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# Condition dependence of testis size in small mammals

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## ABSTRACT

Sperm competition is a common phenomenon across the animal kingdom and is recognized as a major factor in the sexual selection of males. Intraspecific variation in testis size (ejaculate investment) has been implicated as an important factor in male reproductive success because larger testes produce higher quality ejaculates. Sexual selection theory predicts that traits associated with male–male competition or female mate choice must be costly and heritable to confer an evolutionary advantage to those individuals carrying such traits. Because ejaculate production can be costly, we tested the prediction that testis size is condition dependent using morphological data from three species of small mammals. In addition, subordinate males, which are less likely to copulate with a female at the optimal time for achieving fertilization, should invest more heavily in ejaculates than dominant males. Thus we also predicted that small males should invest more heavily in ejaculates than large males. In all three species, testis size was positively related to body condition (size-corrected body mass) and independent of body length. These results suggest that males in good condition are capable of investing more in ejaculates than males in poor condition, and are consistent with the costly nature of ejaculate production. In addition, the results are consistent with other traits that are condition dependent and that serve as honest signals of male quality, whether in the context of male–male competition or female mate choice.

*Keywords*: body condition, ejaculates, rodents, sexual selection, sperm competition.

## INTRODUCTION

Sperm competition is a common phenomenon across the animal kingdom and is recognized as a major factor in the sexual selection of males (for reviews, see Birkhead and Parker, 1997; Birkhead and Møller, 1998). If a female copulates with more than one male, the sperm from these males will compete within the female's reproductive tract to fertilize the egg(s). Game theory models predict that ejaculate investment should increase with the risk and intensity of sperm competition (reviewed in Parker, 1998), and thus, because the testes produce sperm and reflect investment in ejaculates, one of the predicted consequences of sperm competition is that the testes should be relatively large when the likelihood of sperm competition is high.

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This prediction has proved true across multiple taxa in many comparative analyses, including mammals (Harcourt *et al.*, 1981; Ginsberg and Rubenstein, 1990; Heske and Ostfeld, 1990), birds (Møller, 1991; Møller and Briskie, 1995) and fish (Stockley *et al.*, 1997). Similar predictions have been made with respect to intraspecific variation in testis size, although support for these predictions is not consistently supported by the data (e.g. Rising, 1987; Ribble and Millar, 1992).

In mammals, sperm competition influences penis morphology, ejaculate parameters and the behaviour of many species (Ginsberg and Huck, 1989; Møller and Birkhead, 1989; Gomendio *et al.*, 1998). Intraspecific variation in testis size (ejaculate investment) has been implicated as an important factor in male reproductive success because larger testes produce higher quality ejaculates (number of sperm, ejaculate volume and motility) and have higher rates of sperm production (Ginsburg and Huck, 1989; Stockley and Purvis, 1993; Gomendio *et al.*, 1998). Thus males with larger testes are assumed to have higher reproductive success than males with small testes in species with sperm competition. Although interspecific patterns of testis size and sperm morphometry have often been examined in the context of sperm competition, an understanding of intraspecific variation in these traits is critical to our understanding of ejaculate investment and sperm competition.

Sexual selection theory predicts that traits associated with male–male competition or female mate choice must be costly and heritable to confer an evolutionary advantage to those individuals carrying such traits (Andersson, 1994). Thus, males that are capable of bearing the costs of expensive traits are likely to be more successful at male–male competition or at attracting females. There is evidence that the fitness consequences of variation in testis size are substantial – males with large testes have higher reproductive success than those with small testes (Preston *et al.*, 2003; Schulte-Hostedde and Millar, 2004). The costs associated with ejaculate production are not trivial (Olsson *et al.*, 1997; Olsson and Madsen, 1998), and thus only males in good condition may be capable of producing high-quality ejaculates. In addition, an hypothesis developed by evolutionarily stable strategy (ESS) modelling suggests that subordinate males, which are less likely to copulate with a female at the optimal time for achieving fertilization, should invest more heavily in ejaculates than dominant males (Parker, 1990). In this way, small subordinate males can compensate for their reduced likelihood of successfully fertilizing a female by maximizing their investment in sperm. If testis size is correlated with ejaculate investment, then large dominant males should have relatively small testes compared with small subordinate males (Stockley and Purvis, 1993).

Here we use three species of small mammals – deer mice (*Peromyscus maniculatus*), red-backed voles (*Clethrionomys gapperi*) and bushy-tailed woodrats (*Neotoma cinerea*) – to test the predictions that (1) testis size is dependent on male body condition and thus males in good condition have large testes, and (2) small, subordinate males will have larger testes than large, dominant males.

## METHODS

We used data from bushy-tailed woodrats, deer mice and red-backed voles, all collected from the Kananaskis Valley, Alberta, in the Front Ranges of the Rocky Mountains (51°N, 115°W). All animals used in the analyses were breeding adult males producing sperm.

Woodrats (19 males) were collected in the summer of 1984–1985 using Conibear kill-traps (Hickling *et al.*, 1991). Total body length (including tail), tail length and length of the left testis were measured (all to the nearest millimetre) and each body frozen. Deer mice (72 males) and red-backed voles (66 males) were collected from early May to late August

1987 using snap traps baited with a small string soaked in aromatic oils that was tied to the treadle (Millar *et al.*, 1990). Total body length (including tail), tail length and length of the left testis (all to nearest mm) were measured and each body frozen (Millar 1987; Millar *et al.*, 1990).

We used body length (calculated by subtracting tail length from total body length) as an index of body size because other studies have used this index (e.g. Heske and Ostfeld, 1990; Levenson, 1990). We used body mass corrected for body size (residual mass) as an index of condition (Schulte-Hostedde *et al.*, 2001). Finally, left testis length was used as an index of the size of the testes because testis length is highly correlated with the mass of both testes in deer mice (Ribble and Millar, 1992). In addition, left testis length is highly correlated with testis mass in woodrats ($n = 10$, $r = 0.79$, $P = 0.006$). All measurements (testis length, body mass and body length) were log-transformed before statistical analysis to render the data normal and produce linear relationships.

### Statistical analysis

We used multiple regression for each species to examine the effects of body mass and body length on testis length. We interpreted the partial correlation coefficient of body mass as the independent effect of size-corrected body mass (condition) on testis length, and the partial correlation coefficient of body length as the independent effect of mass-corrected body length on testis length. The estimate of condition is akin to using residuals from a regression of body mass on body size as an index of condition (Schulte-Hostedde *et al.*, 2001).

## RESULTS

We found significant evidence of condition-dependence of testis size in all three species (Fig. 1a–c). Among deer mice, multiple regression of body mass and body length on testis length ($F_{2,69} = 6.90$, $r^2 = 0.167$, $P = 0.02$) yielded a significant effect of condition (size corrected for body length) on testis size (partial $r = 0.388$, $P < 0.001$). There was no effect of body length on testis size (partial $r = 0.003$, $P = 0.98$). Similar results were found in red-backed voles ($F_{2,63} = 11.55$, $r^2 = 0.268$, $P < 0.001$; condition partial $r = 0.442$, $P < 0.001$; body length partial $r = -0.089$, $P = 0.5$) and bushy-tailed woodrats ($F_{2,16} = 2.78$, $r^2 = 0.258$, $P = 0.092$; condition partial $r = 0.465$, $P = 0.05$; body length partial $r = -0.33$, $P = 0.177$).

## DISCUSSION

Testis size in all three species of small mammals was condition dependent – that is, males in good condition had larger testes than males in poor condition. Because testis size is associated with the intensity of sperm competition in mammals (Gomendio *et al.*, 1998), these results suggest that males in good condition are capable of investing more in ejaculates than males in poor condition, and are consistent with the costly nature of ejaculate production (Olsson *et al.*, 1997; Olsson and Madsen, 1998). Similar results have been found in yellow-pine chipmunks, *Tamias amoenus* (Schulte-Hostedde and Millar, 2004), and dung beetles, *Onthophagus taurus* (Simmons and Kotiaho, 2002). Because males with large testes tend to sire more offspring than males with small testes under sperm competition (Preston *et al.*, 2003; Schulte-Hostedde and Millar, 2004), positive covariation between testis size and body condition probably results in significant fitness benefits for individual males in good condition. Deer mice and red-backed voles both have a promiscuous mating system in which females mate with multiple males,

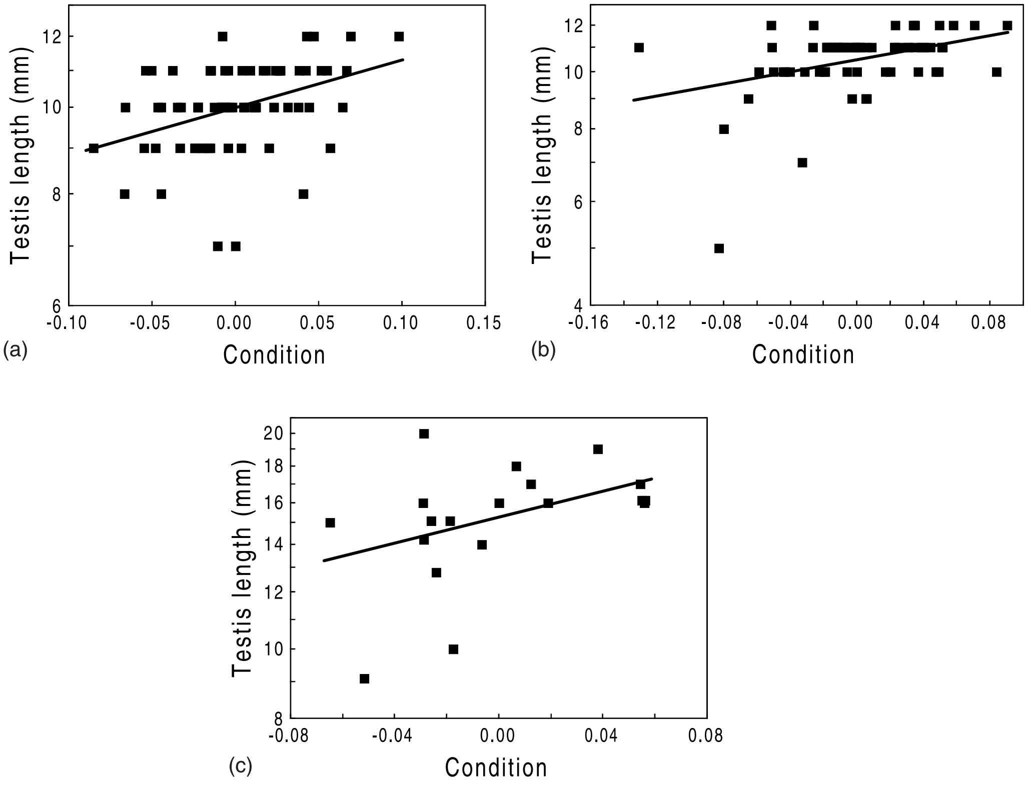

**Fig. 1.** Correlations with fitted regression lines between body condition (residual body mass) and testis size for (a) deer mice, (b) red-backed voles and (c) bushy-tailed woodrats. $P < 0.05$ for all three species. The y-axis is on a $\log_{10}$ scale.

resulting in sperm competition (Heske and Ostfeld, 1990; Ribble and Millar, 1992; Gomendio *et al.*, 1998). Bushy-tailed woodrats, on the other hand, show no evidence of multiple paternity (Topping and Millar, 1998) and females do not appear to have more than one mate during an oestrous period (Topping and Millar, 1999), suggesting that sperm competition is absent in this species. Thus, condition dependence of testis size may occur regardless of the presence of sperm competition, lending support to the idea that ejaculates are inherently energetically expensive to produce.

The lack of an effect of body length on testis size indicates that males do not differentially invest in ejaculates based on size. This result is inconsistent with previous results indicating that small male yellow-pine chipmunks tended to invest more heavily in ejaculates than large males (Schulte-Hostedde and Millar, 2004). Other size-based differences in testis size are evident in many fish species with alternative mating tactics (e.g. Neff *et al.*, 2003). The lack of size-based differences in testis size may be due to differences in mating systems among chipmunks and the three species of rodents examined here, as well as the absence of alternative behavioural tactics that may facilitate the evolution of any differences in testis size.

Condition dependence of traits associated with male–male competition is a fundamental prediction of sexual selection theory (Andersson, 1994). Indeed, structures associated with male–male combat such as horns and antlers are often condition dependent. The antlers of male red deer (*Cervus elaphus*) are largest in males of good condition (Clutton-Brock *et al.*, 1982), as are the horns of bighorn sheep, *Ovis canadensis* (Festa-Bianchet *et al.*, 2004). Similarly, male mountain goats (*Oreamnos americana*) in good condition have highly symmetrical horns relative to males in poor condition (Côté and Festa-Bianchet, 2001). Given that structures that mediate competition between males appear to be generally condition dependent (Andersson, 1994), it should not be surprising that the size of structures associated with ejaculate production (the testes) and sperm competition are also condition dependent.

Signals that are used as honest indicators of individual quality are also often condition dependent, particularly in the context of good genes models of sexual selection (Andersson, 1994). For example, the train length of peacocks, *Pavo cristatus* (Møller and Petrie, 2002), the ultra-violet coloration of blue grosbeaks, *Guiraca caerulea* (Keyser and Hill, 1999), and the exaggerated eye-span of the stalk-eyed fly, *Cyrtodiopsis dalmanni* (Cotton *et al.*, 2004), are all ornamental traits that are most exaggerated in males in good condition. A female is thus able to select males that can confer the genes associated with good condition to her offspring, assuming that condition is heritable (Merilä *et al.*, 2001; Simmons and Kotiaho, 2002). Cryptic female choice may also interact with ejaculate traits if these traits are condition dependent. For example, good sperm models of polyandry suggest that females may gain indirect benefits from multiple matings if male condition is genetically correlated with ejaculate quality. By mating with multiple males and facilitating sperm competition, a female can ensure that her eggs are fertilized by high-quality sperm (Yasui, 1997). Males that produce sperm with traits correlated with condition, for example sperm length (Pitnick *et al.*, 1999), will therefore have a selective advantage.

Alternatively, females might use the condition-dependent signal associated with the testes themselves, rather than the signal associated with ejaculate quality, to evaluate mate quality. Many mammals, including rodents, carry the testes externally in a scrotum. It has been suggested that the scrotum has evolved as a display or signalling device (Werdelin and Nilsonne, 1999); indeed, in some taxa the scrotum is not only large but distinctively coloured (Gerald, 2001). Thus, if the size of the external testes/scrotum is condition dependent, females may assess the size of the testes and/or scrotum and make reproductive decisions based on this assessment.

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