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

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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

gross estimate of true variation among the mating systems of the taxa involved. 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

144 therefore an ideal taxonomic group with which to contrast rates of evolutionary
145 divergence in pre- and post-copulatory traits.
146 Here we estimated the rates of phenotypic divergence in body size, one pre-
147 copulatory trait (horn length) and two post-copulatory traits (testes mass and sperm
148 length) among 16 species of *Onthophagus* that can be included in a molecular
149 phylogeny of the genus, and for which we have phenotypic data. Given knowledge of
150 the relative rates of evolution of pre- and post-copulatory traits, we then ask whether
151 patterns of covariation between weapons and testes among onthophagine beetles are
152 consistent with expectations from Parker *et al.*'s (2013) general sperm competition
153 model for pre-copulatory male-male competition.
154

155 ## *Materials and Methods*

156 ### DATA

157 Beetles were collected in the field from fresh cattle droppings. Males were washed in
158 fresh water before placing them into plastic containers with dry paper towel where
159 they were held for 24 hours before dissection. This procedure reduced variation in
160 body weight that might be due to variation in hydration and/or gut content. Beetles
161 were weighed and the maximum width of the pronotum measured with digital
162 callipers. The length of the horn was measured under a dissection microscope using
163 an eye-piece graticule. For species with multiple horns, we measured the length of the
164 largest or most exaggerated horn (horn morphologies can be viewed in figure 4 of
165 Emlen et al. 2005b). Horn length has been widely used as a measure of horn size in
166 studies of onthophagines; it has been shown to predict fight outcome (Emlen 1997;
167 Moczek and Emlen 2000) and to be under positive selection (Hunt and Simmons
168 2001). We recognise that our simple measure of length is likely to underestimate

169 variation in horn morphology among species and that our estimates of the rate of
170 divergence in horns may therefore be conservative. Testes were dissected and
171 weighed to the nearest 0.01mg. Sperm were retrieved from the reproductive tracts of
172 recently mated females following the procedures of Simmons and Kotiaho (2002).
173 Thus, females were dissected on collection and their bursa copulatrix examined for
174 the presence of a spermatophore. Spermatophores were removed and their contents
175 smeared onto a clean dry microscope slide. Sperm were viewed at x100 magnification
176 under light field, and sperm that showed no signs of damage (the head and tail were
177 clearly visible, see figure 1b in Werner and Simmons 2011) were selected for
178 measurement using the linear measurement tool in the Optimus Image Analysis
179 package (Media Cybernetics, Silver Spring, MD). We measured 8.8±0.3 (range 1-13)
180 sperm per individual and took the average. Data on pre- and postcopulatory traits
181 were collected from a number of field sites in Australia and South Africa over a
182 period of two decades. Therefore, sample sizes vary depending on the trait of interest.
183 Summary data used in the comparative analysis, including sample sizes from each
184 species for each trait, are provided in Table S1 of the online supplementary material.
185

186 **PHYLOGENY**

187 We constructed a molecular phylogeny based on four nuclear and three mitochondrial
188 genes for 33 species of dung beetles from the genus *Onthophagus* (Supplementary
189 Material Figure S1). Nuclear genes included the nuclear ribosomal subunit 28S (219
190 bp), ARD1-like protein a (Ard1a, 352 bp), arginine methyltransferase 1 (Art1, 441 bp)
191 and neurofibromin 1 (Nf1, 672 bp), and mitochondrial genes included the ribosomal
192 submit 16s (588 bp), cytochrome c oxidase subunit I (COI, 612 bp) and subunit II
193 (COII, 545bp). Sequences were extracted from GeneBank using Geneious (v.8.1.6,

194 Biomatters Ltd., Kearse et al. 2012), aligned using the MUSCLE (Edgar 2004) plugin
195 for Mesquite (v.3.03, Maddison and Maddison 1999), and used to reconstruct
196 phylogenetic relationships among species in a Bayesian framework (see
197 Supplementary Material Table S2 for accession numbers). The best-fit nucleotide
198 substitution models were determined for each of the aligned sequences using
199 jModelTest (v.2.1.7, Darriba et al. 2012) by comparing Akaike Information Criterion
200 (AIC) values for three substitution models. The best-fit nucleotide substitution
201 models, which were subsequently used during phylogeny construction, were GTR+Γ
202 for 16s, HKY+Γ+I for 28s and Art1, HKY+ Γ for Ard1a, and GTR+Γ+I for Nf1, COI
203 and COII. However, when constructing the phylogeny using jModelTest best-fit
204 models, the Bayesian chain for COI and COII failed to converge. Therefore, for COI
205 and COII we simplified the nucleotide substitution models to GTR, which led to
206 convergence of the Bayesian chain.
207 Phylogenies were constructed using BEAST and BEAUTi (v.1.8.2,
208 Drummond et al. 2012) using unlinked substitution models for each of the seven
209 genes included, a relaxed uncorrelated lognormal clock, and a Yule speciation
210 process. The ucld.mean prior was set to a uniform distribution with an initial value of
211 0.033 and an upper and lower value of 2 and 0, respectively. As fossil dates were not
212 available for the species examined we did not specify root or node dates in our
213 analyses. Following operational adjustments and sensitivity testing we performed a
214 final Markov Chain Monte Carlo (MCMC) simulation using a chain length of 30
215 million generations with parameters logged every 3000 generations. The program
216 Tracer (v.1.6.0, Drummond et al. 2012) was used to assess convergence of the
217 Bayesian chain and to ensure adequate model mixing by assessing the effective
218 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 *a* 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 λ 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 λ, which estimates the impact of the phylogeny on the covariance in the model residuals. Likelihood ratio tests were used to determine if λ 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 λ 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 *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, σ^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 (σ^2_{obs}) and contrasts the likelihood values of observed models,

294 which assume traits evolve at distinct rates, with an evolutionarily constrained model
295 where all traits evolve at a common evolutionary rate (σ^2_{common}). Likelihood ratio tests
296 are then used to compare the observed and common evolutionary models, with
297 significant differences supporting the hypothesis that traits are evolving at different
298 evolutionary rates.

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

309 For linear measurement, which compared three traits, we used pairwise
310 comparisons between all combinations of traits to determine which traits were
311 evolving at different evolutionary rates. All models presented in the main text
312 converged using the L-BFGS-B optimization function recommended in Adams' code
313 (see Table S3 for cases when different optimization functions were required to
314 achieve model convergence). For all models we assumed evolutionary covariation in
315 the observed evolutionary rate matrix among traits, which is generally the case among
316 phenotypic traits (Adams 2013) and recent theoretical models assume covariation in
317 allocation of resources to pre- and post-copulatory traits (Parker et al. 2013).
318 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: β=0.12, t=0.43, p=0.67; body mass: β=-0.02, t=-0.07, p=0.95; OU model: α=1.01, testes mass: β=0.12, t=0.42, p=0.68; body mass: β=-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: β=1.98, t=2.25, p=0.04; body mass: β=-0.90, t=-1.30, p=0.22; OU model: α=9.22, testes mass: β=1.97, t=2.25, p=0.04; body mass: β=-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

393 be responsible for the evolution of size and shape of male weaponry (Caro et al. 2003;
394 Bro-Jørgensen 2007), no study has yet formally quantified the rates of phenotypic
395 evolution in animal weapons. Horn length is under positive directional selection in
396 onthophagine dung beetles, providing a competitive advantage in disputes over access
397 to females (Emlen 1997; Moczek and Emlen 2000) that translates into an increased
398 reproductive fitness (Hunt and Simmons 2001). We found that horn length in these
399 beetles has diverged faster than a linear measure of body size and sperm length. Our
400 analysis thereby illustrates how the weapons of sexual selection, like ornaments, can
401 be subject to accelerated evolutionary rates of phenotypic divergence.
402 In contrast, testes mass and sperm length showed reduced rates of phenotypic
403 divergence compared to body size. These results suggest that sexual selection on male
404 weaponry during pre-copulatory contest competition is stronger than sexual selection
405 on ejaculate expenditure arising from post-copulatory sperm competition. We might
406 therefore expect male allocation to pre-copulatory traits to be prioritised over
407 allocation to post-copulatory traits (Parker et al. 2013), and there is within species
408 evidence from studies of *O. taurus* to support this expectation (Simmons and Buzatto
409 2014). In general, within species studies of a broad variety of taxa have found that
410 exposing males to sperm competition risk results in increased expenditure on the
411 ejaculate (delBarco-Trillo 2011; Kelly and Jennions 2011). In *O. taurus* however,
412 horned males exposed to rivals during their early reproductive development increased
413 their allocation to body mass rather than testes growth, which was unaffected by
414 exposure to rival males (Simmons and Buzatto 2014). Moreover, horned males show
415 condition dependence in strength but not testes mass while minor males show
416 condition dependence in testes mass but not strength (Knell and Simmons 2010).
417 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;

Wilkinson and Taper 1999; Fromhage and Kokko 2014). Indeed, differences in 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

493 evidence from a variety of taxa that support the notion that sperm competition does
494 favor the evolution of increased sperm length (reviewed in Simmons and Fitzpatrick
495 2012). However, for the onthophagines included in this analysis evolutionary
496 increases in relative testes mass were not associated with evolutionary increases in
497 sperm length, suggesting that sperm competition is not driving increases in sperm
498 length among these beetles. Interestingly, males with shorter sperm have been
499 reported to have a selective advantage in sperm competition in *O. taurus,* and this
500 advantage is mediated by the size of the female sperm storage organ (García-
501 González and Simmons 2007). Thus, cryptic female choice in *O. taurus* selects for
502 decreasing sperm length (Simmons and Kotiaho 2007). Selection for increased sperm
503 length under sperm competition opposed by selection for decreased sperm length
504 from cryptic female choice could generate net stabilizing selection on sperm length,
505 which would be manifest by particularly slow rates of phenotypic evolution, which
506 seems to be the case for sperm length across the onthophagine phylogeny.
507 In conclusion, we show that weapons used in male-male combat can exhibit
508 patterns of accelerated phenotypic divergence in much the same way as male
509 ornaments used by females in mate choice. Among onthophagine dung beetles, pre-
510 copulatory traits exhibit faster rates of evolutionary divergence than do post-
511 copulatory traits suggesting that pre-copulatory male-male competition exerts the
512 stronger sexual selection on males. Parker *et al.*'s (2013) general model for pre-
513 copulatory male-male competition predicts that where male fitness depends strongly
514 on success in male-male competition, males should trade investment in testes for
515 increased weaponry, and there is growing evidence that male expenditure on weapons
516 is associated with lower expenditure on testes (Fitzpatrick et al. 2012; Buzatto et al.
517 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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705

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

711

712

	Blomberg's K		Pagel's λ			
Trait	***K***	***p*-value**	**λ**	**lnL λ**	**lnL λ = 0 (*p*-value)**	**lnL λ = 1 (*p*-value)**
Sperm length	0.57	0.34	<0.001	15.65	15.65 (1.0)	13.63 (0.04)
Horn length	0.57	0.36	<0.001	-8.40	-8.40 (1.0)	-10.62 (0.04)
Pronotum width	0.87	0.04	0.93	7.87	6.39 (0.09)	7.80 (1.0)
Testes mass	0.81	0.08	0.91	-4.79	-5.81 (0.15)	-4.88 (1.0)
Body mass	0.91	0.02	0.99	-7.75	-9.65 (0.05)	-7.75 (1.0)

713

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, σ^2, selection strength parameter, α, 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 compares model fits, for each trait we report the value of delta AICc, Δ_i, and the Akaike weights, ω_i, which indicate the strength of evidence for each model. $\Delta_i < 2$ indicates statically equivalent models of evolution (Burnham and Anderson 2002).

	Brownian Motion Model					Ornstein-Uhlenbeck Model					Early Burst Model				
Trait	σ^2	**lnL**	**AICc**	Δ_i	ω_i	α	**lnL**	**AICc**	Δ_i	ω_i	a	**lnL**	**AICc**	Δ_i	ω_i
Sperm length	0.21	13.63	-22.33	1.35	0.31	58.96	15.84	-23.68	0.00	0.62	-1.04^{-6}	13.63	-19.26	4.43	0.07
Horn length	4.30	-10.62	26.15	1.88	0.27	53.66	-8.14	24.28	0.00	0.68	-1.02^{-6}	-10.62	29.23	4.95	0.06
Pronotum width	0.43	7.80	-10.68	0.00	0.55	21.12	8.84	-9.67	1.00	0.33	-1.03^{-6}	7.80	-7.60	3.08	0.12
Testes mass	2.10	-4.88	14.68	0.00	0.54	22.85	-3.80	15.60	0.92	0.34	-1.06^{-6}	-4.88	17.76	3.08	0.12
Body mass	3.01	-7.75	20.42	0.00	0.61	17.94	-7.05	22.09	1.67	0.26	-1.01^{-6}	-7.75	23.49	3.08	0.13

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 covariacne 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

	σ^2_{obs}			σ^2_{common}			$Log(L_{obs})$	$Log(L_{common})$	LRT	P	AIC_{obs}	AIC_{common}
	Sperm	Horn	Pronotum	Sperm	Horn	Pronotum						
Sperm	0.21	-	-	1.09	-	-	16.47	2.10	28.73	**<0.001**	-20.93	3.80
Horn	0.42	4.30	-	0.32	1.09	-						
Pronotum	0.16	0.78	0.43	0.19	0.29	1.09						
			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

	σ^2_{obs}		σ^2_{common}									
	Testes	Body	Testes	Body								
Testes	2.10	-	2.61	-	1.52	-2.95	8.93	**0.003**	4.97	11.90		
Body	2.42	3.01	2.05	2.61								

Figure 1. Estimates of the observed evolutionary rates (σ^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.