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SPERM COMPETITION GAMES BETWEEN SNEAKS AND GUARDS: A COMPARATIVE ANALYSIS USING DIMORPHIC MALE BEETLES

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Sperm competition is widely recognized as a pervasive force of sexual selection. Theory predicts that across species increased risk of sperm competition should favor an increased expenditure on the ejaculate, a prediction for which there is much evidence. Sperm competition games have also been developed specifically for systems in which males adopt the alternative male mating tactics of sneaking copulations or guarding females. These models have not yet been tested in a comparative context, but predict that: across species male expenditure on the ejaculate should increase with increasing probability of a sneak mating; within species, sneaks should have the greater expenditure on the ejaculate; and the disparity in expenditure between sneaks and guards should be greatest in species with moderate risk of a sneak mating, and decline toward parity in species with low or high risk. Beetles in the genus *Onthophagus* are often characterized by dimorphic male morphologies that reflect the alternative mating tactics of sneak (minor males) and guard (major males). We conducted a comparative analysis across 16 species of male dimorphic onthophagines, finding that testes size increased across species with increasing frequency of the minor male phenotype. Minor males generally had the greater testes size, but across species the disparity between morphs was independent of the frequency of minor males. We present data on testes allometry from two populations of *O. taurus* that have undergone genetic divergence in the frequency of minor males. Consistent with the comparative analysis, these data support the notion that the relative frequency of sneaks in the population influences male expenditure on the ejaculate.

KEY WORDS: Alternative mating tactics, comparative analysis, *Onthophagus*, sperm competition, testis size.

Sperm competition is widely recognized as a potent force of sexual selection, favoring adaptations in male behavior, morphology, and physiology that contribute to competitive fertilization success (Parker 1970; Birkhead and Møller 1998; Simmons 2001). Considerable effort has been made toward developing a game theory approach with which to predict how male ejaculation strategies should respond to variation in sperm competition risk, the probability that females will mate with more than one male so that sperm compete for fertilizations (Parker 1990a,b, 1998; Parker et al. 1997; Parker and Ball 2005). Sperm competition games assume that ejaculates are costly so that males must trade expenditure on gaining fertilizations with expenditure on gaining additional matings, and that males obtain fertilizations in proportion to the relative numbers of sperm they ejaculate (Parker 1998). The models predict that across species, increased risk of sperm competition should favor the evolution of increased male expenditure on the ejaculate, a prediction for which there is now considerable evidence from a variety of taxa (Gage 1994; Harcourt et al. 1995; Hosken 1997; Stockley et al. 1997; Byrne et al. 2002; Pitcher et al. 2005). There is also evidence to suggest that variation in sperm competition risk can influence ejaculation strategies within species.

In many species males adopt alternative mate securing tactics in which males who are unsuccessful in contest competition sneak copulations with females guarded by their competitively superior conspecifics (Gross 1996). Parker (1990b) analyzed a sperm competition game specific to alternative mate securing strategies. Here, by nature of their mating tactic, sneaks are always subject to sperm competition whereas guards are subject to sperm competition with average probability determined by the frequency of sneaks in the population. Across species, the model predicts that as the probability of a sneak mating or the frequency of sneaks increases, so ejaculate expenditure should increase. This parallels the cross-species prediction generated by the general models. Within species however, the models predict that sneaks should have the greater ejaculate expenditure, and that the disparity between sneaks and guards should decrease across species at high sneak frequency, because guards will be subject to sperm competition with the same high certainty when sneaks are common (Parker 1990b; Gage et al. 1995). There is some evidence from species of fish with alternative mating tactics that sneaks can have a higher expenditure on their ejaculate than guards (Gage et al. 1995; Vladic and Järvi 2001; Neff et al. 2003). However, the sneak–guard model has not yet been tested within a comparative framework.

Many species of dung beetle in the genus *Onthophagus* exhibit a suite of behavioral and morphological features that characterize alternative mating tactics (Emlen et al. 2005a). Some males have enlarged horns on the head and/or pronotum (major males), and compete for and guard females (Emlen 1997; Moczek and Emlen 2000). Major males also assist females by collecting and carrying dung from the surface to the brood mass (Cook 1990; Hunt and Simmons 1998b, 2000). Other males are small and have only rudimentary horns, often resembling females (minor males). These minor males do not assist females in brood provisioning, rather they sneak into breeding burrows to copulate with guarded females (Hunt and Simmons 2002). Females readily mate with both major and minor males, and for those species in which it has been studied, sperm numbers at the time of egg deposition appear to be an important determinant of competitive fertilization success (Tomkins and Simmons 2000; Simmons et al. 2004). Ejaculate expenditure also appears to be costly in this genus. Males exposed to females have a reduced life span and mating speed (Kotiaho and Simons 2003; Simmons and Kotiaho 2007), and there is a resource allocation trade-off during development between primary and secondary sexual traits such that males who invest in weapons for competing for females have fewer resources to allocate to their testes for sperm production (Simmons and Emlen 2006). The biology of onthophagines thereby satisfies the underlying assumptions of Parker's (1998) sperm competition games.

In at least one species of onthophagine, *O. binodis*, minor males have been found to have a greater investment in their testes and to produce larger ejaculates than major males (Simmons et al. 1999). Minor male *O. binodis* may pay for their greater ejaculate expenditure via a reduction in courtship rate and mating speed (Simmons and Kotiaho 2007). However, in a second species, *O. taurus*, testis size and ejaculate volume did not differ significantly between minor and major males (Simmons et al. 1999). Simmons et al. (1999) suggested that a resolution to these apparently conflicting findings might lie in the relative frequencies of sneaks and guards that characterize the two species. For the populations used in Simmons et al.'s (1999) study, minor males were twice as common in *O. taurus* than in *O. binodis*, and Parker's (1990b) model predicts that the disparity in ejaculate expenditure between sneaks and guards should depend on the frequency of sneaks in the population. Here we conduct the first comparative analysis of the sneak–guard model, exploiting widespread variation in the relative frequencies of sneaks and guards among species of *Onthophagus*. We also provide a within species test of the prediction that the frequency of sneaks in a population should influence the disparity in ejaculate expenditure between sneaks and guards, by comparing patterns of testes investment among populations of *O. taurus* that have been introduced into locations outside their natural range, and have undergone subsequent evolutionary divergence in the relative frequencies of sneaks and guards.

Methods

COMPARATIVE ANALYSIS AMONG SPECIES

The genus *Onthophagus* is a large genus of the family Scarabaeidae, with over 2000 species currently described (Howden and Cartwright 1963; Matthews 1971; Zunino 1972, 1979; Howden and Gill 1993). The expression of male horn morphology appears to be evolutionarily labile, with repeated gains and losses of alternative mating tactics (Emlen et al. 2005a). We collected data for 16 species of male dimorphic *Onthophagus* from three continents, Australia, Africa, and North America (Table 1). In Australia and North America beetles were sampled from recently deposited cattle dung, whereas in Africa they were sampled from pit traps baited with fresh cattle dung.

Beetles were first washed in fresh water, before placing them for 24 h into a clean dry container containing a paper towel. In this way we reduced any potential variation in body weight that might have arisen from variation in gut content or hydration. We measured pronotum width to the nearest 0.01 mm using digital callipers, and weighed each beetle to an accuracy of 0.01 mg.

Table 1. Phenotypic characteristics and collection locations of populations of Onthophagines used in the comparative analysis.

Onthophagus sp.	Body weight (mg±SE)	Testes weight (mg±SE)	*N*	Proportion minor males	Source
alcyonides	80.1±2.9	2.03±0.08	28	0.17	Nelspruit, RSA
auruginosis	26.8±1.4	0.60±0.07	23	0.20	Nelspruit, RSA
australis	120.5±2.9	3.64±0.07	84	0.47	Warrnambool, VIC
binodis	111.2±3.3	2.86±0.12	78	0.32	Walpole, WA
cribripennis	41.6±1.5	1.19±0.09	19	0.45	Nelspruit, RSA
fuliginosus	103.5±4.3	3.68±0.13	47	0.41	Warrnambool, VIC
gazella	117.8±3.8	3.14±0.09	56	0.34	Armidale, NSW
haagi	85.1±4.3	2.14±0.10	20	0.32	Walpole, WA
hecate	43.0±3.5	1.29±0.10	20	0.29	Raleigh, USA
nigriventris	154.3±5.2	4.33±0.10	24	0.48	Hawaii, USA
nodulifer	63.0±2.5	1.56±0.08	38	0.21	Darwin, NT
nuchicornis	33.5±1.7	0.73±0.08	23	0.10	Missoula, USA
rupicapra	21.4±0.9	0.85±0.04	23	0.61	Geraldton, WA
sloanei	378.7±17.3	5.97±0.20	33	0.17	Exmouth, WA
taurus	72.1±1.7	2.32±0.07	113	0.62	Margaret River, WA
vermiculatus	33.53±1.3	1.03±0.05	29	0.21	Pemberton, WA

RSA, Republic of South Africa; VIC, Victoria, Australia; WA, Western Australia; NT, Northern Territory, Australia; NSW, New South Wales, Australia.

Beetles were then dissected and their testes removed and weighed to an accuracy of 0.01 mg (Table 1).

We identified major and minor males based on the scaling relationship between pronotum width and horn length using the methods of Eberhard and Gutierrez (1991). Briefly, the pronotum width at which there is a change in linear slope of horn length on pronotum width (the switch point) is identified using the model

$$Y = \beta_o + \beta_1 X + \beta_2 (X - X^\circ) D + \beta_3 D + \varepsilon \qquad (1)$$

where Y and X are horn length and pronotum width respectively, X° is the switch point delineating major and minor male morphologies, $D = 1$ unless $X < X^\circ$ in which case $D = 0$; $\beta_{1\text{–}3}$ are regression coefficients, and ε is a random component (Eberhard and Gutierrez 1991). Detailed descriptions of the application of this method to *Onthophagus* can be found in Eberhard and Gutierrez (1991) and Hunt and Simmons (1998a). Importantly, this method provides good separation of alternative reproductive tactics, as indicated by the brood provisioning behavior of males (Hunt and Simmons 2000). All males smaller than the switch point were classed as minor males whereas those larger than the switch point were classed as major males. To maximize the accuracy of our estimate of the switch point, when available we used a larger sample of beetles than those collected for testes measurements. However, the proportion of sneaks in the population sampled for testes measurement was taken as the proportion of that sample that was smaller than the switch point.

We examined the allometric relationships between testes weight and body weight for each species in our comparative analysis. Reduced (standardized) major axis regression slopes and intercepts, and their 95% confidence intervals were calculated from the log-transformed values of body weight and testes weight using SMATR software (Falster et al. 2006; Warton et al. 2006). Standardized rather than major axis regression was used because of the greater precision associated with the calculation of the confidence bands (Warton et al. 2006), which in many cases could not be estimated for the major axis regression parameters. Conventional ANCOVA has been recommended for the comparison of slopes and elevations of testis allometry across alternative mating tactics (Tomkins and Simmons 2002); however, because both X and Y are measured with error, model II regression procedures are more appropriate for our allometric data. The SMATR procedure we followed is identical in principle to ANCOVA, hence we first tested for differences in the reduced major axis slope of the testes allometry between the male morphs within each species. Where there was no significant heterogeneity in slope between male morphs, SMATR was used to compare the intercepts, or elevations, of the testes allometries of minor and major males (Falster et al. 2006; Warton et al. 2006). This provides a test of the difference in testes weight between major and minor males standardized to a common body size, in the same way that marginal means are returned from ANCOVA.

POPULATION VARIATION WITHIN SPECIES

Between 1969 and 1984 the CSIRO Division of Entomology introduced into Australia 52 species of dung beetles as part of a biocontrol program to reduce bush flies that breed in cattle dung (Tyndale-Biscoe 1996). Several of these introductions have resulted in the establishment of large populations, including *O. taurus* (originating from Turkey and Greece). *Onthophagus taurus* was also introduced into North America in the early 1970s (Fincher and Woodruff 1975). Since their introductions, North American and Australian populations of *O. taurus* have undergone significant evolutionary divergence in the position of the switch point that delineates alternative mating tactics, such that minor males are more common in the high-density populations found in Australia than in the low-density populations found in North America (Moczek et al. 2002; Moczek and Nijhout 2003). We sampled populations of *O. taurus* from Margaret River, Western Australia, and from Raleigh, North Carolina, and measured pronotum width, body weight, and testes weight as described above. We used SMATR to compare the testes allometries between populations, and to contrast testes sizes of major and minor males within the different populations.

Results

COMPARATIVE ANALYSIS AMONG SPECIES

Descriptive statistics for the 16 species of *Onthophagus* available for our comparative analysis are presented in Table 1. Weights were log transformed, and the proportion of minor males arcsine square-root transformed prior to statistical analyses. We analyzed variation in testes weight among species using a General Linear Model (GLM) with log testes weight as the dependent variable, and log body weight and the arcsine square-root transformed proportion of minor males as the predictor variables. The whole model was significant, explaining 97% of the among species variation in testes weight ($F_{(2,13)} = 182.07$, $P < 0.0001$). Not surprisingly larger species had larger testes ($F_{(1,13)} = 354.50$, $P < 0.0001$), but after accounting for this variation due to species body size, species with a greater proportion of minor males had larger testes ($F_{(1,13)} = 18.26$, $P = 0.0009$; Fig. 1A).

Of the species used in our analysis, all but *O. rupicapra* and *O. nodulifer* were included in a recent molecular phylogeny of 48 *Onthophagus* species and three out-groups (Emlen et al. 2005a,b). Based on this phylogeny, we used the software package CAIC (Purvis and Rambaut 1995) to calculate phylogenetically independent contrasts in log body weight, log testes weight, and the arcsine square-root proportion of minor males. The analysis returned 13 evolutionary contrasts. The relationships reported in our cross-species analysis were robust to control for phylogeny. A GLM forced through the origin, with contrasts in log body weight and arcsine proportion minor males as predictor variables, explained a significant proportion of the variation in contrasts in log testes weight (whole model $F_{(2,10)} = 504.61$, $P < 0.0001$). Evolutionary increases in body size were associated with evolutionary increases in testes size ($F_{(1,10)} = 225.12$, $P < 0.0001$), and after controlling for evolutionary increases in body size, evolutionary increases in the proportion of minor males were associated with evolutionary increases in testes size ($F_{(1,10)} = 226.23$, $P < 0.0001$; Fig. 1B). Slopes of the regressions of absolute values of the evolutionary contrasts on estimated nodal values did not differ significantly from zero (Ps ≥ 0.239) satisfying CAIC's evolutionary assumptions (Purvis and Rambaut 1995).

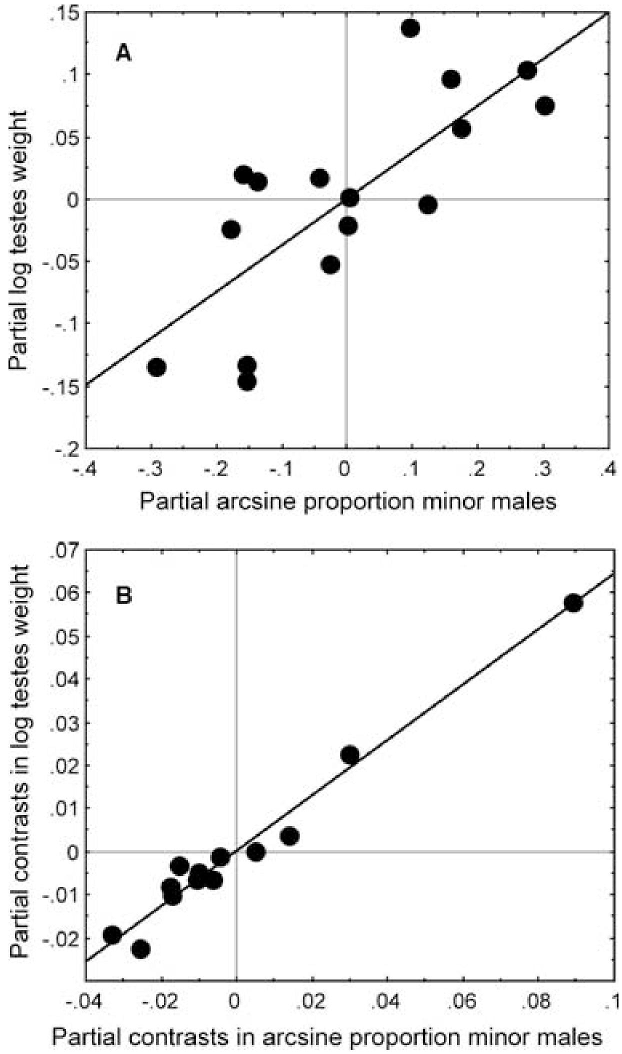

Figure 1. Among species association between testes size and the proportion of males adopting the alternative mating tactic of sneaking (minor males) in the dung beetle genus *Onthophagus*. (A) Effect of arcsine-transformed proportion of minor males on log testes weight after controlling for the effect of log body weight across 16 species of *Onthophagus*. (B) Comparative analysis using independent contrasts. Again, partial contrasts control for contrasts in log body weight.

There was no significant heterogeneity in the testes allometry between minor and major males within 15 of the 16 species studied (Table 2). The only species for which there was a significant change in slope between morphs was *O. rupicapra* where minor males had a shallower slope than major males. The homogeneity

Table 2. Testes allometry ($\log_{10}$ testes weight regressed on $\log_{10}$ body weight) for minor and major male morphs of 16 species of Onthophagine dung beetles.

Species	Minors				Majors				Slope homogeneity	
	n	Slope	Intercept	*P*	*n*	Slope	Intercept	*P*	−2log/λ	*P*
O. alcyonides	5	1.850	−3.049	0.972	22	1.314	−2.210	0.006	0.275	0.607
O. aeruginosis	5	−5.725	6.199	0.376	18	3.329	−5.109	0.017	0.851	0.382
O. australis	16	1.027	−1.472	0.001	68	0.983	−1.502	0.000	0.045	0.819
O. binodis	27	2.354	−4.061	0.032	51	2.350	−4.512	0.000	0.000	0.996
O. cribripennis	8	2.200	−3.451	0.706	11	3.940	−6.443	0.103	1.265	0.257
O. fuliginosus	15	1.388	−2.107	0.056	32	1.051	−1.573	0.000	1.008	0.305
O. gazella	17	0.866	−1.227	0.002	38	1.285	−2.208	0.018	2.586	0.110
O. haagi	5	−0.660	1.536	0.971	15	1.366	−2.368	0.608	1.102	0.340
O. hecate	4	0.604	−0.945	0.144	11	0.970	−1.462	0.014	0.937	0.343
O. nigriventris	18	0.773	−1.047	0.030	5	1.087	−1.782	0.694	0.281	0.590
O. nodulifer	12	1.209	−1.896	0.586	26	1.837	−3.186	0.002	1.326	0.254
O. nuchicornis	5	−3.49	1.503	0.664	18	8.858	−5.401	0.004	1.855	0.175
O. rupicapra	14	0.989	−1.384	0.002	8	2.570	−3.583	0.402	4.164	0.047
O. sloanei	13	1.968	−4.048	0.002	33	1.303	−2.631	0.003	2.596	0.119
O. taurus	67	2.182	−3.591	0.000	46	1.667	−2.827	0.005	2.272	0.123
O. vermiculatus	4	−2.071	2.810	0.740	25	−1.604	2.475	0.746	0.107	0.747

Slopes and intercepts were calculated using the reduced major axis technique. Homogeneity of slopes was tested by the likelihood-ratio test proposed by Warton et al. (2006).

of slopes between morphs allows us to compare the elevations of the testes allometry (Warton et al. 2006) in major and minor males when a common slope is fitted to the data (Table 3). In 10 of 16 species there was a significant difference in testes investment (elevation) between the male morphs (Table 3). The within species difference in elevation between male morphs was significantly biased towards greater testes investment in minor males (Fig. 2; one sample *t*-test with $H_0 = 0$ difference, $t_{15} = -3.240$,

Table 3. Common testes allometries ($\log_{10}$ testes weight regressed on $\log_{10}$ body weight) and 95% confidence intervals (CI), and tests for differences in the elevations of slopes between the male morphs of 16 species of Onthophagine dung beetles.

Species	*n*	*P*	Slope	LowCI	UppCI	Interc	LowCI	UppCI	Δ elevation	Wald^1	*P*
O. alcyonides	27	0.000	1.056	0.771	1.444	−1.701	−2.338	−1.063	−0.118	5.404	0.020
O. aeruginosis	23	0.001	2.729	1.931	3.856	−4.186	−5.555	−2.817	−0.426	1.4	0.237
O. australis	84	0.000	0.722	0.613	0.850	−0.939	−1.184	−0.693	−0.116	22.812	0.000
O. binodis	78	0.062	1.679	1.345	2.095	−2.991	−3.754	−2.227	−0.459	61.016	0.000
O. cribripennis	19	0.020	2.173	1.425	3.313	−3.464	−4.994	−1.935	−0.204	3.291	0.070
O. fuliginosus	47	0.000	0.854	0.719	1.014	−1.152	−1.447	−0.857	−0.102	7.138	0.008
O. gazella	55	0.000	0.736	0.596	0.909	−1.025	−1.347	−0.703	−0.140	15.124	0.000
O. haagi	20	0.714	−0.851	−1.368	−0.529	1.956	1.149	2.763	−0.248	11.171	0.001
O. hecate	15	0.001	0.957	0.663	1.381	−1.451	−2.032	−0.870	0.059	1.327	0.249
O. nigriventris	23	0.001	0.715	0.508	1.007	−0.926	−1.471	−0.381	−0.029	0.809	0.368
O. nodulifer	38	0.001	1.142	0.861	1.515	−1.866	−2.452	−1.281	−0.215	9.692	0.002
O. nuchicornis	23	0.010	5.402	3.705	7.875	−3.392	−4.628	−2.115	−0.321	1.855	0.175
O. rupicapra	22	0.000	1.112	0.848	1.458	−1.543	−1.945	−1.141	−0.018	0.385^2	0.542
O. sloanei	46	0.000	1.010	0.792	1.287	−1.837	−2.465	−1.208	−0.220	20.653	0.000
O. taurus	113	0.000	1.405	1.198	1.648	−2.251	−2.667	−1.835	−0.200	27.726	0.000
O. vermiculatus	29	0.733	1.328	0.904	1.951	−2.016	−2.812	−1.220	0.323	10.794	0.001

Slopes and intercepts were calculated using the reduced major axis technique. Differences in the elevation of the testes allometry between morphs were tested using likelihood ratios (Warton et al. 2006). This technique is equivalent to ANCOVA, but uses the reduced major axis slopes to compare the intercepts of the two groups. "Δ elevation" is the change in the elevations of the morphs (major – minor), hence negative values indicate greater investment by minor males.^{1}df = 1, ^{2}*F*-ratio (df = 1,20) rather than W calculated using SMATR ver. 1 because ver. 2 could not calculate this value.

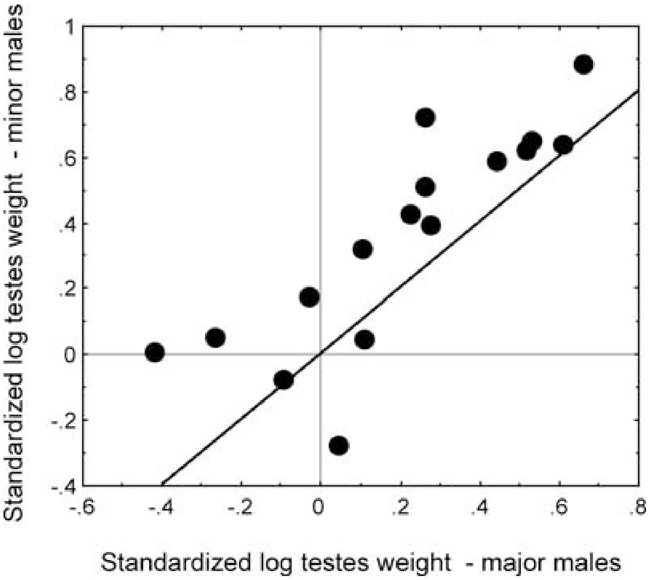

Figure 2. Log-transformed testes weight for major males plotted against log testes weight for minor males for 16 species of *Onthophagus*. Log testes weights are standardized to the mean body weight for each species using SMATR (see text for details).

$P = 0.005$). However, across species the disparity (Δ elevation in Table 3) in testes investment between major and minor males was not associated with the proportion of minor males, either before ($F_{(1,14)} = 0.37$, $P = 0.555$) or after control for phylogeny ($F_{(1,11)} = 0.34$, $P = 0.569$).

POPULATION VARIATION WITHIN SPECIES

The scaling relationships between body size and horn length for Western Australian and North American populations of *O. taurus* are depicted in Figure 3A. These populations have diverged with respect to the switch point between major and minor males, and common garden rearing has shown that this divergence has a genetic basis (Moczek et al. 2002). The switch points calculated according to equation (1) were at a pronotum width of 5.24 for the West Australian population and 4.85 for the North American population. Thus, males switch to the major male phenotype at a larger body size in Western Australia so that a significantly higher proportion of males adopt the minor or sneak mating tactic (WA 107 of 172, or 62%; USA 37 of 171, or 22%; Monte Carlo Exact $P < 0.0001$) (see also Moczek et al. 2002).

There was significant heterogeneity in the testes allometries across Western Australian and North American populations of *O. taurus* ($\chi^2_1 = 55.66$, $P = 0.001$; Fig. 3B). The North American population had a shallower slope (b {95% CI} = 0.52 {0.48, 0.67}). Within this population, the slopes for major and minor males were homogeneous ($\chi^2_1 = 0.07$, df = 1, $P = 0.804$) and there was a significant difference in the elevation of the testes allometries of minor and major males ($\text{Wald}_1 = 31.38$, $P < 0.001$), minors having relatively larger testes (intercept: major males, −1.182; minor males, −1.061). The disparity in testes sizes between major and minor males (Δ elevation −0.12) was thus half that found in the population from Western Australia (Table 3).

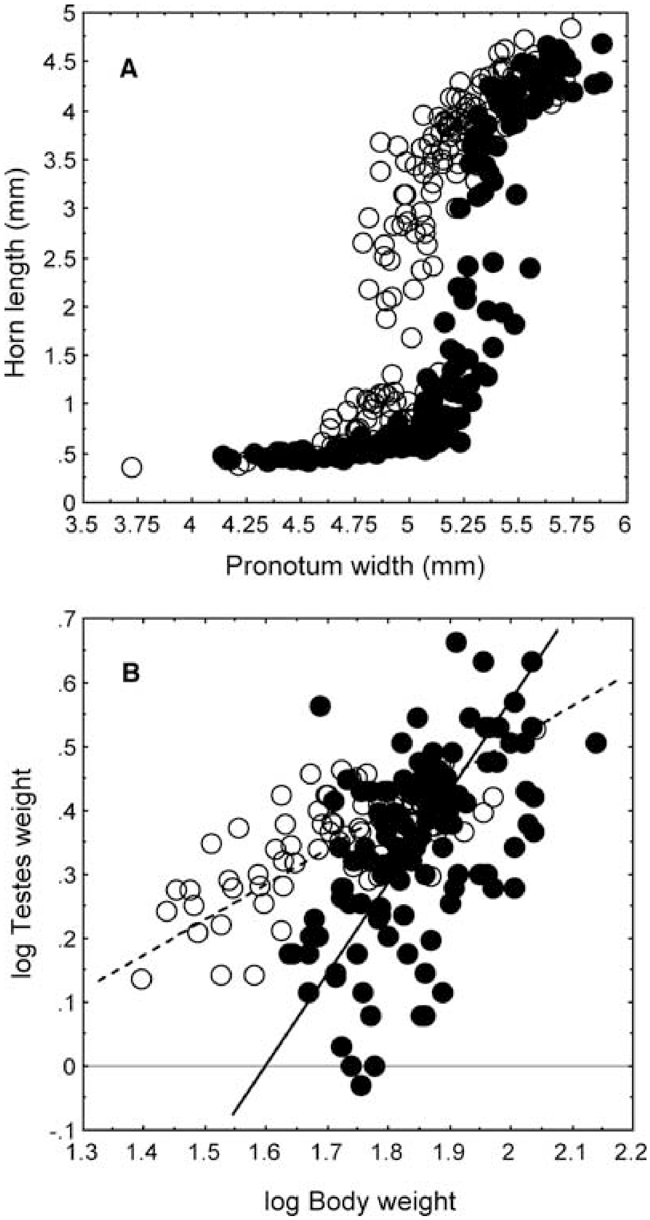

Figure 3. Population variation in the frequency of alternative mating tactics and its effects on testes size in *Onthophagus taurus*. (A) Scaling relationships between body size and horn length in Western Australia (solid symbols) and North America (open symbols) (reproduced from Moczek et al. 2002). (B) Testes allometries in Western Australia (solid symbols) and North America (open symbols). Reduced major axis slopes calculated using SMATR (see text for details).

Discussion

Parker's theoretical analysis of sperm competition games between sneaks and guards predicts that across species, male expenditure on the ejaculate should increase with the probability of a sneak mating (Parker 1990b), or the frequency of males in the population that adopt the sneaking tactic (Gage et al. 1995). In the beetle genus *Onthophagus*, many species exhibit dimorphic male morphology; minor males adopt the alternative mating tactic of sneaking copulations with females that are guarded by major males. The proportion of minor males in a species thus provides a direct measure of the relative frequency of sneaks, and an indirect measure of the probability of a sneak mating occurring. Our comparative analysis of male dimorphic onthophagines showed that, consistent with Parker's (1990b) sneak–guard models, testes size increased with the proportion of minor males. The probability of a sneak mating is directly analogous to the risk of sperm competition in Parker's (1997, 1998) general sperm competition games. These general models have garnered much support, with positive associations between sperm competition risk and testes size being reported across butterflies (Gage 1994), fish (Stockley et al. 1997), frogs (Byrne et al. 2002), birds (Pitcher et al. 2005), and mammals (Harcourt et al. 1995; Hosken 1997).

Our data also support the second prediction of Parker's (1990b) sneak–guard models, that within species, sneaks should have the greater expenditure on the ejaculate. In general we found that after controlling for differences in body size between male morphs, minor males had relatively larger testes. The models also predict that the disparity in expenditure between sneaks and guards should at first increase as the probability of a sneak mating increases, and then decrease again at high probability (Parker 1990b). This is because at low probability of a sneak mating, guards should expend very little on sperm because they rarely face sperm competition, and sneaks should invest minimally because of the low expenditure by guards. At very high probability of a sneak mating the asymmetry in sperm competition risk between sneaks and guards is low, and both should invest maximally. At moderate probability of a sneak mating, the asymmetry in risk between tactics is at its maximum, and the disparity between sneak and guard expenditure should also be at its maximum. Thus, the models predict that the disparity in expenditure between sneaks and guards should show a curvilinear relationship with increasing probability of a sneak mating. However, we failed to find such a relationship in our comparative data. There was no relationship across species between the disparity in testes size between morphs and the proportion of minor males. The proportion of minor males varied across our sample of species from 10% in *O. nuchicornis* to a maximum of 62% in Western Australian populations of *O. taurus*. It may be that we lacked species in our comparative dataset that characterized extreme values in the parameter distribution of risk. That is, if we sampled only species with moderate probability of sneak mating we would not see the predicted curvilinear relationship between the proportion of minor males and disparity in testes size between morphs. The 52% range in sneak frequencies among species in our analysis would seem sufficient to reflect a range in probability of sneak matings, and we have no reason to believe that the sample of dimorphic onthophagines used in our comparative analysis is unrepresentative; from large museum collections of 35 species of native Australian dimorphic onthophagines, the proportion of minor males ranges from 0.05 in *O. thoreyi* to 0.50 in *O. macrocephalus* and averaged 0.23 ± 0.02 (J. Hunt, unpubl. data). Nevertheless, the possibility remains that for male dimorphic onthophagines the proportion of minor males rarely reaches either the high or low values that are predicted to generate declines in the disparity in testes sizes.

Deviations from the models predictions could arise, however, from violations in their underlying assumptions. The sneak–guard model assumes that the probability of two or more sneak matings is very small and can be disregarded. It is thus assumed that guards only face sperm competition from one sneak male (Parker 1990b). In reality female onthophagines store sperm from many different males, including sneaks and guards involved in previous breeding attempts, so that sperm competition intensity is likely to be far greater than envisaged in the original sneak–guard model. It is not clear how relaxing the assumption of a single sneak mating will influence the predictions of Parker's model.

Comparative analyses can only shed light on association, so that we cannot be confident that the patterns we observe are causal. In contrast, variation in sperm competition risk across populations of the same species provides a powerful test of the selective consequences of sperm competition. Positive covariation in the risk of sperm competition and ejaculate expenditure has been demonstrated across populations of cliff swallows, *Petrochelidon pyrrhonota* (Brown and Brown 2003), leeches, *Helobdella papillornata* (Tan et al. 2004), and experimentally manipulated populations of the moths, *Plodia interpunctella* (Gage 1995) and *Pseudaletia separata* (He and Tsubaki 1992; He and Miyata 1997). The fortuitous cointroduction of *O. taurus* to Australia and North America and the subsequent evolutionary divergence of these populations provides a unique opportunity for us to examine the effects of changes in the proportion of sneaks on testes size within this single species. The position of the switch point between minor and major morphs can respond to directional selection (Emlen 1996), and there has been a genetic divergence in the position of the switch point between North American and West Australian populations of *O. taurus* so that minor males are 40% less common in North America than they are in Western Australia (Moczek et al. 2002; Moczek and Nijhout 2003). We found that the allometric slope of testes size on body size also differed between these populations, being shallower in North America than in Western Australia. As a consequence, the disparity in testes size between morphs in North America was half that found in Western Australia. If a frequency of 60% minor males in Western Australia generates a probability of a sneak mating occurring in the middle region of Parker's (1990b) parameter space, then the differences we observe between North American and Western Australian populations could be interpreted as being consistent with his prediction that the disparity in expenditure on testes size between morphs is influenced by the relative frequencies of alternative mating tactics; the 40% lower proportion of minor males in North America, and thus lower probability of a sneak mating, was associated with a smaller disparity in testes size between major and minor males. Data on the relationship between the proportion of sneaks and the probability of a sneak mating will be useful in the further interpretation of these data.

In conclusion, we have provided the first comparative test of Parker's (1990b) theoretical models involving sperm competition between alternative male mating tactics. Across 16 species of *Onthophagus*, testes size increases with the proportion of males adopting the alternative mating tactic of sneaking copulations with females guarded by major males. Within species, minor males have a greater expenditure on their ejaculate than do major males. Although insects have provided some of the greatest insights into the selective process of sperm competition, surprisingly few comparative analyses have examined the impact of sperm competition on testes size in insects. The majority of support for Parker's sperm competition game models comes from vertebrates. Prior to our study, the only other insect taxa for which there was evidence of a general relationship between sperm competition and testes size were the butterflies (Gage 1994) and fungus growing ants (Baer and Boomsma 2004). Nonetheless, unlike vertebrates, we doubt that positive covariation between sperm competition risk and testes size will prove to be a general phenomenon across insects because of the often complex nature of adaptations for the avoidance of sperm competition (Pitnick 1996; Simmons 2001). For onthophagines, patterns of sperm utilization conform to the assumptions of Parker's models. For other insect taxa, males have often solved the problem of sperm competition through adaptations for sperm removal or displacement, so that the relative numbers of sperm from rival males have little impact on paternity (Simmons 2001). Thus for insects, comparative analyses such as ours need to be grounded on tests of the underlying assumptions of sperm competition game theory.

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