Intraspecific variation in testis size of small mammals: implications for muscle mass

Albrecht I. Schulte-Hostedde, John S. Millar, and Graham J. Hickling

Abstract: Intraspecific variation in testis size is usually interpreted in the context of sperm competition, yet an unconsidered consequence of increased testis size may be an increase in the production of testosterone, which can affect the growth of muscle mass. After muscle mass is corrected for body size, male small mammals have more muscle mass than females, which suggests that it may be a sexually selected trait. An enhanced musculature may have fitness consequences with respect to male mate-searching activities and male–male competition for access to females. We tested the prediction that males with large testes have more muscle mass (measured as lean dry mass) by examining testis size and body composition in three species of small mammals (bushy-tailed wood rat (*Neotoma cinerea*), deer mouse (*Peromyscus maniculatus*), and red-backed vole (*Clethrionomys gapperi*)) from the Kananaskis Valley, Alberta. In all three species, males with relatively large testes had relatively more lean dry mass than males with relatively small testes. This suggests that a secondary consequence of relatively large testes may be a relative increase in muscle mass. Further research should investigate alternative effects of intraspecific variation in testis size on individual fitness within wild populations to gain further insight into sexual selection.

Résumé : La variation intraspécifique de la taille des testicules est généralement prise en compte lors d'études sur la compétition des spermatozoïdes et une conséquence souvent oubliée de l'augmentation de taille des testicules est une augmentation de la production de testostérone qui peut affecter la croissance de la masse musculaire. Après les corrections pour tenir compte de la masse du corps, il s'avère que les petits mammifères mâles ont une masse musculaire supérieure à celle des femelles, ce qui indique que la masse musculaire peut être déterminée par la sélection sexuelle. Une masse musculaire importante peut avoir un impact sur le fitness en affectant les activités de recherche de partenaires sexuels et la compétition entre mâles pour l'accès aux femelles. Nous avons vérifié l'hypothèse selon laquelle les mâles à gros testicules ont une masse musculaire (masse sèche sans les graisses) supérieure, en mesurant les testicules et en étudiant la composition corporelle des mâles de trois espèces de petits mammifères de la vallée de Kananaskis (le néotoma à queue touffue (*Neotoma cinerea*), la souris sylvestre (*Peromyscus maniculatus*) et le campagnol à dos roux (*Clethrionomys gapperi*)). Chez les trois espèces, la masse sèche sans les graisses est plus importante chez les mâles aux testicules relativement gros que chez les mâles aux testicules relativement petits. Cela laisse croire que la possession de testicules relativement gros a pour conséquence secondaire l'augmentation relative de la masse musculaire. Des recherches sur les autres effets de la variation intraspécifique de la taille des testicules sur le fitness individuel au sein des populations naturelles s'imposent avant qu'il ne soit possible de tirer des conclusions sur la sélection sexuelle.

[Traduit par la Rédaction]

Introduction

Variation in testis size is often interpreted in the context of sperm competition, a common phenomenon across the animal kingdom (for reviews see Birkhead and Parker 1997; Birkhead and Møller 1998). Sperm competition occurs when a female mates with multiple males and the sperm from these males compete within the female's reproductive tract to fertilize the egg(s). One of the predicted consequences of sperm competition is that relative testis size should be highest among species which have the highest likelihood of sperm competition. This prediction has been borne out in many comparative analyses across multiple taxa (e.g., mammals, Harcourt et al. 1981; Ginsberg and Rubenstein 1990; Heske and Ostfeld 1990; birds, Møller 1991; Møller and Briskie 1995; fish, Stockley et al. 1997). Tests of these pre-

Received 22 November 2002. Accepted 19 February 2003. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 30 April 2003.

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 doi: 10.1139/Z03-040

Table 1. Body length, wet mass (excluding stomach contents), lean dry mass, testis length, and relative testis length (testis length/body length) (mean ± SD) of bushy-tailed wood rats (*Neotoma cinerea*), deer mice (*Peromyscus maniculatus*), and red-backed voles (*Clethrionomys gapperi*) sampled from the Kananaskis Valley, Alberta.

	Bushy-tailed wood rats	Deer mice	Red-backed voles
Body length (mm)	233.3 ± 15.69	86.0 ± 5.92	97.7 ± 6.65
Wet mass (g)	353.1 ± 68.85	21.2 ± 2.05	25.7 ± 3.50
Lean dry mass (g)	79.3 ± 15.78	4.6 ± 0.64	6.0 ± 0.85
Left-testis length (mm)	15.4 ± 2.60	10.0 ± 1.18	10.6 ± 1.18

dictions with respect to intraspecific variation in testis size have yielded inconsistent results (e.g., Rising 1987; Ribble and Millar 1992).

A heretofore unconsidered consequence of increased testis size independent of sperm competition may be an increase in the production of androgens such as testosterone. Testosterone is produced by the testes and can affect many physiological parameters including the growth of muscle mass (Mann and Lutwak-Mann 1981; Bhasin et al. 1998). Male small mammals have more lean dry mass (predominantly composed of muscle protein), when corrected for body size, than females (Schulte-Hostedde et al. 2001*a*), and this may result from the effects of higher levels of testosterone. The effects of variation in muscle mass are likely to be profound because muscle mass may be a sexually selected trait (Bonnet et al. 1998). Males with an enhanced musculature may have a fitness advantage over males that do not, because a robust musculature likely increases success in male mate-searching activities and male–male competition for access to females.

Alternative consequences of intraspecific variation in testis size have rarely been considered in an evolutionary or ecological context. Here we examine intraspecific variation in testis size and body composition in three species of small mammals: deer mouse (*Peromyscus maniculatus*), bushy-tailed wood rat (*Neotoma cinerea*), and red-backed vole (*Clethrionomys gapperi*). We predict that males with large testes have relatively more muscle mass (measured as lean dry mass) than males with small testes.

Methods

We used data from three species of small mammals (bushy-tailed wood rat, deer mouse, and red-backed vole) all collected in 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.

Wood rats (20 males) were collected in summer of 1984 and 1985 using Conibear kill-traps (Hickling 1987; Hickling et al. 1991). Total body length (including the tail), tail length, and length of the left testis were measured (all to the nearest millimetre) and each body was frozen. Deer mice (73 males) and red-backed voles (64 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 the tail), tail length, and length of the left testis were measured (all to the nearest millimetre) and each body was frozen (Millar 1987; Millar et al. 1990).

Body-composition analysis was performed following Kerr et al. (1982) and Dobush et al. (1985). For deer mice and red-backed voles, whole bodies excluding stomach contents were dried and then ground in a Wiley Mill or Moulinex coffee grinder, and the fat content was determined using petroleum ether in a Soxhlet fat extractor. Wood rat carcasses (excluding stomach contents, skull, and pelt) were ground in a meat grinder and dried. The dried carcass was then ground in a Moulinex coffee grinder. Fat extraction was performed on two 4-g subsamples from each carcass. The fat content of the pelt was determined by soaking the intact pelt in ether for 24 h. Total fat content was calculated as the mean of the two replicate estimates of carcass fat, plus pelt fat (Hickling et al. 1991). For all species, fat extractions were performed in the Department of Zoology, University of Western Ontario, within a year of the carcasses being collected. For all species we calculated water content as the difference between fresh mass (without stomach contents) and the mass of the carcass after drying. Lean dry mass was determined as the mass of the carcass following the removal of fat.

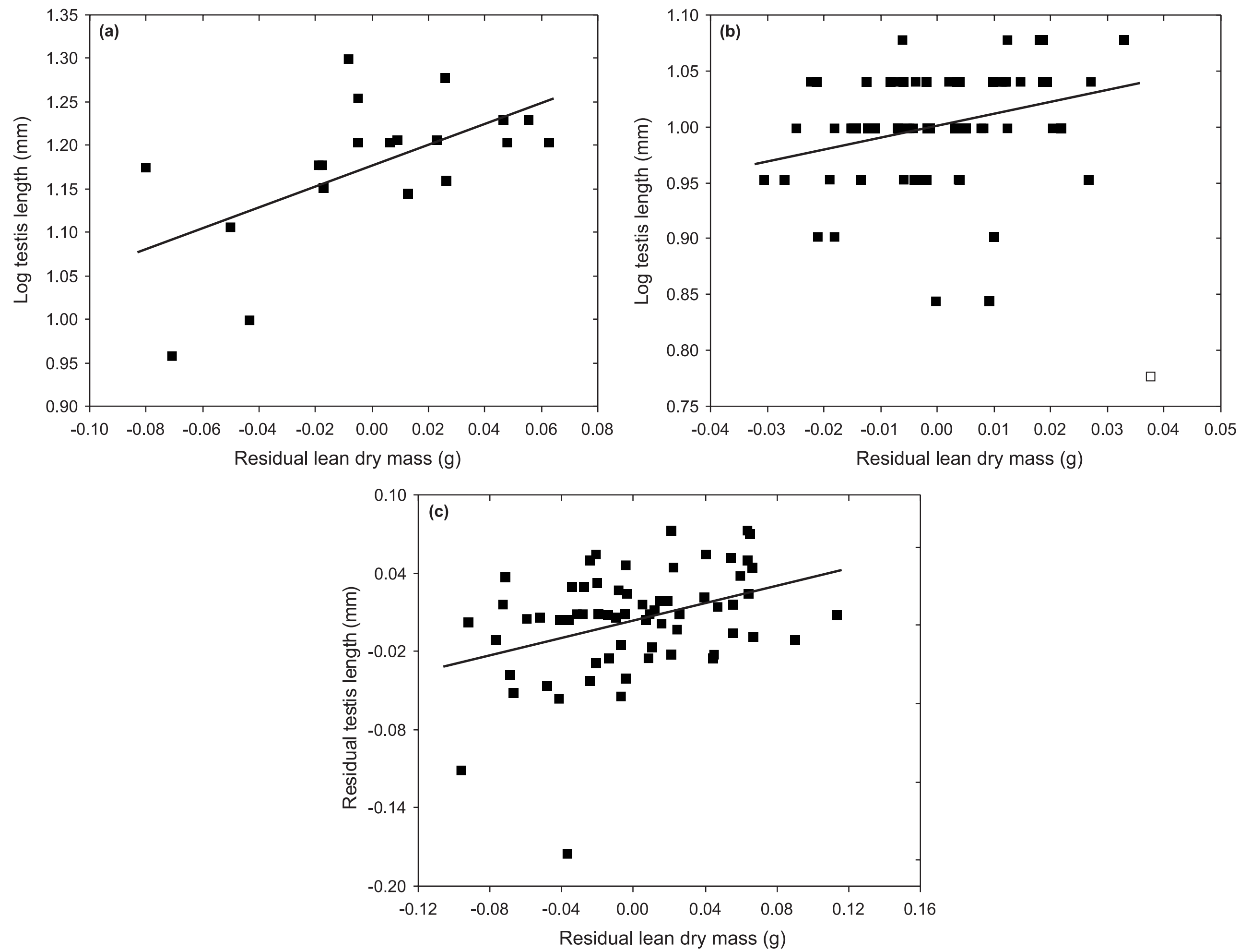
We used body length (calculated by subtracting tail length from total body length), rather than body mass, as an index of body size because of the confounding effects of nutritional condition (Dobson 1992; Schulte-Hostedde et al. 2001*b*), and because this index has been used in other studies (e.g., Heske and Ostfeld 1990; Levenson 1990). Left-testis length was used as an index of testis size because testis length is highly correlated with the mass of both testes in the deer mouse (Ribble and Millar 1992). In addition, left-testis length is highly correlated with testis mass in wood rats (n = 10, r = 0.79, P = 0.006). Finally, we used lean dry mass as an index of muscle mass because the latter is mostly composed of muscle protein (Schulte-Hostedde et al. 2001*a*). All measurements (testis length, lean dry mass, and body length) (Table 1) were log-transformed prior to statistical analysis to remove any allometric effects.

To determine whether testis size was related to lean dry mass, we initially determined whether testis length was significantly related to body length. If there was a significant relationship, we corrected for body size by using the residuals from this regression in further analyses, otherwise we used testis length. We corrected lean dry mass for body size by using residuals from the regression of lean dry mass on body length. We then regressed residual testis length or testis length on residual lean dry mass.

Results

Among wood rats, testis length was independent of body length ($F_{[1,18]}$ = 1.06, r^2 = 0.055, P = 0.32). Lean dry mass

Fig. 1. Regressions between log testis length (bushy-tailed wood rats, *Neotoma cinerea*, and deer mice, *Peromyscus maniculatus*) or residual testis length (red-backed voles, *Clethrionomys gapperi*) and residual lean dry mass for bushy-tailed wood rats (r^2 = 0.349, P < 0.001) (*a*), deer mice (r^2 = 0.09, P = 0.01 after the removal of an outlier (□)) (*b*), and red-backed voles (r^2 = 0.142, P = 0.002) (*c*).



was positively related to body length ($F_{[1,18]}$ = 63.73, r^2 = 0.780, P < 0.001) so we used the residuals from this regression to correct lean dry mass for body length. Male wood rats with larger testes had more lean dry mass than males with smaller testes after we corrected lean dry mass for body size ($F_{[1,18]}$ = 9.66, r^2 = 0.349, P = 0.006) (Fig. 1).

Testis length was independent of body length in deer mice ($F_{[1,71]}$ = 0.29, r^2 = 0.004, P = 0.592). Lean dry mass was positively related to body length ($F_{[1,71]}$ = 6.66, r^2 = 0.086, P = 0.012) so we corrected lean dry mass for body length by using the residuals of this regression. Size-corrected lean dry mass was not related to testis length among male deer mice ($F_{[1,71]}$ = 0.85, r^2 = 0.01, P = 0.36); however, the removal of an outlier yielded a significant positive relationship between testis length and residual lean dry mass ($F_{[1,70]}$ = 6.94, r^2 = 0.09, P = 0.01) (Fig. 1).

Among red-backed voles, testis length was significantly related to body length ($F_{[1,64]}$ = 6.39, r^2 = 0.091, P = 0.014), therefore we corrected testis length for body length by using the residuals of this regression. There was also a significant positive relationship between lean dry mass and body length ($F_{[1,64]}$ = 52.93, r^2 = 0.453, P < 0.001), therefore we again used the residuals from this regression to correct lean dry mass for body length. After we corrected lean dry mass for body size, males with larger testes had relatively more lean dry mass than males with smaller testes ($F_{[1,64]}$ = 10.48, r^2 = 0.142, P = 0.002; Fig. 1).

Discussion

We found evidence that in all three species, males with relatively large testes had relatively more lean dry mass, which is consistent with our initial predictions. Below we address several assumptions upon which our results rest.

The testes produce testosterone, which enhances the growth of lean muscle mass (Bhasin et al. 1998), and there is some evidence that larger testes produce more testosterone (Berndtson and Jones 1989; Kaplan and Mead 1993, 1994; Adam and Findlay 1997). Therefore, the assumption that there is a link between testis size and testosterone level is likely valid, as is the assumption that an increase in testosterone level leads to greater muscle mass. In bushy-tailed wood rats, red-backed voles, and deer mice, testis size was correlated with residual lean dry mass, which suggests that males with relatively large testes had relatively more muscle than males with relatively small testes. Enhanced muscle mass may be advantageous for species engaged in mate-searching activities or male–male combat while guarding estrous females. Interestingly, male mating success appears to be negatively related to male body size in bushy-tailed wood rats (Topping and Millar 1999). It is interesting to speculate whether muscle mass, enhanced by testosterone produced by the testes, is related to male mating success rather than body size per se. Combat may play an important role in male mating success because evidence of wounding due to male–male combat has been found in this species (Hickling 1987). More muscular males may be more likely to successfully procure mates than less muscular males, thus muscle mass may be considered a sexually selected trait.

A final assumption is that variation in lean dry mass reflects variation in muscle mass rather than variation in the other major constituent, ash (derived from the skeletal structure). Most of the lean dry mass is protein derived from muscle. For instance, in water snakes (*Nerodia sipedon*), protein made up, on average, 78% of lean dry mass (Weatherhead and Brown 1996). In muskrats (*Ondatra zibethicus*), approximately 83% of lean dry mass was composed of protein (Virgl and Messier 1992). In both cases, ash composed the lesser proportion of lean dry mass. Nonetheless, variation in lean dry mass may also reflect some variation in skeletal structure (ash).

A concomitant trade-off between a high testosterone level (and perhaps large testes) and its effects on muscle mass is a reduction in immunological functioning (Folstad and Karter 1992). It would therefore be interesting to determine whether males with relatively large testes (and relatively more muscle mass) had higher parasite loads than males with relatively small testes (and relatively less muscle mass), as has been indicated in some birds (e.g., Merilä and Sheldon 1999).

Although an analysis of three species precludes a general conclusion regarding the hypothesized increase in muscle mass due increased androgen levels produced by the testes, it is interesting to consider whether this relationship exists among other taxa. It is clear that more research into intraspecific variation in testosterone levels in wild populations is required. Additionally, any adaptive explanation of variation in testis size, muscle mass, or any other trait assumes that these traits are heritable. Determining whether testis size and muscle mass are heritable in wild populations will help put this study and others like it in proper context.

Acknowledgements

We thank past research assistants who measured the body size and analyzed the body composition of the animals used in this study. R.D. Montgomerie, S.N. Doucet, and T.A.F. Long provided excellent comments on an earlier version of the manuscript. The manuscript was also improved by comments from two anonymous reviewers. This study was supported by an operating grant to J.S.M. from the Natural Sciences and Engineering Research Council of Canada.

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