Sperm competition and the coevolution of pre- and post-copulatory traits: weapons evolve faster than testes among onthophagine dung beetles

Leigh W. Simmons¹,² and John L. Fitzpatrick³

¹Centre for Evolutionary Biology, School of Animal Biology (M092), The University of Western Australia, 35 Stirling Highway, Crawley 6009, WA, Australia
²E-mail: leigh.simmons@uwa.edu.au
³Department of Zoology/Ethology, Stockholm University, Svante Arrhenius väg 18B, SE-10691 Stockholm, Sweden

KEY WORDS: male contest competition, horns, sperm length, ejaculate expenditure, evolutionary divergence, life-history trade-off

Running head: coevolution of pre- and post-copulatory traits
Abstract

Reproductive competition generates episodes of both pre- and post-copulatory sexual selection. Theoretical models of sperm competition predict that as the fitness gains from expenditure on the weapons of male combat increase, males should increase their expenditure on weapons and decrease their expenditure on traits that contribute to competitive fertilization success. Although traits subject to sexual selection are known to have accelerated evolutionary rates of phenotypic divergence, it is not known whether the competing demands of investment into pre- and post-copulatory traits affect their relative rates of evolutionary divergence. We use a comparative approach to estimate the rates of divergence in pre- and post-copulatory traits among onthophagine dung beetles. Weapons evolved faster than body size while testes mass and sperm length evolved more slowly than body size, suggesting that pre-copulatory competition is the stronger episode of sexual selection acting on these beetles. Although horns evolved faster than testes, evolutionary increases in horn length were not associated with evolutionary reductions in testes mass. Our data for onthophagines support the notion that in taxa where males are unable to monopolise paternity, expenditure on both weapons and testes should both be favored.
Males generally have the higher potential reproductive rate and can increase their reproductive success by mating with multiple females (Clutton-Brock and Parker 1992; Parker and Simmons 1996; Kokko et al. 2012). Sexual selection thereby favors traits in males that allow them to monopolise access to females (Darwin 1871; Andersson 1994). As a result, males are often larger than females or bear exaggerated secondary sexual traits that serve as weapons used in direct competition with rival males, and/or as ornaments to attract females and persuade them to mate (Berglund et al. 1996; Emlen 2008). For a variety of reasons females can also benefit from mating with more than one male (Kvarnemo and Simmons 2013) and when females remate, males will face post-copulatory sexual selection in the form of sperm competition over the fertilization of available ova (Parker 1970; Simmons 2001). When a male's competitive fertilization success depends on the number of sperm he has at the site of fertilization relative to other males, sperm competition is expected to favour increased expenditure on sperm production (Parker and Pizzari 2010), an expectation for which there is considerable evidence (Simmons and Fitzpatrick 2012).

Weapons are expensive to produce and deploy, and their expression can depend strongly on the availability of resources (Hunt and Simmons 1997; Kruuk et al. 2002; Cotton et al. 2004). Likewise sperm production can be resource dependent (Simmons and Parker 1992; Amitin and Pitnick 2007; Knell and Simmons 2010; Perry and Rowe 2010) so that males may face a resource allocation trade-off between expenditure on gaining access to females and expenditure on winning fertilizations (Simmons and Emlen 2006; reviewed in Kvarnemo and Simmons 2013). Indeed, negative covariation between male expenditure on pre- and post-copulatory traits is a fundamental assumption underlying game theoretic modelling of the evolution of ejaculate expenditure (Parker and Pizzari 2010). In their most recent iteration of these
models, Parker et al. (2013) considered explicitly how the form of pre-copulatory competition should affect male expenditure on both weapons and testes. As in previous iterations of the models, increasing levels of sperm competition predicted increasing male allocation to sperm production (testes). However, for a given level of sperm competition, as both the number of males competing for each mating opportunity and the payoff in terms of mate acquisition per unit investment in weaponry increase, males are predicted to increase their expenditure on weapons and decrease their expenditure on testes (Parker et al. 2013). In other words, among species or populations where males are able to monopolise females through direct contest competition we might expect to see males trading expenditure on testes for increased weaponry. Indeed, recent comparative studies of marine mammals and howler monkeys have found that male investment in weaponry is negatively correlated with investment in testes size (Fitzpatrick et al. 2012; Dines et al. 2015; Dunn et al. 2015). Moreover, in their taxonomically broad comparative analyses, ranging from acanthocephalan worms to primates, Lüpold et al. (2014) found negative covariation between relative testes mass and the degree of male biased sexual size dimorphism (a trait associated with sexual selection via male-male contest competition in the taxa examined) for taxa where males monopolize access to females. However, patterns of covariation become increasingly positive in taxa where males are unable to monopolize females. Lüpold et al. (2014) suggested that where females can not be monopolized, males might be selected to invest in both weapons and testes, trading increased expenditure in both pre- and post-copulatory traits for some other important life history trait. While these broad taxonomic patterns lend some support to Parker et al.’s (2013) models, Lüpold et al. (2014) stressed caution in their interpretation because the estimate of female monopolization was necessarily a
Studies that explore the relative importance of pre- and post-copulatory traits for male fitness will be required to assess more accurately the role of these episodes of sexual selection in shaping the evolution of male allocation to weapons or testes in any given taxon.

Traits that are subject to sexual selection typically exhibit accelerated rates of phenotypic evolution (Arnqvist 1998; Mendelson and Shaw 2005; Price and Whalen 2009; Gonzalez-Voyer and Kolm 2011; Seddon et al. 2013). Comparing rates of phenotypic evolution among different traits across a phylogeny can thereby provide insight into the relative importance of pre- and post-copulatory traits for male fitness; traits that contribute most to male fitness are expected to show faster rates of phenotypic evolution. Fitzpatrick et al. (2012) thus examined the rates of phenotypic evolution in male biased sexual size dimorphism and relative testes mass among pinnipeds (seals, sea lions and walruses), contrasting species that form harems with those that do not. Sexual size dimorphism was found to have evolved seven times faster among harem forming species, a pattern expected given that body size contributes strongly to a male's ability to monopolize access to harems of females (reviewed in Fitzpatrick et al. 2012). In contrast there was no significant difference in the rates of testes mass evolution, which were low regardless of whether species formed harems or not. This suggests that pre-copulatory sexual selection imposes the greater evolutionary pressure in harem forming species. Consistent with Parker et al.'s (2013) theoretical models, among harem forming species Fitzpatrick et al. (2012) found negative covariation between sexual size dimorphism and relative testes mass. In contrast, for species that do not form harems and where the rate of divergence in sexual size dimorphism was low, there was no significant covariation between sexual
size dimorphism and relative testes mass. In the current study, we use a similar comparative approach to examine rates of phenotypic evolution of pre- and post-copulatory traits across beetles in the genus *Onthophagus*.

Onthophagine dung beetles arrive at fresh animal droppings and excavate breeding tunnels in the soil below. Males typically bear exaggerated weapons in the form of horns that can occur at several locations on the head and/or thorax, and are used as weapons to contest ownership of breeding tunnels and the females nesting within (Emlen et al. 2005b). Horn length contributes strongly to contest outcome, so that weapons are subject to pre-copulatory sexual selection (Emlen 1997; Moczek and Emlen 2000; Hunt and Simmons 2001). In many species of *Onthophagus* a subset of the male population will adopt an alternative mating tactic in which they sneak into breeding tunnels and copulate with nesting females. These so called "minor" males are generally smaller in body size and do not invest in horns (Emlen et al. 2005a). By the nature of their alternative mating tactic, sneaks do not engage in pre-copulatory male combat but are always subject to sperm competition, while horned males are subject to sperm competition with low probability depending on the frequency of sneaks in the population. Consistent with sperm competition theory, comparative analysis has revealed that relative testes size increases across species of *Onthophagus* as the frequency of minor males increases, and within species, males that sneak copulations have larger testes than males that fight for and guard females (Simmons et al. 2007). Experimental evolution studies using *O. taurus* have also documented evolutionary divergence in testes mass and competitive fertilization success among lines subject to sexual selection or enforced monogamy (Simmons and García-González 2008). Collectively, these studies show that both pre- and post-copulatory sexual selection impose significant selection on male onthophagines. *Onthophagus* is
therefore an ideal taxonomic group with which to contrast rates of evolutionary divergence in pre- and post-copulatory traits.

Here we estimated the rates of phenotypic divergence in body size, one pre-copulatory trait (horn length) and two post-copulatory traits (testes mass and sperm length) among 16 species of *Onthophagus* that can be included in a molecular phylogeny of the genus, and for which we have phenotypic data. Given knowledge of the relative rates of evolution of pre- and post-copulatory traits, we then ask whether patterns of covariation between weapons and testes among onthophagine beetles are consistent with expectations from Parker *et al.*'s (2013) general sperm competition model for pre-copulatory male-male competition.

**Materials and Methods**

**DATA**

Beetles were collected in the field from fresh cattle droppings. Males were washed in fresh water before placing them into plastic containers with dry paper towel where they were held for 24 hours before dissection. This procedure reduced variation in body weight that might be due to variation in hydration and/or gut content. Beetles were weighed and the maximum width of the pronotum measured with digital callipers. The length of the horn was measured under a dissection microscope using an eye-piece graticule. For species with multiple horns, we measured the length of the largest or most exaggerated horn (horn morphologies can be viewed in figure 4 of Emlen *et al.* 2005b). Horn length has been widely used as a measure of horn size in studies of onthophagines; it has been shown to predict fight outcome (Emlen 1997; Moczek and Emlen 2000) and to be under positive selection (Hunt and Simmons 2001). We recognise that our simple measure of length is likely to underestimate
variation in horn morphology among species and that our estimates of the rate of divergence in horns may therefore be conservative. Testes were dissected and weighed to the nearest 0.01mg. Sperm were retrieved from the reproductive tracts of recently mated females following the procedures of Simmons and Kotiaho (2002). Thus, females were dissected on collection and their bursa copulatrix examined for the presence of a spermatophore. Spermatophores were removed and their contents smeared onto a clean dry microscope slide. Sperm were viewed at x100 magnification under light field, and sperm that showed no signs of damage (the head and tail were clearly visible, see figure 1b in Werner and Simmons 2011) were selected for measurement using the linear measurement tool in the Optimus Image Analysis package (Media Cybernetics, Silver Spring, MD). We measured 8.8±0.3 (range 1-13) sperm per individual and took the average. Data on pre- and postcopulatory traits were collected from a number of field sites in Australia and South Africa over a period of two decades. Therefore, sample sizes vary depending on the trait of interest. Summary data used in the comparative analysis, including sample sizes from each species for each trait, are provided in Table S1 of the online supplementary material.

**PHYLOGENY**

We constructed a molecular phylogeny based on four nuclear and three mitochondrial genes for 33 species of dung beetles from the genus *Onthophagus* (Supplementary Material Figure S1). Nuclear genes included the nuclear ribosomal subunit 28S (219 bp), ARD1-like protein a (Ard1a, 352 bp), arginine methyltransferase 1 (Art1, 441 bp) and neurofibromin 1 (Nf1, 672 bp), and mitochondrial genes included the ribosomal submit 16s (588 bp), cytochrome c oxidase subunit I (COI, 612 bp) and subunit II (COII, 545bp). Sequences were extracted from GeneBank using Geneious (v.8.1.6,
Biomatters Ltd., Kearse et al. 2012), aligned using the MUSCLE (Edgar 2004) plugin for Mesquite (v.3.03, Maddison and Maddison 1999), and used to reconstruct phylogenetic relationships among species in a Bayesian framework (see Supplementary Material Table S2 for accession numbers). The best-fit nucleotide substitution models were determined for each of the aligned sequences using jModelTest (v.2.1.7, Darriba et al. 2012) by comparing Akaike Information Criterion (AIC) values for three substitution models. The best-fit nucleotide substitution models, which were subsequently used during phylogeny construction, were GTR+\Gamma for 16s, HKY+\Gamma+I for 28s and Art1, HKY+\Gamma for Ardl1a, and GTR+\Gamma+I for Nf1, COI and COII. However, when constructing the phylogeny using jModelTest best-fit models, the Bayesian chain for COI and COII failed to converge. Therefore, for COI and COII we simplified the nucleotide substitution models to GTR, which led to convergence of the Bayesian chain.

Phylogenies were constructed using BEAST and BEAUTi (v.1.8.2, Drummond et al. 2012) using unlinked substitution models for each of the seven genes included, a relaxed uncorrelated lognormal clock, and a Yule speciation process. The ucld.mean prior was set to a uniform distribution with an initial value of 0.033 and an upper and lower value of 2 and 0, respectively. As fossil dates were not available for the species examined we did not specify root or node dates in our analyses. Following operational adjustments and sensitivity testing we performed a final Markov Chain Monte Carlo (MCMC) simulation using a chain length of 30 million generations with parameters logged every 3000 generations. The program Tracer (v.1.6.0, Drummond et al. 2012) was used to assess convergence of the Bayesian chain and to ensure adequate model mixing by assessing the effective sample size (ESS) values for each of the tree statistics. A maximum clade credibility
(MCC) tree was generated using mean node heights and a 40% burn-in using TreeAnnotator (v.1.8.2, Drummond et al. 2012). A consensus phylogeny was viewed using FigTree (v.1.4.2, Drummond et al. 2012) and exported as a Nexus file for analyses. The resulting phylogeny (Figure S1) was largely consistent with previous phylogenies of the genus *Onthophagus* (Emlen et al. 2005b).

**PHYLOGENETIC SIGNAL AND COMPARING EVOLUTIONARY MODELS**

A key assumption of the approach we use below to evaluate rates of phenotypic divergence is that trait evolution follows a Brownian motion process. Therefore, prior to analyses we used two complementary approaches to evaluate trait evolution using the phylogeny generated above. First, for each trait we assessed phylogenetic signal, a measure of how similar closely related species are to one another, using the `phylosig` function in the package *phytools* (Revell 2012) in RStudio v.3.1.2 statistical software (R Development Core Team, 2014). To assess phylogenetic signal we used Blomberg’s *K* (Blomberg et al. 2003), which compares the observed trait variance to a null model assuming traits evolve under Brownian motion, and Pagel’s *λ* (Pagel 1999), which evaluates the phylogenetic dependence of the traits independently. *K* values of 1 indicate traits evolve as expected under a Brownian motion model, while *K* values below or above 1 indicate less or more, respectively, phylogenetic signal than expected under a Brownian motion model. We then tested if *K* values differed significantly from zero. Pagel’s *λ* values range from 0 to 1, indicating no or strong phylogenetic signal, respectively. The maximum-likelihood value of *λ* was then compared statistically using likelihood ratio tests to estimates where *λ* was constrained to 0 or 1. Evaluating phylogenetic signal using Blomberg’s *K* and Pagel’s *λ* produced qualitatively similar results (Table 1), with sperm and horn length...
exhibiting lower phylogenetic signal, while pronotum width, testes mass and body mass exhibited relatively high phylogenetic signal. Thus, three of the five traits examined appear to evolve under a Brownian motion model of evolution.

Second, to contrast different models of evolution directly we used the `fitContinuous` function in the package `geiger` (Harmon et al. 2008) in RStudio v.3.1.2 to compare Brownian motion (BM), Ornstein-Uhlenbeck (OU) and Early-burst (EB) models of evolution for each of the traits (Table 2). For all traits, the OU model did not converge using the default bounds in `geiger`. Therefore, for all OU models the maximum value in the `bounds` argument in `fitContinuous` was increased to 10, which facilitated model convergence. Sample size corrected AICc comparisons of evolutionary models revealed that for all traits the BM model and OU model were statistically indistinguishable ($\Delta_i < 2$, see Table 2).

The results presented in Table 2 clearly show that BM model of trait evolution is the best model, or statistically indistinguishable from alternative models, for every trait assessed in our analyses. Therefore all subsequent analyses assessed the rate of phenotypic evolution under a Brownian motion model of evolutionary change. However, because Blomberg’s K and Pagel’s $\lambda$ values suggested poor fit with BM models for sperm length and horn length we caution that the evolutionary divergence results for linear measures may be less robust than those for mass measures. Therefore, we refrain from interpreting the magnitude of the differences in rates of phenotypic diversification and focus instead on the broad patterns revealed from our analyses of linear traits.
PHYLOGENETIC LINEAR MODELS

To control for shared ancestry when assessing evolutionary relationships among continuous variables, the relationships between traits were assessed using phylogenetically controlled generalized least-squares (PGLS) models (Freckleton et al. 2002) in the APE package of RStudio v.3.1.2, using the molecular phylogeny constructed above. All data were log10-transformed to linearize relationships. Multiple regression PGLS models were used to assess the association between horn length (and sperm length) and body size corrected testes mass (a commonly used proxy measure for sperm competition, Simmons and Fitzpatrick 2012). We used PGLS models to estimate the phylogenetic scaling parameter $\lambda$, which estimates the impact of the phylogeny on the covariance in the model residuals. Likelihood ratio tests were used to determine if $\lambda$ values differed significantly from 0, indicating no phylogenetic dependence, and 1, indicating phylogenetic dependence of the traits. Significance values for likelihood ratio tests are presented in subscript following $\lambda$ values comparing 0 and 1, respectively. However, because BM and OU models of evolution for sperm length and horn length we statistically indistinguishable we also performed PGLS regressions assuming an OU model of evolution by specifying an OU error structure in the model using $\text{corMartins}$ function.

COMPARING EVOLUTIONARY RATES

To compare evolutionary rates of phenotypic divergence among species we used a recently developed likelihood approach that allows the evolutionary Brownian rate parameter, $\sigma^2$, of multiple traits to be directly compared on a phylogeny (Adams 2013). This method determines the observed rate of evolutionary divergence in phenotypic traits ($\sigma^2_{\text{obs}}$) and contrasts the likelihood values of observed models,
which assume traits evolve at distinct rates, with an evolutionarily constrained model where all traits evolve at a common evolutionary rate ($\sigma^2_{\text{common}}$). Likelihood ratio tests are then used to compare the observed and common evolutionary models, with significant differences supporting the hypothesis that traits are evolving at different evolutionary rates.

We compared the evolutionary rate of three log10-transformed linear measures (sperm length, horn length and pronotum width) and two log10-transformed mass measures (testes mass and body mass) using the R code provided in Appendix 2 of Adams (2013). Adams (2013) warned that differences in trait scales can dramatically impact estimates of evolutionary rates as per-unit changes in trait values, and by extension evolutionary rates, are influenced by measurement units. Therefore, all data were log10-transformed prior to estimating evolutionary rates to create unit-less variables (Adams 2013). However, we separated our analyses of linear length and mass measures because variance is expected to be higher in higher dimensional traits, making comparisons between lengths and weights problematic (Houle 1992).

For linear measurement, which compared three traits, we used pairwise comparisons between all combinations of traits to determine which traits were evolving at different evolutionary rates. All models presented in the main text converged using the L-BFGS-B optimization function recommended in Adams’ code (see Table S3 for cases when different optimization functions were required to achieve model convergence). For all models we assumed evolutionary covariation in the observed evolutionary rate matrix among traits, which is generally the case among phenotypic traits (Adams 2013) and recent theoretical models assume covariation in allocation of resources to pre- and post-copulatory traits (Parker et al. 2013).

Although we consider models assuming trait covariation to be more biologically
intuitive (see Adams 2013), we also explored models assuming no trait covariation in
the observed evolutionary rate matrix (i.e. the off-diagonal of the evolutionary rate
matrix is set to zero) and present these models in Table S3. The model outputs are
largely similar when assuming trait covariation or independent evolution in the
observed rate matrices (discussed in Table S3). Evolutionary rate comparisons can
also incorporate intra-specific trait covariance and measurement error (i.e. error
around the mean), two parameters that can influence the effect estimates in
phylogenetic models (Adams 2013), into the common evolutionary rates model.
Therefore, when contrasting evolutionary rates of mass measures (testes and body
mass) we incorporated both log10-standard error and intra-specific covariance values
(Table S4) into our analyses. However, we only incorporated measurement error, not
intra-specific trait covariance, in models assessing linear traits as sperm length data
were obtained from different, and fewer, individuals than pronotum width and horn
length. To investigate the impact of intra-specific covariance on length measures we
ran a separate model only assessing horn and pronotum length to compare
evolutionary rates while incorporating measurement error and intra-specific trait
covariance, which revealed qualitatively similar results to those presented in the main
text (Table S5).
Finally, while alternative reproductive tactics and their associated male
dimorphisms are a common feature of the onthophagine mating system, they are not
ubiquitous across the genus. Our analysis included both male dimorphic and
monomorphic species (Table S1) and the presence of minor males that sneak
copulations in some species but not others may affect the rates of evolutionary
divergence in pre- and post-copulatory traits. We therefore looked for variation in
evolutionary rates between monomorphic and dimorphic taxa (Table S6). Body size
tended to diverge more slowly among dimorphic taxa but effect sizes were very small. Importantly there were no significant differences in evolutionary rates between monomorphic and dimorphic taxa for testes mass, sperm length or horn length (Table S6) so that the presence of minor males are unlikely to affect the results reported here.

Results

We detected significant differences in evolutionary rates among the linear and mass measures assessed (Figure 1, Table 3). Likelihood ratio tests revealed that sperm length, horn length and pronotum width were not evolving at a common evolutionary rate (Figure 1a, Table 3a). Rather, pairwise comparisons revealed that the rate of evolutionary change in horn length was significantly faster than pronotum width, which in turn evolved at a significantly faster rate than sperm length. The results obtained using likelihood ratio tests were supported by comparisons of AIC between observed and common evolutionary models (Table 3a). We obtained largely similar results using models that assumed traits evolved independently, with horn length evolving faster than sperm length and pronotum width (Supplementary Material Table S3).

In addition, testes mass and body mass evolved at different evolutionary rates in the trait covariance model (Figure 1b, Table 3b), with the rate of body mass evolution being significantly faster than testes mass. In contrast the model assessing testes and body mass that assumed trait independence did not detect a significant difference in evolutionary rates between these mass measures (Table S3). However, as we argue above, the model assuming trait covariance is more appropriate for comparing evolutionary rates of testes and body mass (see also Adams 2013).
Since the inclusion of both dimorphic and monomorphic species in our analyses could increase within-species variance, we also examined the rates of phenotypic divergence among monomorphic species exclusively (n=7 species, see Table S1). Despite the reduction in sample size, these analyses revealed similar results to our analyses of the whole dataset. Specifically, sperm length in monomorphic species evolved slower than horn length and pronotum width (Table S7). Body mass in monomorphic species evolved faster than testes mass, however this difference was no longer significant with the greatly reduced sample size (Table S7).

There was no evolutionary relationship between sperm length and testes mass when assessed while controlling for body mass (BM model: $\lambda^{1.0.43}$, testes mass: $\beta=0.12$, t=0.43, p=0.67; body mass: $\beta=-0.02$, t=-0.07, p=0.95; OU model: $\alpha=1.01$, testes mass: $\beta=0.12$, t=0.42, p=0.68; body mass: $\beta=-0.02$, t=-0.07, p=0.95). We detected a positive significant evolutionary relationship between horn length and testes mass, corrected for body size (BM model: $\lambda^{1.0.003}$, testes mass: $\beta=1.98$, t=2.25, p=0.04; body mass: $\beta=-0.90$, t=-1.30, p=0.22; OU model: $\alpha=9.22$, testes mass: $\beta=1.97$, t=2.25, p=0.04; body mass: $\beta=-0.90$, t=-1.30, p=0.22).

**Discussion**

Traits that are subject to sexual selection exhibit accelerated evolutionary rates of phenotypic divergence (Gonzalez-Voyer and Kolm 2011). Previous studies on the rates of phenotypic divergence have focused on male sexual traits that serve as ornaments in mate choice (Mendelson and Shaw 2005; Price and Whalen 2009; Gonzalez-Voyer and Kolm 2011; Seddon et al. 2013), or on male genitalia (Arnqvist 1998). While weapons clearly show all the signs of rapid divergent evolution (Emlen 2008), and comparative analyses of bovids and cervids show that sexual selection can
be responsible for the evolution of size and shape of male weaponry (Caro et al. 2003; Bro-Jørgensen 2007), no study has yet formally quantified the rates of phenotypic evolution in animal weapons. Horn length is under positive directional selection in onthophagine dung beetles, providing a competitive advantage in disputes over access to females (Emlen 1997; Moczek and Emlen 2000) that translates into an increased reproductive fitness (Hunt and Simmons 2001). We found that horn length in these beetles has diverged faster than a linear measure of body size and sperm length. Our analysis thereby illustrates how the weapons of sexual selection, like ornaments, can be subject to accelerated evolutionary rates of phenotypic divergence.

In contrast, testes mass and sperm length showed reduced rates of phenotypic divergence compared to body size. These results suggest that sexual selection on male weaponry during pre-copulatory contest competition is stronger than sexual selection on ejaculate expenditure arising from post-copulatory sperm competition. We might therefore expect male allocation to pre-copulatory traits to be prioritised over allocation to post-copulatory traits (Parker et al. 2013), and there is within species evidence from studies of *O. taurus* to support this expectation (Simmons and Buzatto 2014). In general, within species studies of a broad variety of taxa have found that exposing males to sperm competition risk results in increased expenditure on the ejaculate (delBarco-Trillo 2011; Kelly and Jennions 2011). In *O. taurus* however, horned males exposed to rivals during their early reproductive development increased their allocation to body mass rather than testes growth, which was unaffected by exposure to rival males (Simmons and Buzatto 2014). Moreover, horned males show condition dependence in strength but not testes mass while minor males show condition dependence in testes mass but not strength (Knell and Simmons 2010).

These patterns of phenotypic variation make adaptive sense for horned and minor
male *O. taurus* who specialise on pre-copulatory or post-copulatory male-male competition respectively.

In their comparative analysis of pinnipeds, Fitzpatrick *et al.* (2012) found accelerated rates of phenotypic divergence in male biased sexual size dimorphism and negative covariation between sexual size dimorphism and testes mass, but only among harem forming species where males are able to monopolize access to females.

Variation in the monopolizability of females may provide a general explanation for variation in the direction of covariation between weapons and testes across a diversity of taxa (Lüpold *et al.* 2014). Given the faster rates of phenotypic divergence of weapons among onthophagine dung beetles and the importance of weapons for controlling access to breeding tunnels and the females nesting within, we might expect, all else being equal, to see negative covariation between weapons and testes (Parker *et al.* 2013). Studies of *O. nigriventris* have shown that males do indeed suffer a resource allocation trade off between weapons and testes; males prevented from developing horns grew larger and had relatively larger testes than males allowed to grow horns (Simmons and Emlen 2006). However, among species, we found the pattern of covariation between weapons and testes to be positive rather than negative.

How can we reconcile these observations?

First inferring trade-offs among traits from correlations between them, either at the intra- or interspecific level, can be difficult. When variance in acquisition of resources exceeds variance in allocation of those resources to life history traits such as weapons and testes, species better able to acquire resources will have a larger energy budget to allocate to both traits, generating a positive correlation between them (van Noordwijk and de Jong 1986). Second, males may have a fixed reproductive energy budget that they allocate to both weapons and testes, with correlated investment in
these traits trading against somatic maintenance (Parker et al. 2013). Indeed, allocation to both weapons and testes may be necessary when males are unable to monopolise females (Lüpold et al. 2014).

Lüpold et al. (2014) argued that a general shift in the direction of covariation between weapons and testes, from negative in taxa where female monopolization is complete to positive in taxa where females can not be monopolized, might be expected because in the former male reproductive success depends only on a male's ability to control access to females, while in the latter it will depend both on a male's ability to compete for mating opportunities and for fertilizations. The occurrence of alternative mating tactics in some onthophagines compromises the ability of males to monopolize access to paternity. Sneaks can occur in high frequency and shared paternity of broods means that males successful in defending breeding tunnels from other horned males will nonetheless loose paternity to sneaks that mate undetected in the breeding tunnels (Tomkins and Simmons 2000; Simmons et al. 2004). The occurrence of alternative mating tactics in onthophagines suggests that post-copulatory sexual selection, even if weaker than pre-copulatory sexual selection, should favor males able to allocate resources to testes. Indeed, evolutionary increases in the frequency of minor males are associated with evolutionary increases in relative testes mass among species (Simmons et al. 2007). Even in species that lack male dimorphism, males will also be subject to sperm competition because females travel between dung deposits carrying sperm from previous mates in their sperm stores. Thus, male onthophagines are unlikely to be able to monopolise paternity, favouring male investment in both weapons and testes.

The weapons and ornaments of sexual selection typically evolve condition dependence, and exhibit patterns of positive allometry (Simmons and Tomkins 1996;
underlying allometries between weapons and reproductive traits could explain the patterns of evolutionary divergence observed in our study, particularly if weapons and reproductive traits exhibit positive and negative allometric relationships, respectively. In their comparative analysis of 25 species of *Onthophagus*, Simmons & Emlen (2006) found that species with the strongest positive horn allometries had the most canalised patterns of testes growth, suggesting that strong pre-copulatory sexual selection on weapons is associated with an evolutionary response that protects testes from resource allocation trade-offs. Canalisation of testes investment might also constrain their evolutionary divergence and account for the relatively low rate of phenotypic divergence across the onthophagine phylogeny when compared with weapons. If male onthophagines do not trade weapons for testes, then life-history theory demands that they must trade their co-expenditure on weapons and testes for some other life history trait. Indeed, across onthophagines there is evidence that males trade expenditure on weapons for expenditure on antennae, eyes or wings, depending on horn location (Emlen 2001), and within species studies of *O. taurus* suggest that horn growth may prolong development and reduce survival, requiring an up-regulation of immune function and increased risk of pre-adult mortality (Hunt and Simmons 1997; Cotter et al. 2008).

Sperm length showed the slowest rate of phenotypic divergence of all the linear traits measured. This is in marked contrast to a recent study of birds in which sperm length was shown to exhibit patterns of accelerated phenotypic divergence (Rowe et al. 2015). Specifically, the rate of divergence in sperm length among birds was found to be positively associated with relative testes mass, suggesting that post-copulatory sexual selection may be responsible for sperm length divergence. There is
evidence from a variety of taxa that support the notion that sperm competition does favor the evolution of increased sperm length (reviewed in Simmons and Fitzpatrick 2012). However, for the onthophagines included in this analysis evolutionary increases in relative testes mass were not associated with evolutionary increases in sperm length, suggesting that sperm competition is not driving increases in sperm length among these beetles. Interestingly, males with shorter sperm have been reported to have a selective advantage in sperm competition in *O. taurus*, and this advantage is mediated by the size of the female sperm storage organ (García-González and Simmons 2007). Thus, cryptic female choice in *O. taurus* selects for decreasing sperm length (Simmons and Kotiaho 2007). Selection for increased sperm length under sperm competition opposed by selection for decreased sperm length from cryptic female choice could generate net stabilizing selection on sperm length, which would be manifest by particularly slow rates of phenotypic evolution, which seems to be the case for sperm length across the onthophagine phylogeny.

In conclusion, we show that weapons used in male-male combat can exhibit patterns of accelerated phenotypic divergence in much the same way as male ornaments used by females in mate choice. Among onthophagine dung beetles, pre-copulatory traits exhibit faster rates of evolutionary divergence than do post-copulatory traits suggesting that pre-copulatory male-male competition exerts the stronger sexual selection on males. Parker *et al.'s* (2013) general model for pre-copulatory male-male competition predicts that where male fitness depends strongly on success in male-male competition, males should trade investment in testes for increased weaponry, and there is growing evidence that male expenditure on weapons is associated with lower expenditure on testes (Fitzpatrick et al. 2012; Buzatto et al. 2015; Dines et al. 2015). However, our data for onthophagines support the notion that
where males are unable to monopolise paternity, they may be selected to invest in
both weapons and testes, generating positive associations between pre- and post-
copulatory traits and trade-offs with alternative life-history traits (Lüpold et al. 2014).

Acknowledgements

We thank Dean Adams for advice on implementing his procedures. This work was
supported by the Australian Research Council (LWS) and a University of Western
Australia Research Collaboration Award (LWS & JLF).

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Table 1. Evaluation of phylogenetic signal in each of the traits examined. Phylogenetic signal was evaluated using Blomberg’s $K$ and Pagel’s $\lambda$. $K$ values and $\lambda$ values of 1 are indicative of trait evolution following a Brownian motion model. For Blomberg’s $K$, significant $p$-values indicate phylogenetic signal in the trait. For Pagel’s $\lambda$, the maximum likelihood estimate of $\lambda$ was compared using likelihood ratio test to models were $\lambda=0$ and $\lambda=1$. Significant $p$-values indicate if $\lambda$ differs significantly from models assuming no phylogenetic signal ($\lambda=0$) or strong phylogenetic signal ($\lambda=1$). All analyses were conducted using log10-transformed data.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Blomberg’s $K$</th>
<th>$p$-value</th>
<th>$\lambda$</th>
<th>lnL $\lambda$</th>
<th>lnL $\lambda = 0$ ($p$-value)</th>
<th>lnL $\lambda = 1$ ($p$-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm length</td>
<td>0.57</td>
<td>0.34</td>
<td>&lt;0.001</td>
<td>15.65</td>
<td>15.65 (1.0)</td>
<td>13.63 (0.04)</td>
</tr>
<tr>
<td>Horn length</td>
<td>0.57</td>
<td>0.36</td>
<td>&lt;0.001</td>
<td>-8.40</td>
<td>-8.40 (1.0)</td>
<td>-10.62 (0.04)</td>
</tr>
<tr>
<td>Pronotum width</td>
<td>0.87</td>
<td>0.04</td>
<td>0.93</td>
<td>7.87</td>
<td>6.39 (0.09)</td>
<td>7.80 (1.0)</td>
</tr>
<tr>
<td>Testes mass</td>
<td>0.81</td>
<td>0.08</td>
<td>0.91</td>
<td>-4.79</td>
<td>-5.81 (0.15)</td>
<td>-4.88 (1.0)</td>
</tr>
<tr>
<td>Body mass</td>
<td>0.91</td>
<td>0.02</td>
<td>0.99</td>
<td>-7.75</td>
<td>-9.65 (0.05)</td>
<td>-7.75 (1.0)</td>
</tr>
</tbody>
</table>
Table 2. Comparison of model parameters and fit for each trait examined under Brownian motion (BM), Ornstein-Uhlenbeck (OU) and Early-burst (EB) evolutionary models. The Brownian rate parameter, \( \sigma^2 \), selection strength parameter, \( \alpha \), and rate of evolutionary change parameter, \( a \), are presented for the BM, OU and EB models, respectively. For all models the maximum likelihood estimates (lnL) and sample size corrected Akaike Information Criterion (AICc) values are presented. Models were compared using the AICc values presented in the table. To compare model fits, for each trait we report the value of delta AICc, \( \Delta_i \), and the Akaike weights, \( \omega_i \), which indicate the strength of evidence for each model. \( \Delta_i < 2 \) indicates statistically equivalent models of evolution (Burnham and Anderson 2002).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Brownian Motion Model</th>
<th>Ornstein-Uhlenbeck Model</th>
<th>Early Burst Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \sigma^2 )</td>
<td>lnL</td>
<td>AICc</td>
</tr>
<tr>
<td>Sperm length</td>
<td>0.21</td>
<td>13.63</td>
<td>-22.33</td>
</tr>
<tr>
<td>Horn length</td>
<td>4.30</td>
<td>-10.62</td>
<td>26.15</td>
</tr>
<tr>
<td>Pronotum width</td>
<td>0.43</td>
<td>7.80</td>
<td>-10.68</td>
</tr>
<tr>
<td>Testes mass</td>
<td>2.10</td>
<td>-4.88</td>
<td>14.68</td>
</tr>
<tr>
<td>Body mass</td>
<td>3.01</td>
<td>-7.75</td>
<td>20.42</td>
</tr>
</tbody>
</table>
Table 3. Comparisons of evolutionary rates assuming trait covariance in the observed rate matrices of (a) length measures (sperm and horn length and pronotum width) incorporating measurement error and (b) mass measures (testes and body mass) measurement error and intra-specific trait covariance in models. The observed and common rate matrices are shown. The Log Likelihood values for the observed ($L_{obs}$), and common models ($L_{common}$), Log-Likelihood-Ratio tests (LRT) comparing models of observed rates with evolutionarily constrained models where all traits evolve at a common rate, p values, and AIC values for the observed ($AIC_{obs}$) and common ($AIC_{common}$) models are presented. LRT and AIC comparisons of pairwise trait analyses are also shown for linear measures.

(a) length measure comparisons

<table>
<thead>
<tr>
<th></th>
<th>$\sigma^2_{obs}$</th>
<th>$\sigma^2_{common}$</th>
<th>Log($L_{obs}$)</th>
<th>Log($L_{common}$)</th>
<th>LRT</th>
<th>P</th>
<th>AIC$_{obs}$</th>
<th>AIC$_{common}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm</td>
<td>0.21</td>
<td>1.09</td>
<td>16.47</td>
<td>2.10</td>
<td>28.73</td>
<td>&lt;0.001</td>
<td>-20.93</td>
<td>3.80</td>
</tr>
<tr>
<td>Horn</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pronotum</td>
<td>0.16</td>
<td>0.19</td>
<td>1.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pairwise analyses:
- sperm vs. horn: 5.47 -4.01 18.96 <0.001 -2.94 14.02
- horn vs. pronotum: 0.64 -4.53 10.34 0.001 6.71 15.06
- sperm vs. pronotum: 22.97 12.04 21.86 <0.001 -37.94 -18.08

(b) mass measure comparisons

<table>
<thead>
<tr>
<th></th>
<th>$\sigma^2_{obs}$</th>
<th>$\sigma^2_{common}$</th>
<th>Log($L_{obs}$)</th>
<th>Log($L_{common}$)</th>
<th>LRT</th>
<th>P</th>
<th>AIC$_{obs}$</th>
<th>AIC$_{common}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testes</td>
<td>2.10</td>
<td>2.61</td>
<td>1.52</td>
<td>-2.95</td>
<td>8.93</td>
<td>0.003</td>
<td>4.97</td>
<td>11.90</td>
</tr>
<tr>
<td>Body</td>
<td>2.42</td>
<td>2.05</td>
<td>2.61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Estimates of the observed evolutionary rates ($\sigma^2$) of phenotypic divergence across the *Onthophagus* phylogeny of three linear (a) and two mass (b) traits with their 95% confidence intervals. Evolutionary rates were assessed using log10-transformed variables.