as outlined above, we need to consider the way in which a scrotal testis differs from one that is primitively abdominal. Temperature is the most obvious difference, with the organs within the scrotum (testis and epididymis) functioning optimally at ca. 4°C below abdominal temperature. It should be noted that the lower temperature only takes effect in the pre-pubertal, pubertal and adult stages of testis development (i.e. spermatogenesis, sperm maturation and storage), but not in the earlier stages, such as the establishment of testicular architecture and the principal compartments of testis and epididymis. These are established prior to the descent of the testes into a scrotum, which occurs in some species (e.g. rats) only shortly before puberty begins. In modern mammals, the most sensitive steps with regard to temperature appear to be the maintenance of spermatogonial stem cells and the survival of gametes through meiosis and spermatid differentiation (Setchell 1998; Ivell 2007). Furthermore, genes expressed by the epididymal epithelium, which are essential for sperm maturation and storage, appear to be exquisitely sensitive to small changes in temperature (Pera *et al.* 1996), lending weight to the hypothesis that the mammalian evolution of the epididymis as a sperm maturation and storage organ is possibly one of the most important driving forces in scrotal evolution (Bedford 1978).

1.3 *Tracking the evolution of the scrotum via mammalian phylogeny*

There exists a huge amount of literature wherein various hypotheses on the adaptive function of the scrotum are discussed in detail. In this study, however, we primarily ask *how* the scrotum has evolved during the phylogenetic history of mammals. Methods of comparative biology represent a powerful tool for testing hypotheses on the function of particular characters, i.e. organs or behavioural patterns. The output of these methods is critically dependent on a correct determination of the plesiomorphic and apomorphic (derived) state of the character under study. Few papers have focused on the evolution of the scrotum from a phylogenetic perspective (Williams and Hutson 1991; Werdelin and Nilsonne 1999). Nowadays, the phylogeny of mammals is better understood and major relationships between lineages seem to be corroborated and epistemologically reliable. As such, it is slightly surprising that *no* paper dedicated to the evolutionary history of the scrotum has been published since the influential entry of Werdelin and Nilsonne (1999); this is even more surprising when we consider how much the views about mammalian phylogeny have changed in the past decade. Here, we partially follow the attempt of Werdelin and Nilsonne (1999) to differentiate between three different character states: testicles non-descended (testicondy), testicles descended but ascrotal, and testicles descended and scrotal. Despite the fact that testicles in Marsupialia are also descended and scrotal, the position of the marsupial scrotum differs from that of Eutheria; as such, we have rather classified the marsupial condition as a separate character state. Accordingly, four different character states are mapped on the current phylogeny of mammals, and the consequences for the evolution of the scrotum and testicular descent are discussed. Finally, we propose a hypothesis of the evolutionary history of the scrotum that is contrary to the previous hypothesis of Werdelin and Nilsonne (1999), which considered the descended scrotal state as the primitive condition for Mammalia.

2. Methods

2.1 *Material and character coding*

Data on the development of the scrotum and testicular position among various groups of mammals have been taken from the literature. Among the important references which deserve mention are the works of Ottow (1955), Freeman (1990), Williams and Hutson (1991), and Werdelin and Nilsonne (1999). Based on the work of Werdelin and Nilsonne (1999), we have used a 'four character state model' for our description of the evolution of the scrotum and testicular descent: (1) *testicondy* [T]: the testicles are positioned close to the kidney and the scrotum is not present; (2) *descended ascrotal* [DA]: the testicles have descended but no scrotum is developed, (3) *descended scrotal* [DS]: the testicles have descended to the scrotum and the scrotum is fully developed (pendulous or otherwise); (4) marsupial

[M]. The situation in the group Marsupialia needs a special category due to the difference in position of the scrotum with respect to the penis. In eutherians, the scrotum is posited behind the penis, i.e. *postpenial* scrotum (following craniocaudal direction) whereas the marsupial scrotum lies in front of the penis (*prepenial* scrotum). Testicular descent appears to be similar in both the groups, but there are many differences in anatomy, timing and hormonal versus genetic control of scrotal development (Hutson *et al*. 1988; Shaw *et al*. 1990; Griffiths *et al*. 1993; Coveney *et al*. 2002). The character state 2 [DA] may be further subdivided into two conditions (i) testicles remain inside the abdominal cavity, and (ii) testicles outside the abdominal cavity, i.e. subcutaneous testicles. As we focus primarily on the evolution of the scrotum, rather than the phases of descent, this refinement of testicular position was not taken into account. See Werdelin and Nilsonne (1999) for a discussion of how this coding matches the description of diversity in the development of testicular descent presented in the works of other authors: especially the six-character model of Carrick and Setchell (1977), which was also adopted by Freeman (1990).

2.2 *Tree topology*

The phylogenetic relationships of mammalian taxa were obtained from recently published mammalian phylogenies (Bininda-Emonds *et al*. 2007; Wildman *et al*. 2007). The tree topology that has currently garnered the most support roots Eutheria (Placentalia) between Boreoeutheria and Atlantogenata (Afrotheria + Xenarthra) (Waters *et al*. 2007; Wildman *et al*. 2007; Prasad *et al*. 2008). Nevertheless, other recent examinations have found support for a root between Afrotheria and Exafroplacentalia (Boreoeutheria + Xenarthra) (Bininda-Emonds *et al*. 2007; Nikolaev *et al*. 2007). It should also be mentioned that some recent papers have included testicondy in analyses that support Afrotheria (Seiffert 2007; Asher and Lehmann 2008). For these reasons, we decided to represent both the Atlantogenata and Afrotheria hypotheses while drawing the mammalian tree (figure 1).

The trees presented in this paper have been constructed in order to examine the distribution of the particular character states (1–4) in the context of mammalian phylogeny. All eutherian taxa are depicted at an ordinal level but only the taxa with diversified development of the scrotum and testicular descent have been further broken down into subordinal levels (as in the case of Laurasiatheria). Marsupialia and Monotremata have not been subdivided, neither to ordinal nor to lower levels, because the testicular position is uniform in each of these groups, descended scrotal (prepenial) and testicondy, respectively. Generally, a more detailed mapping of the character states on the

lower taxonomic levels would be redundant in cases where the situation regarding testicular position and scrotal development is uniform within all members of a particular taxon. The trees were drawn using the software Treeview, version 1.6.6. (*http://taxonomy.zoology.gla.ac.uk/rod/treeview.html*) and Dendroscope (Huson *et al. 2007).*

3. Results

Our results of mapping the four character states on the present-day phylogenetic tree of mammals are diagrammatically depicted in figures 1 and 2. Figure 1 shows the relationships among different mammalian orders. Figure 2 focuses more closely on the situation within the clade Laurasiatheria, because this group shows a particularly high diversity in testicular position and development of the scrotum.

The four basic character states of scrotal and testicular development are distinguished: (1) testicondy (T); (2) descended ascrotal (DA); (3) descended scrotal (DS); (4) marsupial (M). The monotremes, as the most basal group of living mammals, have testes positioned close to the kidney. This condition, called testicondy (*sensu* primary testicondy), is usually considered to be the primitive character state within mammals. In marsupials, we find a well-developed scrotum that is, however, prepenial; this contrasts with the postpenial scrotum of the vast majority of placental mammals (Eutheria). The situation within the groups Monotremata and Marsupialia is uniform for all subgroups in each of these groups, so that this is not really informative with regard to which condition is primitive or derived for mammals. In contrast, placental mammals show a highly diversified picture, thus potentially providing much information on the evolution of the scrotum.

Testicondy (understood as primary testicondy) occurs only within Afrotheria. Moreover, almost all representatives of Afrotheria have testicondy, the exception being Tubulidentata, which shows a descended but ascrotal condition (DA). The Afrotheria is the most basal clade on the phylogenetic tree of placental mammals, which may suggest that testicondy represents the original ancestral state of all Eutheria, and perhaps of all mammals – if we consider the presence of testicondy in every single species of Monotremata. It is generally more probable that testicondy is the ancestral state of mammals and the prepenial scrotum of marsupials evolved as a novel structure; this contrasts with the scenario in which the scrotal condition is original for all mammals and only monotremes and afrotherians have independently regained testicondy. If the fully developed scrotum had undergone evolutionary loss, the testicles would probably remain close to the ventral side of the abdominal wall. The repeated loss of testicular descent, i.e. holding the testicles back in the position close to the kidney and dorsal abdominal wall, seems to be evolutionarily improbable
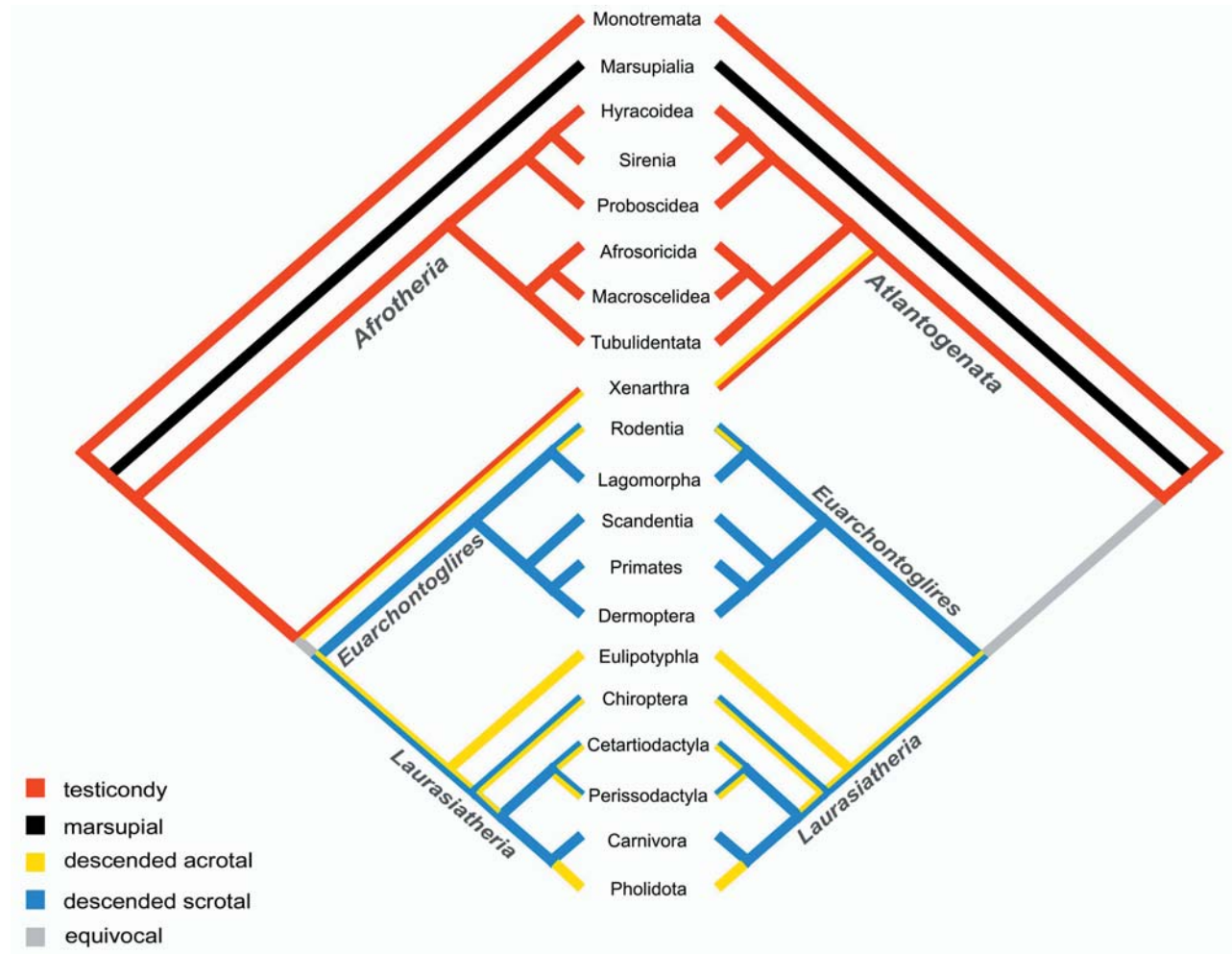
Figure 1. Two representations of the mammalian tree showing the relationships among the orders; left –Afrotheria hypothesis, right – Atlantogenata hypothesis. Both dendrograms trace the evolutionary trajectories of four character states represented by different colours: testicondy – red, marsupial – black, descended ascrotal – yellow, descended scrotal – blue, equivocal situation – grey.

and functionally useless. Loss of the scrotum has occurred several times in the evolution of mammals; however, this was usually a transition from DS to DA, not from DS to T (e.g. the situation within Laurasiatheria). Moreover, the particular instance of the descended ascrotal condition in Tubulidentata seems to originate from testicondy, i.e. by partial descent of the testicles towards the ventral abdominal wall, and represents rather a derived condition within Afrotheria.

In Xenarthra, we find the beginnings of testicular descent (Mickoleit 2004: 485). Herein the descended testes, located between the bladder and rectum, reach the pelvic region but do not pass through the inguinal canal to a position close to the ventral body wall (Starck 1995: 214). Note that Xenarthra represent a group with a substantially derived morphology; the pelvis is compact, prolonged and dorsoventrally flattened, the kidneys are moved caudally to reach the pelvic cavity (Mickoleit 2004: 485). As such, testicular descent here may

be connected with the movement of the kidneys and other inner structures. The position of the testes in this case is thus different from the descended ascrotal situation found in some members of the Laurasiatheria character state DA and rather represents an intermediate state between testicondy and descended ascrotal. In Atlantogenata (Afrotheria + Xenarthra), we find either testicondy or a partially descended but ascrotal position. Irrespective of whether the Afrotheria or the Atlantogenata hypothesis will garner more support in the future, the eutherian (non-marsupial) scrotum evolved soon after the origin of Boreoeutheria, but before the split into Laurasiatheria and Euarchontoglires, which is about 100 Myr ago – according to the time scale in Bininda-Emonds *et al*. (2007).

Special interest is deservedly given to Laurasiatheria wherein we find the most diversified situation as to testicular position and development of the scrotum among

all mammals (*see* figure 2). The monophyletic clades in which the condition of scrotal evolution is the same within all subgroups are depicted in ordinal or subordinal levels (symbolized by capitals). This is the case of Eulipotyphla, Megachiroptera, Microchiroptera, Ruminantia, Cetacea and Pholidota; all of which comprise representatives with
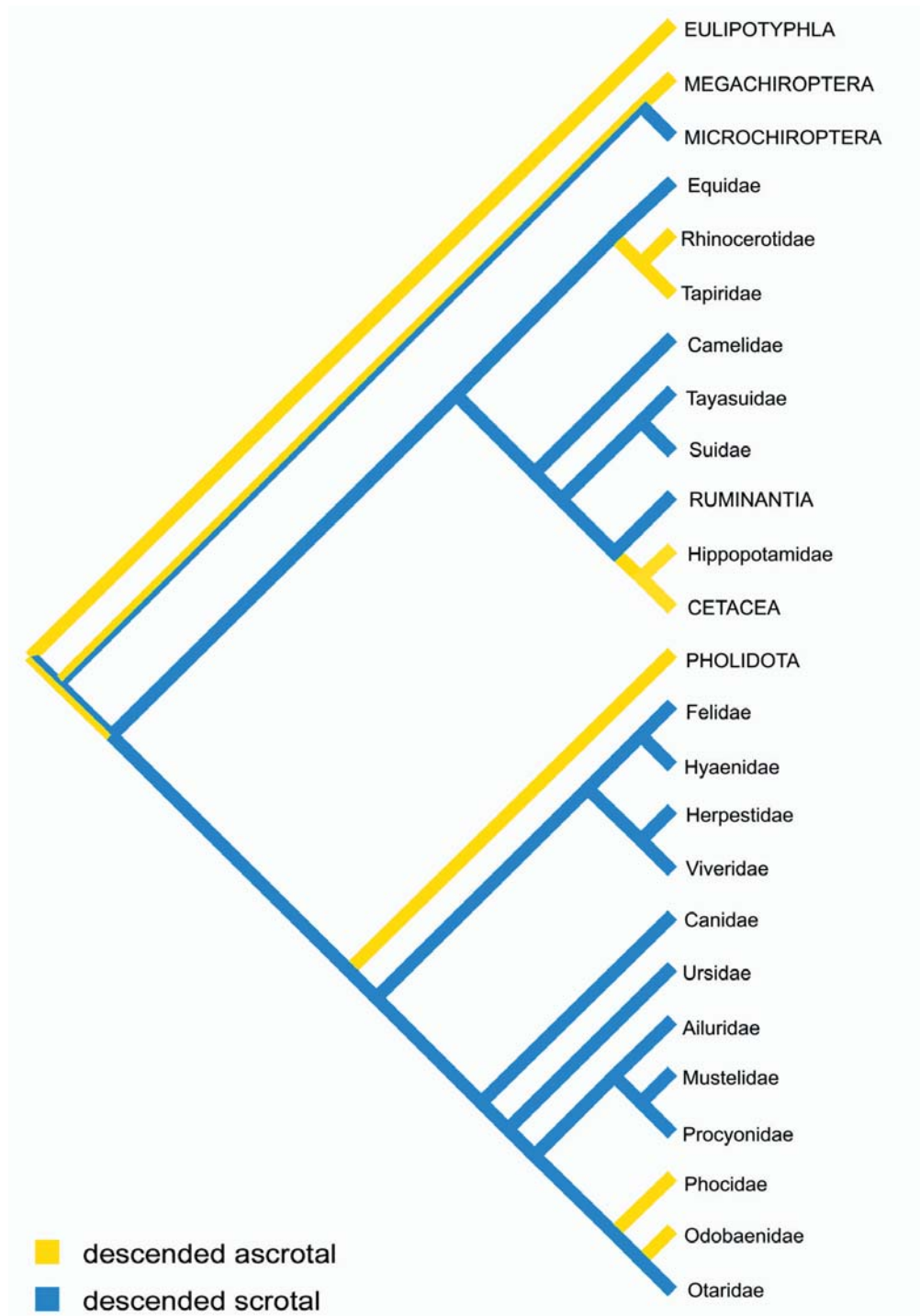
Figure 2. Representation of the Laurasiatheria tree showing the relationships among families, suborders and orders: the monophyletic clades in which the scrotal position is the same within all subgroups are depicted in ordinal or subordinal levels (written in capitals): the presumed evolutionary history of each of two character states is marked in yellow – descended ascrotal, and blue – descended scrotal.

a descended ascrotal state, except for Microchiroptera and Ruminantia, which have testes descended into a well-developed scrotum. Eulipotyphla, as the most basal order of the clade Laurasiatheria, comprises representatives with descended but ascrotal testes. Hence, theoretically, the descended but ascrotal testicular position may be considered as the primitive condition for Laurasiatheria. However, this is an inappropriate overgeneralization. Consider especially some members of the orders Cetartiodactyla (all cetaceans) and Carnivora – walruses (Odobaenidae) and seals (Phocidae) – that inhabit an aquatic environment. Here the situation represents a derived character state due to adaptations to the water environment. Within the 'cooling hypothesis', this is usually explained by the invention of an alternative cooling solution provided by the pampiniform plexus, which also has other functions such as the local transfer of hormones (Einer-Jensen and Hunter 2005), and/or by life in the aquatic environment itself (Rommel *et al*. 1995). Filtering out these more or less apparent adaptations to an aquatic environment – i.e. the situation found in all cetaceans and some pinnipeds – the descended scrotal condition is pervasive among Laurasiatheria. What is, then, the primitive condition for Laurasiatheria? Does the descended ascrotal condition represent the primitive or the derived character state? If the descended ascrotal (DA) condition is taken for plesiomorphy then the descended scrotal state (DS = synapomorphy) had to originate ten times independently within Laurasiatheria. Conversely, if the descended scrotal condition (DS) represents plesiomorphy, then loss of the scrotum has occurred six times. Comparing 10 independent origins against 6 independent losses of the scrotum speaks clearly for the latter scenario; note also that the repeated loss of the structure is generally more probable than its evolutionary reappearance. Presumably, the 'testicles descended into a scrotum' (DS) seems to be the plesiomorphic condition for Laurasiatheria. This is suggested in spite of the fact that no scrotum is developed (DA) in Eulipotyphla (the most basal clade of Laurasitheria). The most plausible explanation is that the scrotum was lost early in the evolutionary history of Eulipotyphla. This is in agreement with the conjecture mentioned above, that the eutherian scrotum appeared in evolution soon after the origin of Boreoeutheria. This hypothesis also supports the fact that the vast majority of Euarchontoglires, the sister group of Laurasiatheria, have descended testicles encased in a developed scrotum (DS). Caviomorph rodents (Caviidae, Chinchillidae) represent the only exception, showing the descended but ascrotal condition (DA), which certainly represents a derived state.

4. Discussion

The results from mapping four character states on the phylogeny of mammals support the traditional view on evolution of the scrotum and testicular descent, i.e. the scrotum as a derived feature and testicondy as the ancestral condition. The scrotum has appeared in the evolution of mammals at least twice in Marsupialia and Boreoeutheria. Moreover, the scrotum has been lost by many taxa, especially within the group Laurasiatheria, wherein the distribution of scrotal and ascrotal species is the most varied among all mammals. In the following discussion we focus on the evolutionary trajectory of scrotal development and testicular descent, and conclude with a brief reconsideration of the adaptive significance of the scrotum.

4.1 *Evolutionary scenario of testicular descent and scrotal development*

The position of the testes close to the kidney represents the ontogenetically primary condition termed testicondy. Werdelin and Nilsonne (1999: 67) argued that '*the hypothesis that testicondy is primitive requires the independent evolution of a scrotum in at least four groups: Marsupialia, Rodentia, Primates + Chiroptera and the group including Ferungulata (Perissodactyla + Carnivora) and Artiodactyla (including Cetacea)*.' In contrast, according to our results, i.e. mapping the same character states on the recent mammalian tree, the hypothesis that testicondy is the plesiomorphic character state requires the independent origin of scrotum only in Marsupialia and Boreoeutheria. Testicondy also occurs very basally in the mammalian tree (Monotremata, Afrotheria), and the eutherian (i.e. non-marsupial) scrotum does not appear until the origin of Boreoeutheria (Laurasiatheria + Euarchontoglires).

Therefore, we suggest the following evolutionary scenario: testicondy represents the ontogenetically, as well as phylogenetically, initial condition for mammals. Testicular descent and the development of the scrotum appears very soon in the evolutionary history of mammals, probably with the appearance of marsupials, 147.7 Myr ago (Bininda-Emonds *et al*. 2007). The scrotum, however, did not evolve in the lineage leading to Atlantogenata (or alternatively to Afrotheria), in which the testes are pervasively in the ontogenetically primary (testicondy in all Afrotheria except for Tubulidentata) or partially descended (Xenarthra) position. The eutherian scrotum evolved soon after the origin of Boreoeutheria (Laurasiatheria + Euarchontoglires about 100 Myr ago); about 45–50 Myr separate the hypothetical origins of the marsupial scrotum from that of the Boreoeutheria. The descended scrotal position is supposed to be plesiomorphic for all Boreoeutheria. This is significant in Euarchontoglires where the scrotum is well developed in practically all orders; only in caviomorph rodents have the testes undergone descent, but the scrotum did not develop. Despite the fact that Eulipotyphla is evolutionarily the oldest group of Laurasiatheria, the

scrotum therein is not developed and was probably lost early after the origin of Eulipotyphla. During the evolutionary history of Laurasiatheria, the scrotum disappears six times in some terminal taxa: (1) Eulipotyphla (shrews, moles and hedgehogs); (2) Rhinocerotidae (rhinoceros) + Tapiridae (tapirs); (3) Cetacea (whales) + Hippopotamidae (hippopotamus); (4) Pholidota (pangolins); (5) Phocidae (seals); (6) Odobaenidae (walruses). There might be also a particular developmental reason why the diversity of testicular position in Laurasiatheria is more likely due to repeated scrotal loss rather than repeated *de novo* evolution. The inhibition of specific gene expression in the male foetuses of scrotal species, as occurs in some mutant strains of mice, could easily account for the reversal to a descended ascrotal situation, and seems to be mechanistically a much easier process than the repeated *de novo* development of a scrotum. Generally, evolution has proceeded from testicondy through the descended but ascrotal to the descended scrotal condition. There are, however, two exceptions to this simplified view: (1) the scrotum of marsupials appeared very early in mammalian evolution, (2) the scrotum has been reduced in many terminal taxa within Boreoeutheria.

### 4.2	*Adaptive significance of the scrotum and testicular descent*

There are several hypotheses that have been advanced to explain the selective pressures that have led to a scrotal testis. The difficulty is always to avoid the tautology of suggesting that the differences we see in the modern mammalian scrotum might have been causally linked to its evolution. We also need to understand the physiological starting position of early mammals, which had only recently acquired the major innovations of viviparity, lactation and a constant higher core body temperature. Viviparity in particular demanded mechanisms for ensuring that sperm reaching the site of fertilization in the oviduct synchronized functionally, spatially and temporally with the presence of a fertilizable egg. This required adaptations to female endocrinology, to the male gamete and to reproductive behaviour. In particular, it led to a need for sperm storage in the male, as well as to a process of sperm maturation which could hold the maturing sperm indefinitely in an almost finished state, but which completed the maturation process only when in the female tract close to a fertilizable oocyte (i.e. capacitation and hyperactivation). This has led to the evolution of the modern epididymis as a sperm maturation and storage organ.

The evolution of viviparity also led to a reduction in the number of female gametes produced, increasing thereby their individual value, and hence a stronger conservative selection pressure on their physiology. This shifted the emphasis to the male gamete as the generator of genetic variation and

thus the engine of evolution. In modern mammals, genetic variation is introduced into the male gamete by meiosis and recombination, but also by allowing acceptable levels of retroviral activity, as well as naturally occurring mutations caused by irradiation and free radical-induced DNA damage, with the worst products being eliminated by *in vivo* selective mechanisms. While a scrotal testis might encourage more irradiation-induced mutations, a cooler scrotal temperature might prevent these from becoming excessive (Short 1997). Particularly during epididymal storage of sperm, a cooler temperature might be very beneficial in reducing further DNA damage, once the testicular generation of genetic variation is complete.

Along with this, the invention of a scrotum might accelerate the genetic and phenotypic changes in particular mammalian lineages and thus be in part responsible for their fast speciation (cf. species selection – competition between evolutionary lines for higher speciation rate). This implies that scrotal groups should show higher taxic diversity than ascrotal ones. If we compare the diversification rates from Early Eocene onward, when rapid net diversification (evolutionary explosions) of major lineages began, we see that all testicond goups – Monotremata, Afrotheria and Xenarthra – show markedly lower levels of net diversification than Boreoeutheria and marsupials (Bininda-Emonds *et al.* 2007). This indicates that the evolutionary origin of the scrotum possibly played an important role in mammalian radiation and that the evolutionary persistence of a scrotum may have been favoured by species selection.

Considering the cooling hypothesis, there seem to be particular mechanistic reasons why a higher temperature is inconvenient for sperm production. Higher temperatures increase aromatase function causing the conversion of testosterone into oestrogen; testosterone is required for efficient sperm maturation (Bilinska *et al.* 2003). The evolutionary appearance of the scrotum could solve this problem by maintaining a lower temperature and thus also the correct ratios of both hormones (Gilbert and Eppel 2009: 52).

Recently, another convincing argument has been put forward (Gallup *et al.* 2009); that by storing sperm at a temperature significantly below the female core body temperature, there is an automatic temperature trigger of ca +5°C upon direct ejaculation into the female tract. Together with the changed electrolyte and endocrine environment, this might provide a definitive trigger for those events essential for converting mature but stored sperm into activated sperm able to fertilize an egg in the female tract. This hypothesis has the added attraction of also accounting for the small but significant elevation seen in female core body temperature at the beginning of the fertile period in some species (e.g. humans).

While reduced temperature can account for the progressive evolution firstly of descended and subcutaneous

testes and then a scrotal testis independently in marsupials and eutherian mammals, it also explains why the loss of the scrotum in certain aquatic species is always accompanied by a modified blood circulation which maintains a lower testicular and epididymal temperature, for example, in cetaceans.

Nevertheless, our results also provide support for what can be referred to as the 'galloping hypothesis' (Frey 1991; Chance 1996), which is absent in an otherwise thorough discussion by Werdelin and Nilsonne (1999). Testicular descent is a risky and developmentally demanding process that potentially endangers the genetic contribution to future generations; even when not taking into consideration the reduced protection of the extra-abdominal testes encased in a vulnerable scrotum. Frey (1991) suggested that the disadvantages of testicular descent and development of the scrotum would be outweighed by a strong selective advantage, which is a faster mode of locomotion, namely, the gallop. Frey argued: '*the strong flexions and extensions of the vertebral column during gallop should cause intense fluctuations of intra-abdominal pressure. Fluctuations of intra-abdominal pressure severely impede continuous flow of blood in the abdominal veins. Periodically reduced venous drainage resulting in fluctuations of intra-testicular pressure would impair the process of spermiohistogenesis, which is dependent on an absolutely constant pressure within the testis*' (Frey 1991). This problem was solved by displacement of the testes out of the abdominal cavity. A similar explanation of testicular externalization comes from Chance (1996), who argued that the male reproductive tract has no sphincter and its content may be thus expelled by the concussive peritoneal pressure that is produced during galloping, jumping or similar modes of locomotion. The descent of the testes and the origin of a scrotum could thus represent an adaptation that reduces this fluctuation of peritoneal pressure and prevents the loss of seminal fluid. The successive increase of intra-abdominal and intra-gastric pressure during walking, trotting and galloping has been experimentally recorded in horses (Lorenzo-Figueras and Merritt 2002).

The distribution of the scrotal taxa on the phylogenetic tree corresponds to the distribution of mammals with a variety of physiologically demanding modes of terrestrial locomotion (galloping, jumping and leaping). Every such mammal occurs within Marsupialia or Boreoeutheria. Moreover, mammals with testicondy, grouped in Afrotheria and Monotremata, do not gallop. If we focus on Laurasiatheria in more detail (figure 2), we see that all groups that lack a scrotum (character state DA) either do not gallop or inhabit an aquatic environment (shrews, moles, hedgehogs, cetacean, hippos, pangolins, seals and walruses), except for rhinos and tapirs which can gallop, but this can be explained by the presence of subcutaneous testes in both of

these groups; testes which lie in a scrotal-like sac external to the abdominal wall (Ottow 1955). Importantly, subterranean mammals underwent a convergent loss of scrotum (Burda 2003; Nevo 1999). In the groups of Boreoeutheria that do not gallop, the scrotum loses some of its adaptive importance, hence the loss of the scrotum was preferred by selection pressure to keep the testes better protected from the external environment. There are, however, some groups that do not gallop and still retain a scrotum, e.g. primates, some marsupials, microbats, etc. In such cases, the scrotum may be preserved due to a secondary role of this organ such as signalling function (Portmann 1952; Kleisner 2008); this can be true for many primates (e.g. Cercopithecidae) and marsupials (e.g. *Marmosa*, *Caluromys*), in which the scrotum is brightly coloured and visible. In a separate instance, the intra-abdominal pressure of Microchiroptera could fluctuate widely either during active flight or, alternatively, due to strong sagittal flexions of the vertebral column during feeding or landing (Frey 1991). The same problem in birds could have been solved by the functional co-option of the posterior pulmonary extensions of bird respiratory systems (abdominal and caudal thoracic air sacs), which may possibly compensate for the intra-abdominal pressures (and also serve as a cooling device). Alternatively, in some groups of mammals, the presence of a scrotum does not represent any significant disadvantage as it is protected from damage by a particular lifestyle, and thus selective pressure preferring its loss may be weak.

Probably a combination of all three selective pressures suggested by the cooling, the galloping and behavioural signalling hypotheses satisfactorily explains the diversity in testicular position and development of the scrotum in mammals.

5. Conclusions

Testis is a Latin word that signifies 'witness' or 'spectator' as mentioned by Setchell in the introduction to his book *Mammalian testis* (Setchell 1978). Short (1997) referred to the testis as the witness of the mating system. For us, the externalization of the testes is a witness of common descent with various degrees of modification, which allows not only dichotomies but also similarities to arise during the evolutionary process. We conclude that: (1) testicondy represents the plesiomorphic character state for Mammalia, because of the lack of testicular descent in all Monotremata and almost all Afrotheria; (2) the scrotum evolved twice during the evolutionary history of mammals within Marsupialia and Boreoeutheria; (3) the distribution of mammals with a fast mode of locomotion (gallop) is aligned with the distribution of the scrotal life forms on the phylogenetic tree of mammals; at the same time, repeated loss of the scrotum in many taxa within Laurasiatheria occurs

in groups that do not gallop. In particular, we see a loss of the scrotum accompanying other corporeal streamlining in many aquatic taxa, albeit with the development of intra-abdominal gonadal cooling systems. These findings support multiple selection pressures leading to the evolution of a scrotal testis, including a need for controlled cooling below core body temperature, the protection against fluctuation of intra-abdominal pressure due to galloping, and the opportunity to develop exaggerated, honest, intraspecific signals.

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