CURRENT ISSUES - PERSPECTIVES AND REVIEWS

# The Evolution of Large Testes: Sperm Competition or Male Mating Rate?

Karim Vahed* & Darren J. Parker†

* Biological Sciences, School of Sciences, Faculty of Education, Health & Sciences, University of Derby, Derby, UK
† School of Biology, University of St Andrews, St Andrews, UK

**(Invited Review)**

**Correspondence**
Karim Vahed, School of Sciences, Faculty of
Education, Health & Sciences, University of
Derby, Kedleston Road, Derby DE22 1GB, UK.
E-mail: k.vahed@Derby.ac.uk

## Abstract

A positive relationship across species between the extent to which females mate with more than one male and relative testes mass has been demonstrated in a wide range of vertebrate taxa and certain insects. At least two hypotheses, which are not necessarily mutually exclusive, could account for this pattern: (1) the numerical sperm competition hypothesis, which assumes that larger testes enable the male to transfer more sperm to each female, giving the male an advantage in sperm competition and (2) the male mating rate hypothesis, which proposes that larger testes allow the male to produce a greater number of (potentially smaller) ejaculates to engage in frequent copulations with different females. Of these hypotheses, the former has won broad acceptance, while the latter has tended to be dismissed. Here, we argue that the lines of evidence commonly used to support the numerical sperm competition hypothesis in favour of the male mating rate hypothesis are not as clear cut or generally applicable as they are purported to be and that, consequently, the male mating rate hypothesis cannot be excluded with confidence on the basis of the current evidence. Furthermore, some evidence, such as the finding that ejaculate mass and/or sperm number is negatively correlated with testes mass across species in some insects and that larger testes in *Drosophila* can evolve in response to an increase in the number of females available for mating in the laboratory, provides support for the male mating rate hypothesis. Further work is needed to disentangle the relative effects of these selective pressures on the evolution of testes size.

## Introduction

That relative testes size correlates positively, across species, with the extent to which females mate with more than one male might be described as an evolutionary rule. Such a relationship has been demonstrated in mammals (Harcourt et al. 1981, 1995; Kenagy & Trombulak 1986; Møller 1988a; Ginsberg & Rubenstein 1990; Heske & Ostfeld 1990; Kappeler 1997; Hosken 1997, 1998; Ramm et al. 2005; Soulsbury 2010; but see Iossa et al. 2008), birds (Møller 1988b, 1991a; Birkhead & Møller 1992; Møller & Briskie 1995; Pitcher et al. 2005), frogs (Kusano et al. 1991; Jennions & Passmore 1993; Prado & Haddad 2003; Byrne et al. 2002), fish (Stockley et al. 1997; Fitzpatrick et al. 2009; but see Pyron 2000; Kvarnemo & Simmons 2004) and several insect taxa (Gage 1994; Pitnick 1996; Baer & Boomsma 2004; Demary & Lewis 2007; Katvala et al. 2008; Vahed et al. 2011). At least two hypotheses, which are not necessarily mutually exclusive, could account for this pattern: (1) the numerical sperm competition hypothesis (reviewed in Birkhead & Møller 1998; Simmons 2001) and (2) the male mating rate (or

sperm depletion) hypothesis (Short 1979; Cartar 1985; Møller 1991a; Jennions & Passmore 1993; Parker & Ball 2005; Fromhage et al. 2008; Parker & Pizzari 2010). The numerical sperm competition hypothesis assumes that larger testes produce more sperm per ejaculate, giving the male an advantage in numerical sperm competition (reviewed in Parker 1998; Parker & Pizzari 2010). The male mating rate hypothesis proposes that larger testes allow the male to produce a greater number of (potentially smaller) ejaculates to engage in frequent copulations (Parker & Ball 2005; Fromhage et al. 2008; Parker & Pizzari 2010). An increase in the extent of multiple mating in females will, after all, lead not only to an increase in the risk and/or intensity of sperm competition, but since it take two sexes to mate, will also tend to entail an increase in the potential number of mating opportunities available for the male (Svärd & Wiklund 1989; Jennions & Passmore 1993; Parker & Ball 2005; Fromhage et al. 2008; Reuter et al. 2008; Parker & Pizzari 2010). At the population level, all else being equal, male mating rate and the level of sperm competition are perfectly confounded, such that a doubling of one involves a doubling of the other (Parker & Pizzari 2010).

Over the last 30 yr, the numerical sperm competition hypothesis has won broad acceptance, while the arguably less interesting male mating rate hypothesis, although occasionally acknowledged, has tended to be dismissed or neglected in studies pertaining to the evolution of testes size (e.g. Harcourt et al. 1981; Ginsberg & Rubenstein 1990; Møller 1991a; Birkhead & Møller 1992; Gage 1994; Møller & Briskie 1995; Stockley et al. 1997; Kappeler 1997; Gomendio et al. 1998; Hosken 1997, 1998; Byrne et al. 2002; Ramm et al. 2005; Calhim & Birkhead 2006; Fitzpatrick et al. 2009; Lüpold et al. 2009; Ramm & Stockley 2010; Immler et al. 2011; but see also Blanckenhorn et al. 2004; Parker & Ball 2005; Fromhage et al. 2008; Reuter et al. 2008 in which male mating rate has been taken into account). For example, several studies have used relative testes mass as an index of the level of sperm competition, neglecting to mention that it could equally represent an index of male mating rate (e.g. Gage & Freckleton 2003; Gomendio et al. 2006; Lüpold et al. 2009; Iossa et al. 2008; Immler et al. 2011).

## Arguments Traditionally Used to Favour the Numerical Sperm Competition Hypothesis

Some of the arguments commonly used to support the numerical sperm competition hypothesis in favour of the male mating rate hypothesis are as follows (e.g. Møller & Briskie 1995; Stockley et al. 1997; Calhim & Birkhead 2006; Parker & Pizzari 2010; Wedell & Hosken 2010; Immler et al. 2011): (1) there is a positive correlation across species between the relative size of the testes and the relative number of sperm per ejaculate (e.g. Møller 1988a,b, 1989; Stockley et al. 1997); (2) there is a positive correlation across species between the relative size of the testes (and/or degree of polyandry) and the rate of sperm production (e.g. Møller 1989; Lüpold et al. 2009; Ramm & Stockley 2010) and size of the male's sperm reserves (e.g. Birkhead et al. 1993); (3) males in the laboratory adjust the number of sperm per ejaculate or testes size in response to an increase in the perceived level of sperm competition (reviewed in Parker et al. 1997; Kelly & Jennions 2011); (4) in species with alternative reproductive tactics in males, testes are larger in 'sneaker' than in 'dominant' male morphs (reviewed in Simmons et al. 1999; Oliveira et al. 2008; Taborsky & Neat 2010); (5) experimental evolution experiments in the laboratory have demonstrated a change in testes size over successive generations in laboratory populations in which the level of sperm competition has been manipulated (reviewed in Crudgington et al. 2009; Edward et al. 2010); and (6) testes are not proportionately larger in polygynous species in which males have either extreme harem-defence or lek-based mating systems than they are in monogamous species (e.g. Harcourt et al. 1981; Møller 1991a; Birkhead & Møller 1992). While such evidence is, on the face of it, very persuasive, some of these lines of evidence may not be as clear cut or as generally applicable as they are purported to be and may not actually exclude the male mating rate hypothesis.

### (1) Interspecific Relationships Between Testes Size and the Number of Sperm Per Ejaculate

While the existence of a positive relationship between relative testes size and relative sperm number is an assumption of the numerical sperm competition hypothesis (reviewed in Parker 1998; Parker & Pizzari 2010), the male mating rate hypothesis, if anything, predicts the opposite: if sperm are limited, males may be selected to transfer less sperm per mating to maximise the number of females inseminated (Parker & Ball 2005; Fromhage et al. 2008). A positive relationship between relative testes size and relative sperm number across species has been reported in mammals, birds and fish (e.g. Møller

1988a,b, 1989; Stockley et al. 1997; Fitzpatrick et al. 2009; Immler et al. 2011). However, in vertebrates, sperm counts need to be interpreted with caution (Birkhead 1998). Data on sperm per ejaculate in nature are difficult to obtain for these taxa; hence, sperm counts have been obtained by artificial means such as electroejaculation (Møller 1988a), manual extrusion of ejaculates (Møller 1988b; Stockley et al. 1997; Immler et al. 2011) or counts of sperm taken directly from the testes (Fitzpatrick et al. 2009). Such techniques could easily lead to overestimates of the number of sperm per ejaculate by stripping out sperm reserves that might, in reality, have been used over a series of successive copulations, potentially involving different females (Birkhead 1998).

A positive correlation across species between the relative size of the testes and sperm number per ejaculate and/or ejaculate mass does not occur in certain insect taxa including bushcrickets (Tettigoniidae) (Vahed et al. 2011), *Drosophila* species (Pitnick 1996; see also Immler et al. 2011) and Bruchid beetles (Katvala et al. 2008). In bushcrickets, while the relationship between testes size and sperm number across species was not significant, the trend was negative and close to significance (Vahed et al. 2011; K. Vahed, unpubl. data). For example, the sperm count for *Platycleis affinis* (largest testes, at 13.8% male body mass) was about three times *smaller* than that for *Gampsocleis glabra* (smallest testes, at 1% male body mass), even though males of both species were of similar body mass (Vahed et al. 2011). Furthermore, ejaculate mass was significantly smaller in species with larger testes (Vahed et al. 2011) and in more polyandrous species (Vahed 2006; Vahed et al. 2011). Across *Drosophila* species, the relationship between an index of sperm number based on sperm extracted from the testes and relative testes mass was non-linear, peaking at intermediate testes size then decreasing with testes mass (Immler et al. 2011). The association of large testes with lower sperm counts was even more pronounced when based on the actual number of sperm per ejaculate (Pitnick 1996). These data from studies of insects would appear to support the male mating rate hypothesis rather than the sperm competition hypothesis.

There are many differences in the reproductive biology of vertebrates and insects that could lead to differences in the nature of the relationship between testes size and sperm numbers across species. Simmons (2001) cautioned that in insects, a simple 'cause and effect' relationship between female mating frequency and testes size or sperm number might

not be expected because of the diversity of sperm competition mechanisms. For one thing, it seems probable that numerical sperm competition (the 'raffle' mechanism of sperm competition) is likely to be more prevalent in many vertebrate taxa than it is in insects (Birkhead & Møller 1998; Simmons 2001; Immler et al. 2011). Furthermore, while most models of the evolution of testes and ejaculate size assume that these traits in males respond to changes in mating patterns in females (Parker 1998; Parker & Ball 2005), in many insects, the causal relationship might be reversed (Simmons 2001). In Tettigoniidae, for example, the association of large ejaculates with a lower degree of polyandry across species could be caused by non-sperm ejaculate substances produced by the accessory glands that affect female receptivity to further copulations (Vahed 2006, 2007; Vahed et al. 2011). In *Drosophila* species, the association of lower sperm counts per ejaculate with a higher level of polyandry might occur because females are sperm limited in such species and therefore need to mate more frequently to maintain fertility (Pitnick 1996; Simmons 2001). Whatever the cause/effect nature of the relationship between polyandry and ejaculate size, it is nevertheless clear that in bushcrickets and *Drosophila*, the species with the largest testes do not transfer more sperm per ejaculate and instead appear to be adapted to allocate sperm to a greater number of females (Pitnick 1996; Pitnick & Markow 1994; Vahed et al. 2011).

A negative correlation between relative testes size and the number of sperm per ejaculate across species could result from a macro-evolutionary trade-off between sperm size and sperm numbers (Pitnick 1996; Simmons 2001; Immler et al. 2011). After all, an increase in sperm numbers is not the only way of increasing sperm competitiveness; an increase in the level of sperm competition might also select for traits such as sperm size, amongst other things (reviewed in Simmons 2001; Ramm & Stockley 2010; Immler et al. 2011). Evidence suggests that across species, larger testes can be associated with longer sperm (e.g. Gage 1994; Pitnick 1996; Morrow & Gage 2000; Fitzpatrick et al. 2009; Immler et al. 2011; but see Stockley et al. 1997; Gage & Freckleton 2003). Immler et al. (2011) provided evidence for a macro-evolutionary trade-off between sperm size and the number of sperm in sperm reserves in male birds and in *Drosophila*. The nature of this trade-off appeared to be different in the two taxa: in *Drosophila*, species with the longest sperm (and largest testes) produced relatively fewer sperm (see also Pitnick 1996), while in birds, sperm number appeared

to be maximised at the expense of sperm length (Immler et al. 2011). However, such a trade-off does not appear to be the full explanation for the small numbers of sperm allocated *per ejaculate* in *Drosophila* species with large testes: the absolute number of sperm extracted from the testes was still relatively high in species with large testes such as *Drosophila hydei* (compare testicular sperm in Fig. 1 with sperm per ejaculate in Table 2 in Pitnick 1996). Males of such species, however, do not transfer all of their testicular sperm per mating. Instead they prudently allocate this over successive copulations (Pitnick 1996; Wedell et al. 2002). This is consistent with selection to increase the number of females inseminated.

## (2) Interspecific Relationships Between Testes Size (and/or the Degree of Polyandry) and the Rate of Sperm Production and Sperm Reserves in Males

The size of the testes, of course, is not perhaps the trait that is the main target of selection here, but instead the density of sperm producing tissue and the rate of sperm production (Schärer & Vizoso 2007; Lüpold et al. 2009; Ramm & Stockley 2010). Evidence suggests that these factors do seem to increase with relative testes size and the extent of multiple mating in females, across taxa (e.g. in mammals: Møller 1989; Ramm & Stockley 2010; in birds: Lüpold et al. 2009). This has been cited as evidence to support the numerical sperm competition hypothesis (Møller 1989; Parker et al. 1997; Lüpold et al. 2009; Ramm & Stockley 2010), yet it could equally support the male mating rate hypothesis, allowing males to replenish sperm reserves rapidly to capitalise on extra mating opportunities.

In species with large testes and/or species in which females mate multiply, males are clearly adapted for a high frequency of mating: they often have large sperm reserves and allocate sperm over successive copulations (Møller 1989; Birkhead et al. 1993; Pitnick & Markow 1994; Pitnick 1996; Wedell et al. 2002). For example, male sheep have reserves for up to 95 successive copulations (Møller 1989), and male birds store sperm in specialised cloacal protuberances, the relative size of which correlates positively with relative testes mass and male mating frequency (Birkhead et al. 1993). In frogs, species with very large testicles such as *Chiromantis xerampelina* have an exceptionally high mating frequency in males in comparison with other anurans (Jennions & Passmore 1993). In *Drosophila* species with large testes such as *D. hydei*, males do not transfer all of

their sperm per mating and have a very high mating frequency, mating with up to ten different females in a single morning (Markow 1985; Pitnick 1996; Pitnick & Markow 1994). In bushcrickets, males of more polyandrous species have much shorter recovery times between successive copulations (Vahed 2007). Similarly, in butterflies, males of species in which females are more polyandrous are better adapted to mate more than once and are able to replenish ejaculate mass more rapidly (Svärd & Wiklund 1989; Bissoondath & Wiklund 1996).

Obviously a distinction needs to be made here between repeated copulations that occur within pairs and multiple copulations involving different females. In the former case, an increased mating rate in males can be equated with an increase in the number of sperm inseminated in each female (and/or an increased chance that the male's sperm will occupy a favoured position or be deposited at the correct time for use in fertilisation) and thus with the likely success of the male in sperm competition (Ginsberg & Huck 1989; Birkhead & Møller 1992; Birkhead 1998). Such frequent re-mating within pairs has been documented in various mammals and birds and in some insects (reviewed in Ginsberg & Huck 1989; Birkhead 1998; Birkhead et al. 1993; Simmons 2001). In the case of frequent copulation involving a series of different females, on the other hand, such behaviour cannot be viewed as an adaptation to sperm competition. In the case of butterflies and bushcrickets, the increased mating propensity of males of species in which females are more polyandrous appears to facilitate frequent copulation involving a series of different females as opposed to frequent copulation within pairs (Svärd & Wiklund 1989; Bissoondath & Wiklund 1996; Vahed 2007). Therefore, although it has been cited as evidence to support the numerical sperm competition hypothesis (e.g. Møller 1991b; Parker et al. 1997; Gomendio et al. 1998; Simmons 2001), the increased mating propensity of males of such species would seem to offer much stronger support to the male mating rate hypothesis.

## (3) The Effects of an Increase in the Perceived Level of Sperm Competition on the Number of Sperm Per Ejaculate or Testes Size

There is a body of evidence to suggest that males can adjust the number of sperm per ejaculate in direct response to an increase in the perceived level of sperm competition (reviewed in Wedell et al. 2002; Parker & Pizzari 2010; Kelly & Jennions

2011). While such evidence is not directly relevant to the evolution of larger testes, it does give an indication of the selective pressures that could shape ejaculate allocation on a broader scale. Several studies have compared the number of sperm transferred per mating in an isolated pair with that of a pair in the presence of one or more competing males. According to a recent meta-analysis (Kelly & Jennions 2011), there is strong evidence from such studies involving a range of taxa that ejaculate size increases when males are exposed to a single rival (although the effect of more than one rival on ejaculate size overall was not significant). This is interpreted as evidence that males allocate sperm in response to an increase in the risk of sperm competition (i.e. the probability that sperm competition will occur), in line with theoretical expectations (Parker et al. 1997; Parker 1998; Wedell et al. 2002; Parker & Pizzari 2010; Kelly & Jennions 2011). While this evidence is undoubtedly very strong, it is conceivable that males might be adjusting the number of ejaculated sperm in response to the level of perceived future mating opportunities, rather than the level of sperm competition: situations in which a rival male is present during mating could indicate that the sex ratio is male biased, thus there would be less benefit to the male of conserving sperm supplies for future mating opportunities.

Some studies have demonstrated a developmental alteration of investment in sperm production in response to social cues (such as rearing density) that could be linked to the level of sperm competition (reviewed in Parker et al. 1997; Kelly & Jennions 2011). For example, Gage (1995) found that Indian meal moths (*Plodia interpunctella*) reared at high larval density subsequently produced larger testes than those reared at lower density. Although this has been interpreted as supporting predictions of sperm competition theory (Gage 1995; Parker et al. 1997), it could equally support the male mating rate hypothesis: at high population density, the availability of mates is likely to be greater than at low population density; hence, there would be greater potential for males to engage in a multiple mating strategy.

## (4) Differences in Testes Size Between Sneaker and Dominant Males in Species with Alternative Reproductive Tactics

In some species, two or more distinct male morphs exist with alternative reproductive tactics: dominant (or 'bourgeois') males invest in traits linked to driv-

ing away other males and attracting females, while sneaker (or 'parasitic') males tend to be smaller, less well armed and attempt to fertilise eggs of females that have been attracted to the dominant males (reviewed in Oliveira et al. 2008; Taborsky & Neat 2010). Relative to male body mass, testes tend to be larger in sneaker males in, for example, several species of fish and certain dung beetles (reviewed in Simmons et al. 1999; Taborsky & Neat 2010), and sneaker males may produce a greater density of sperm per ejaculate (Neff et al. 2003). This is consistent with the predictions of sperm competition theory because sneaker males are always subject to sperm competition by the dominant male they are attempting to usurp, while dominant males will generally be subject to a lower level of sperm competition, although this depends upon the number of sneaks in the population (Gross 1982; Parker 1990; Simmons et al. 1999; Neff et al. 2003). The difference in relative (but not in absolute) testes size between dominant and sneaker male morphs does not, on the other hand, appear to support the male mating rate hypothesis because some studies indicate that sneaker males have fewer mating opportunities in comparison with dominant males (e.g. Neff et al. 2003; Sato et al. 2004; Taborsky & Neat 2010; but see Schuster & Wade 1991). The generality of this pattern requires formal testing, however. Unlike the differences in relative testes mass between male morphs, the relationship between relative testes mass and male body mass within male morphs does lend some support to the male mating rate hypothesis: in bluegill sunfish (*Lepomis macrochirus*), for example, within 'dominant' (or 'parental') male morphs, larger males obtain more mating opportunities but experience a lower risk of sperm competition and such males invest proportionately more in their testes (Neff et al. 2003; see also Blanckenhorn et al. 2004).

Although the differences in relative testes size between sneaker and dominant males are consistent with the numerical sperm competition hypothesis, the level of sperm competition is not the only variable that is likely to differ between males of the different morphs: the level of pre-mating sexual selection and the level of parental care shown by the male are confounding factors because they are likely to be greater in the case of the dominant male morphs (Simmons et al. 1999; Taborsky & Neat 2010). The relatively smaller testes in such males could therefore be the result of a trade-off between resources allocated to testes size and those allocated to armaments, ornaments, territory defence and

parental care. Such a trade-off between weapon size and testes size has been demonstrated in dung beetles (Simmons & Emlen 2006).

## (5) The Effect of Altering the Level of Sperm Competition on Testes Size as Demonstrated by Experimental Evolution Experiments in the Laboratory

Several studies have demonstrated a decrease in testes size over successive generations in laboratory populations in which monogamy was enforced in comparison with laboratory populations in which females were mated with multiple males, for example in the dung fly *Scathophaga stercoraria* and the dung beetle *Onthophagus taut-us* (Hosken & Ward 2001; Hosken et al. 2001; Simmons & Garcia-Gonzalez 2008; reviewed in Crudgington et al. 2009; Edward et al. 2010). While these studies have been interpreted as demonstrating that sperm competition selects for larger testes, in monogamous lines, the mating rate of males is likely to have been lower than in natural populations. Furthermore, in some of these studies (e.g. Hosken & Ward 2001; Simmons & Garcia-Gonzalez 2008), both males and females in the polyandrous lines experienced an elevated number of copulations in comparison with those in the monogamous lines. Therefore, selection for a male's ability to engage in multiple copulations could also have been at play, although Hosken & Ward (2001) dismissed this possibility because the natural mating frequency of males in the field in their study species was higher than in their 'polyandrous' group. Nevertheless, the possibility that the removal of the opportunity to engage in multiple copulations could have been a factor in the evolution of smaller testes in the monogamous lines cannot be excluded.

One way to disentangle the relative effects of sperm competition and male mating rate on the evolution of testes size in studies involving experimental evolution is to examine the effect of altering the sex ratio in species in which females will mate with more than one male (Reuter et al. 2008; Crudgington et al. 2009): with male-biased sex ratios, the level of sperm competition will be high, but the potential mating frequency of males will be low. Conversely, with increasingly female-biased sex ratios, the level of sperm competition will be lower and the potential mating frequency of males will be higher (Reuter et al. 2007). Recent studies of this type have provided support for the male mating rate hypothesis rather than the sperm competition hypothesis: Crudgington et al. (2009) found no effect of male-biased sex ratios on the evolution of testes mass in laboratory populations of *Drosophila pseudoobscura*, while Reuter et al. (2008) found that male *Drosophila melanogaster* in lines with a strongly female-biased sex ratio (10:1) evolved larger testes than males in either less female-biased or monogamous lines. The level of pre-mating sexual selection may be a confounding factor in such studies, however (Engqvist & Reinhold 2005): increasing the male bias of a population's sex ratio might increase the level of sperm competition, but it will also increase the pre-mating components of sexual selection. Increasing the female bias of a population's sex ratio, on the other hand, might relax pre-mating sexual selection. Traits selected for by inter- or intra-sexual selection prior to mating could potentially trade-off with testes size (Engqvist & Reinhold 2005).

## (6) Testes Size in Polygynous Species

Frequently cited evidence against the male mating rate hypothesis (and in favour of the numerical sperm competition hypothesis) is that in polygynous species in which males either defend harems and/or mate in leks (groups of displaying males), the potential mating frequency of males should be relatively high (although skewed in favour of selected males), yet it has been reported that the testes are not significantly larger than in monogamous species and are smaller than in species in which females mate with multiple males (in primates: Harcourt et al. 1981, 1995; Møller 1988a; in Birds: Møller 1991a; Birkhead & Møller 1992). For example, in primates with harem-based mating systems such as gorillas (*Gorilla gorilla*), the testes are much smaller than in species with multimale mating systems such as the common chimpanzee (*Pan troglodytes*), in which several males copulate with each female that comes into oestrus within the social group (Short 1979; Harcourt et al. 1981, 1995). Yet, it could be that the mating frequency of males in harem-forming species is not as high as in species with multimale mating systems, especially if the number of females available for mating within the harem at any given time is small and/or harem size is small. In gorillas, each female is only sexually receptive for one to 2 d in the 32-d reproductive cycle (Nadler 1980; Harcourt et al. 1980), while female chimpanzees are receptive for 10 d in a similar oestrus cycle (Harcourt et al. 1980). Furthermore, in Gorillas, the mean harem size in the field (about 2, Clutton-Brock & Harvey 1977; or 3.5, Breuer et al. 2010) is considerably smaller than

the number of females likely to be available for mating within a social group of chimpanzees (Nishida 1997). For example, the mean number of partners per male recorded within just a 3-mo period in a social group of chimpanzees was 5.5, and the number of adult females in the social group can be considerably higher than this (Nishida 1997; see also Goodall 1986).Thus not only do male chimpanzees copulate more frequently per female, crucially they also copulate with a greater number of females than do male gorillas (Short 1979; Nadler 1980; Harcourt et al. 1980; Breuer et al. 2010). This pattern is also repeated on a wider scale: in Harcourt et al.'s (1995) comparative study of testes size and mating system in 58 primate species, social groups in species with multimale mating systems consistently contained more adult females (more than ten females per group) than those with single-male mating systems (less than ten females per group).

In birds, comparative studies have demonstrated that testes *are* relatively larger in species classified as having 'polygynous' mating systems than in species with 'solitary and monogamous' mating systems (although they are not as large as those of 'polyandrous' species; Cartar 1985; Møller 1988b, 1991a; Birkhead & Møller 1992; Pitcher et al. 2005). This has been cited as evidence to support the numerical sperm competition hypothesis because males of polygynous bird species tend to lack paternity guards such as mate guarding and repeated mating with the same female and 'extra-pair' copulations by females are relatively high (Møller 1988b; Birkhead & Møller 1992; Møller & Briskie 1995). The difference in relative testes size between monogamous and polygynous bird species, however, is also predicted by the male mating rate hypothesis (Cartar 1985). Evidence against the male mating rate hypothesis, on the other hand, comes from studies that have reported that in species of bird with lek-based mating systems, the testes are not relatively larger than in monogamous species (Møller 1991a; Birkhead & Møller 1992). A more recent comparative study, which was based on a much larger sample size than previous studies, however, reported that relative testes size *is* significantly larger in species with lek-based mating systems than in monogamous species (Pitcher et al. 2005).

In birds, the male mating rate hypothesis has also been dismissed because one study (Møller 1991a) found no significant relationship, across taxa, between the rate of copulation in males and the relative mass of the testes (Birkhead & Møller 1992; Calhim & Birkhead 2006). This evidence would appear to be very weak, however, because copulation rates in males in the study by Møller (1991a) were based on assumptions rather than on actual data. Møller's (1991a) conclusions also appear to contrast with those of Birkhead et al. (1993), who found that in species in which males copulate frequently, males have larger sperm reserves, and that species with larger sperm reserves have relatively larger testes. Obviously, however, a distinction needs to be made between the mating rate of males within pairs and with different females (see section 2). In studies of birds (e.g. Møller 1991a), it is not always clear which is being referred to. Overall, the grounds for dismissing the male mating rate hypothesis for the evolution of testes size in birds would not appear to be strong.

## Conclusions

While sperm competition is undoubtedly an important selective force in the evolution of large testes, the role of the confounding factor of selection for increased mating rate in males cannot be excluded with confidence on the basis of the current evidence. Furthermore, there are several lines of evidence to support the male mating rate hypothesis such as the finding that ejaculate mass and/or sperm number per ejaculate is smaller in certain insects with larger testes (e.g. Pitnick 1996; Vahed et al. 2011), that males of species with a high level of polyandry are better adapted to mate at a higher frequency (e.g. Svärd & Wiklund 1989; Bissoondath & Wiklund 1996; Vahed 2007) and that larger testes can evolve in response to an increase in the number of females available for mating (e.g. Reuter et al. 2008). Further studies are needed to disentangle the relative effects of selection for increased mating rate in males and the level of sperm competition on the evolution of testes size. Given that, at the population level, the level of sperm competition and male mating rate are perfectly confounded (Parker & Pizzari 2010), disentangling the effects of these selective pressures will obviously not be straight forward.

One way to distinguish the numerical sperm competition and the male mating rate hypotheses for the evolutionary enlargement of testes in comparative studies is that while the former hypothesis generally predicts that the number of sperm transferred per mating will increase with relative testes mass across taxa, the latter potentially predicts the opposite (see section 1). So far, evidence for a negative relationship across taxa between relative testes mass and the number of sperm per ejaculate or ejaculate mass has

only been found in certain insects and not in vertebrates (section 1). This may simply be because the number of sperm per ejaculate is often easier to quantify in insects than it is in vertebrates. More data on the number of sperm transferred in natural copulations or spawning events in vertebrate taxa are needed.

More data are needed on the mating rate of males in selected taxa and the extent to which it occurs within – as opposed to between – pairs. This would enable the predictions to be tested that relative testes mass will increase with the extent to which males mate with different females across taxa (male mating rate hypothesis) or with the level of within-pair copulations (sperm competition *and* mating rate hypotheses, sections 2 and 6). Such data would also enable an assessment to be made as to whether males of species with a higher rate of sperm production and/or larger sperm reserves (section 2) mate more rapidly with a series of different females (mating rate hypothesis) or transfer a greater number of sperm per female (sperm competition hypothesis). The relative mating rate of 'dominant' and 'sneaker' male morphs that differ in testes mass could also be examined further (section 4).

To disentangle the male mating rate and the numerical sperm competition hypotheses, comparative studies ideally need to be designed in which the level of sperm competition across taxa remains constant with increases in the mating rate of males. One potential way of doing this would be to test for a positive relationship between harem size (or more specifically, the rate at which males mate with different females within the harem) and relative testes size in species with 'single-male' mating systems. To our knowledge, this has not been done in mammals, even though data on harem size and testes size are available for a range of taxa (e.g. in primates: Clutton-Brock & Harvey 1977; Harcourt et al. 1981, 1995). One difficulty would be that it would obviously not be safe to assume that males within a harem control exclusive mating access to females within their harems, and quantitative data on the level of sperm competition (e.g. the degree of mixed paternity within the harem) may not be available for a sufficient number of species. A further difficulty would be controlling for the potentially confounding effects of differences in the level of pre-copulatory sexual selection between taxa differing in harem size.

In laboratory studies involving experimental evolution, altering the sex ratio (in species in which females will mate with more than one male) provides a way of disentangling the relative effects of sperm competition and male mating rate on the evolution of testes size (section 5). The potentially confounding effect of differences in the level of pre-copulatory sexual selection between treatments is, however, a problem with such experiments (Engqvist & Reinhold 2005). Experimental evolution experiments could be designed to examine the effect of increased male mating rate on the evolution of testes size in which both the level of sperm competition and the level of pre-copulatory sexual selection are controlled. For example, instead of having free-mating populations, lines differing in mating rate in males could be created by allocating females of the same mating status (i.e. virgins) to males in succession.

The male mating rate and the numerical sperm competition hypotheses are, of course, not mutually exclusive: males could be selected to maximise both the proportion of eggs they fertilise per mating and the number of females inseminated, although if sperm production is limited, there is likely to be a trade-off between these two factors (Parker & Ball 2005; Parker & Pizzari 2010). Asking what factors affect how such a trade-off is resolved and attempting to disentangle the relative effects of these selective pressures on the evolution of testes size and ejaculate traits (e.g. Reuter et al. 2008) would seem to be a more constructive approach than one of simply dismissing the role of selection for male mating rate.

## Acknowledgements

## Literature Cited

Baer, B. & Boomsma, J. J. 2004: Male reproductive investment and queen mating-frequency in fungus-growing ants. Behav. Ecol. **15**, 426—432.

Birkhead, T. R. 1998: Sperm competition in birds: mechanisms and function. In: Sperm Competition and Sexual Selection (Birkhead, T. R. & Møller, A. P., eds). Academic Press, London, pp. 579—622.

Birkhead, T. R. & Møller, A. P. 1992: Sperm Competition in Birds: Evolutionary Causes and Consequences. Academic Press, London.

Birkhead, T. R. & Møller, A. P. (eds) 1998: Sperm Competition and Sexual Selection. Academic Press, London.

Birkhead, T. R., Briskie, J. V. & Møller, A. P. 1993: Male sperm reserves and copulation frequency in birds. Behav. Ecol. Sociobiol. **32**, 85—93.

Bissoondath, C. J. & Wiklund, C. 1996: Male butterfly investment in successive ejaculates in relation to mating system. Behav. Ecol. Sociobiol. **39**, 285—292.

Blanckenhorn, W. U., Hellriegel, B., Hosken, D. J., Jann, P., Altwegg, R. & Ward, P. I. 2004: Does testes size track expected mating success in yellow dung flies? Funct. Ecol. **18**, 414—418.

Breuer, T., Robbins, A. M., Olejniczak, C., Parnell, R. J., Stokes, E. J. & Robbins, M. M. 2010: Variance in male reproductive success of western gorillas: acquiring females is just the beginning. Behav. Ecol. Sociobiol. **64**, 515—528.

Byrne, P. G., Roberts, J. D. & Simmons, L. W. 2002: Sperm competition selects for increased testes mass in Australian frogs. J. Evol. Biol. **15**, 347—355.

Calhim, S. & Birkhead, T. R. 2006: Testes size in birds: quality versus quantity – assumptions, errors, and estimates. Behav. Ecol. **18**, 271—275.

Cartar, R. V. 1985: Testes size in sandpipers. Naturwissenschaften, **72**, 157—158.

Clutton-Brock, T. H. & Harvey, P. H. 1977: Primate Ecology and social organisation. J. Zool. **183**, 1—39.

Crudgington, H. S., Fellows, S., Badcock, N. S. & Snook, R. R. 2009: Experimental manipulation of sexual selection promotes greater male mating capacity but does not alter sperm investment. Evolution **63**, 926—938.

Demary, K. C. & Lewis, S. M. 2007: Male reproductive allocation in fireflies (*Photinus* spp.). Invertebr. Biol. **126**, 74—80.

Edward, D. A., Fricke, C. & Chapman, T. 2010: Adaptations to sexual selection and sexual conflict: insights from experimental evolution and artificial selection. Philos. Trans. R. Soc. B **365**, 2541—2548.

Engqvist, L. & Reinhold, K. 2005: Pitfalls in experiments testing predictions from sperm competition theory. J. Evol. Biol. **18**, 116—123.

Fitzpatrick, J. L., Montgomerie, R., Desjardins, J. K., Stiver, K. A., Kolm, N. & Balshine, S. 2009: Female promiscuity promotes the evolution of faster sperm in cichlid fishes. Proc. Natl Acad. Sci. USA **106**, 1128—1132.

Fromhage, L., McNamara, J. H. & Houston, A. I. 2008: Sperm allocation strategies and female resistance: a unifying perspective. Am. Nat. **172**, 25—33.

Gage, M. J. G. 1994: Associations between body size, mating pattern, testes size and sperm lengths across butterflies. Proc. R. Soc. Lond. B **258**, 247—254.

Gage, M. J. G. 1995: Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. Proc. R. Soc. Lond. B **261**, 25—30.

Gage, M. J. G. & Freckleton, R. P. 2003: Relative testes size and sperm morphometry across mammals: no evidence for an association between sperm competition and sperm length. Proc. R. Soc. Lond. B **270**, 625—632.

Ginsberg, J. R. & Huck, U. W. 1989: Sperm competition in mammals. Trends Ecol. Evol. **4**, 74—79.

Ginsberg, J. R. & Rubenstein, D. I. 1990: Sperm competition and variation in zebra mating behavior. Behav. Ecol. Sociobiol. **26**, 427—434.

Gomendio, M., Harcourt, A. H. & Roldán, E. R. S. 1998: Sperm competition in mammals. In: Sperm Competition and Sexual Selection (Birkhead, T. R. & Møller, A. P., eds). Academic Press, London, pp. 667—751.

Gomendio, M., Martin-Coello, J., Crespo, C., Magaña, C. & Roldan, E. R. S. 2006: Sperm competition enhances functional capacity of mammalian spermatozoa. Proc. Natl Acad. Sci. USA **103**, 15113—15117.

Goodall, J. 1986: The Chimpanzees of Gombe: Patterns of Behaviour. Belknap press (Harvard Univ. Press), Cambridge, MA.

Gross, M. R. 1982: Sneakers, satellites, and parentals: polymorphic mating strategies in North American sunfishes. Z. Tierpsychol. **60**, 1—26.

Harcourt, A. H., Fossey, D., Stewart, K. J. & Watts, D. P. 1980: Reproduction in wild gorillas and some comparisons with chimpanzees. J. Reprod. Fertil. Suppl. **28**, 59—70.

Harcourt, A. H., Harvey, P. H., Larson, S. G. & Short, R. V. 1981: Testes weight, body weight and breeding system in primates. Nature **293**, 55—57.

Harcourt, A. H., Purvis, A. & Liles, L. 1995: Sperm competition: mating system, not breeding season, affects testes size of primates. Funct. Ecol. **9**, 468—476.

Heske, E. J. & Ostfeld, R. S. 1990: Sexual dimorphism in size, relative size of testes and mating systems in North American voles. J. Mammal. **71**, 510—519.

Hosken, D. J. 1997: Sperm competition in bats. Proc. R. Soc. Lond. B **264**, 385—392.

Hosken, D. J. 1998: Testes mass in megachiropteran bats varies in accordance with sperm competition theory. Behav. Ecol. Sociobiol. **44**, 169—177.

Hosken, D. J. & Ward, P. I. 2001: Experimental evidence for testes size evolution via sperm competition. Ecol. Lett. **4**, 10—13.

Hosken, D. J., Garner, T. W. J. & Ward, P. I. 2001: Sexual conflict selects for male and female reproductive characters. Curr. Biol. **11**, 489—493.

Immler, S., Pitnick, S., Parker, G. A., Durrant, K. L., Lüpold, S., Calhim, S. & Birkhead, T. R. 2011: Resolving variation in the reproductive tradeoff between sperm size and number. Proc. Natl Acad. Sci. USA **108**, 5325—5330.

Iossa, G., Soulsbury, C. D., Baker, P. J. & Harris, S. 2008: Sperm competition and the evolution of testes size in terrestrial mammalian carnivores. Funct. Ecol. **22**, 655—662.

Jennions, M. D. & Passmore, N. I. 1993: Sperm competition in frogs: testes size and a 'sterile male' experiment

on *Chiromantis xerampelina* (Rhacophoridae). Biol. J. Linn. Soc. **50**, 211—220.

Kappeler, P. M. 1997: Intrasexual selection and testes size in strepsirhine primates. Behav. Ecol. **8**, 10—19.

Katvala, M., Roenn, J. L. & Arnqvist, G. 2008: Correlated evolution between male ejaculate allocation and female remating behaviour in seed beetles (Bruchidae). J. Evol. Biol. **21**, 471—479.

Kelly, C. D. & Jennions, M. D. 2011: Sexual selection and sperm quantity: meta-analysis of strategic ejaculation. Biol. Rev. **86**, 863—884.

Kenagy, G. J. & Trombulak, S. C. 1986: Size and function of mammalian testes in relation to body size. J. Mamm. **67**, 1—22.

Kusano, T., Toda, M. & Fukuyama, K. 1991: Testes size and breeding systems in Japanese anurans with special reference to large testes in the treefrog *Rhacophorus arboreus* (Amphibia: Rhacophoridae). Behav. Ecol. Sociobiol. **29**, 27—31.

Kvarnemo, C. & Simmons, L. W. 2004: Testes investment and spawning mode in pipefishes and seahorses (Syngnathidae). Biol. J. Linn. Soc. **83**, 369—376.

Lüpold, S., Linz, G. M., Rivers, J. W., Westneat, D. F. & Birkhead, T. R. 2009: Sperm competition selects beyond relative testes size in birds. Evolution **63**, 391—402.

Markow, T. A. 1985: A comparative investigation of the mating system of *Drosophila hydei*. Anim. Behav. **33**, 775—781.

Møller, A. P. 1988a: Ejaculate quality, testes size and sperm competition in primates. J. Hum. Evol. **17**, 479—488.

Møller, A. P. 1988b: Testes size, ejaculate quality, and sperm competition in birds. Biol. J. Linn. Soc. **33**, 273—283.

Møller, A. P. 1989: Ejaculate quality, testes size and sperm production in mammals. Funct. Ecol. **3**, 91—96.

Møller, A. P. 1991a: Sperm competition, sperm depletion, paternal care and relative testes size in birds. Am. Nat. **137**, 882—906.

Møller, A. P. 1991b: Sperm competition and sperm counts: an evaluation of the empirical evidence. In: Comparative Spermatology 20 Years Later (Baccetti, B., ed.). Raven Press, New York, pp. 775—777.

Møller, A. P. & Briskie, J. V. 1995: Extra-pair paternity, sperm competition and the evolution of testes size in birds. Behav. Ecol. Sociobiol. **36**, 357—365.

Morrow, E. H. & Gage, J. G. 2000: The evolution of sperm length in moths. Proc. R. Soc. Lond. B **267**, 307—313.

Nadler, R. D. 1980: Reproductive physiology and behaviour of gorillas. J. Reprod. Fertil. Suppl. **28**, 79—89.

Neff, B. D., Fu, P. & Gross, M. R. 2003: Sperm investment and alternative mating tactics in bluegill sunfish (*Lepomis macrochirus*). Behav. Ecol. **14**, 634—641.

Nishida, T. 1997: Sexual behavior of adult male chimpanzees of the Mahale Mountains National Park, Tanzania. Primates **38**, 379—398.

Oliveira, R. F., Taborsky, M. & Brockmann, H. J. (eds) 2008: Alternative Reproductive Tactics: An Integrative Approach. Cambridge Univ. press, Cambridge.

Parker, G. A. 1990: Sperm competition games: sneaks and extra-pair copulations. Proc. R. Soc. Lond. B **242**, 127—133.

Parker, G. A. 1998: Sperm competition and the evolution of ejaculates: towards a theory base. In: Sperm Competition and Sexual Selection (Birkhead, T. R. & Møller, A. P., eds). Academic Press, London, pp. 3—54.

Parker, G. A. & Ball, M. A. 2005: Sperm competition, mating rate and the evolution of testes and ejaculate sizes: a population model. Biol. Lett. **1**, 235—238.

Parker, G. A. & Pizzari, T. 2010: Sperm competition and ejaculate economics. Biol. Rev. **85**, 897—934.

Parker, G. A., Ball, M. A., Stockley, P. & Gage, M. J. G. 1997: Sperm competition games: a prospective analysis of risk assessment. Proc. R. Soc. Lond. B **264**, 1793—1802.

Pitcher, T. E., Dunn, P. O. & Whittingham, L. A. 2005: Sperm competition and the evolution of testes size in birds. J. Evol. Biol. **18**, 557—567.

Pitnick, S. 1996: Investment in testes and the cost of making long sperm in *Drosophila*. Am. Nat. **148**, 57—80.

Pitnick, S. & Markow, T. A. 1994: Male gametic strategies: sperm size, testes size, and the allocation of ejaculate among successive mates by the sperm-limited fly *Drosophila pachea* and its relatives. Am. Nat. **143**, 785—819.

Prado, C. P. A. & Haddad, C. F. B. 2003: Testes size in Leptodactylid frogs and occurrence of multimale spawning in the Genus *Leptodactylus* in Brazil. J. Herpetol. **37**, 354—362.

Pyron, M. 2000: Testes mass and reproductive mode in minnows. Behav. Ecol. Sociobiol. **48**, 132—136.

Ramm, S. A. & Stockley, P. 2010: Sperm competition and sperm length influence the rate of mammalian spermatogenesis. Biol. Lett. **6**, 219—221.

Ramm, S. A., Parker, G. A. & Stockley, P. 2005: Sperm competition and the evolution of male reproductive anatomy in rodents. Proc. R. Soc. Lond. B **272**, 949—955.

Reuter, M., Linklater, J. R., Lehmann, L., Fowler, K., Chapman, T. & Hurst, G. D. D. 2008: Adaptation to experimental alterations of the operational sex ratio in populations of *Drosophila melanogaster*. Evolution **62**, 401—412.

Sato, T., Hirose, M., Taborsky, M. & Kimura, S. 2004: Size-dependent male alternative reproductive tactics in the shell-brooding cichlid fish *Lamprologus callipterus* in Lake Tanganyika. Ethology **110**, 49—62.

Schärer, L. & Vizoso, D. B. 2007: Phenotypic plasticity in sperm production rate: there's more to it than testes size. Evol. Ecol. **21**, 295—306.

Schuster, S. M. & Wade, M. J. 1991: Equal mating success among male reproductive strategies in a marine isopod. Nature **350**, 608—610.

Short, R. V. 1979: Sexual selection and its component parts, somatic and genital selection, as illustrated by man and the great apes. Adv. Stud. Behav. **9**, 131—158.

Simmons, L. W. 2001: Sperm Competition and its Evolutionary Consequences in the Insects. Princeton Univ. Press, Oxford, UK.

Simmons, L. W. & Emlen, D. J. 2006: Evolutionary trade-off between weapons and testes. Proc. Natl Acad. Sci. USA **103**, 16346—16351.

Simmons, L. W. & Garcia-Gonzalez, F. 2008: Evolutionary reduction in testes size and competitive fertilization success in response to the experimental removal of sexual selection in dung beetles. Evolution **62**, 2580—2591.

Simmons, L. W., Tomkins, J. L. & Hunt, J. C. 1999: Sperm competition games played by dimorphic male beetles. Proc. R. Soc. Lond. B **266**, 145—150.

Soulsbury, C. D. 2010: Genetic patterns of paternity and testes size in mammals. PLoS ONE **5**, e9581 doi: 10.1371/journal.pone.0009581.

Stockley, P., Gage, M. J. G., Parker, G. A. & Møller, A. P. 1997: Sperm competition in fishes: the evolution of testes size and ejaculate characteristics. Am. Nat. **149**, 933—954.

Svärd, L. & Wiklund, C. 1989: Mass and production rate of ejaculates in relation to monandry/polyandry in butterflies. Behav. Ecol. Sociobiol. **24**, 395—402.

Taborsky, M. & Neat, F. C. 2010: Fertilisation mode, sperm competition, and cryptic female choice shape primary sexual characters in fish. In: The Evolution of Primary Sexual Characters in Animals (Leonard, J. L. & Córdoba-Aguilar, A., eds). Oxford Univ. Press, Oxford, pp. 379—408.

Vahed, K. 2006: Larger ejaculate volumes are associated with a lower degree of polyandry across bushcricket taxa. Proc. R. Soc. Lond. B **273**, 2387—2394.

Vahed, K. 2007: Comparative evidence for a cost to males of manipulating females in bushcrickets. Behav. Ecol. **18**, 499—506.

Vahed, K., Parker, D. J. & Gilbert, J. D. J. 2011: Larger testes are associated with a higher level of polyandry, but a smaller ejaculate volume, across bushcricket species (Tettigoniinae). Biol. Lett. **7**, 261—264.

Wedell, N. & Hosken, D. J. 2010: The evolution of male and female internal reproductive organs in insects. In: The Evolution of Primary Sexual Characters in Animals (Leonard, J. L. & Córdoba-Aguilar, A., eds). Oxford Univ. Press, Oxford, pp. 307—331.

Wedell, N., Gage, M. J. G. & Parker, G. A. 2002: Sperm competition, male prudence and sperm-limited females. Trends Ecol. Evol. **17**, 313—320.