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EVOLUTION OF EJACULATES: PATTERNS OF PHENOTYPIC AND GENOTYPIC VARIATION AND CONDITION DEPENDENCE IN SPERM COMPETITION TRAITS

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Abstract.—Sperm competition is widely recognized as a potent force in evolution, influencing male behavior, morphology, and physiology. Recent game theory analyses have examined how sperm competition can influence the evolution of ejaculate expenditure by males and the morphology of sperm contained within ejaculates. Theoretical analyses rest on the assumption that there is sufficient genetic variance in traits important in sperm competition to allow evolving populations to move to the evolutionarily stable equilibrium. Moreover, patterns of genotypic variation can provide valuable insight into the nature of selection currently acting on traits. However, our knowledge of genetic variation underlying traits important in sperm competition is limited. Here we examine patterns of phenotypic and genotypic variation in four sperm competition traits in the dung beetle Onthophagus taurus. Testis weight, ejaculate volume, and copula duration were found to have high coefficients of additive genetic variation (CV_A), which is characteristic of fitness traits and traits subject to sexual selection. Heritabilities were high, and there was some evidence for Y-linked inheritance in testis weight. In contrast, sperm length had a low CV_A, which is characteristic of traits subject to stabilizing selection. Nevertheless, there was little residual variance so that the heritability of sperm length exceeded 1.0. Such a pattern is consistent with Y-linked inheritance in sperm length. Interestingly, we found that testis weight and sperm length were genetically correlated with heritable male condition. This finding holds important implications for potential indirect benefits associated with the evolution of polyandry.

Key words.—Copula duration, ejaculate volume, genetic variance, sperm competition, sperm length, testis size.

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Sperm competition, the competition between sperm of two or more males for the fertilization of a given set of ova (Parker 1970, 1998), is widely recognized as a pervasive force generating sexual selection on males (see reviews in Smith 1984; Birkhead and Møller 1992, 1998; Simmons 2001). Over the last decade, Parker and his colleagues have developed a series of game theory models to predict how selection via sperm competition might influence the evolution of ejaculates (Parker 1990a,b, 1998; Parker et al. 1997). Their analyses predict that across species, the evolutionarily stable ejaculate expenditure should increase both with the risk (the average probability that females in the population will mate with more than one male) and intensity (the average number of males competing for a given set of ova) of sperm competition (reviewed in Parker 1998). General support for this prediction comes from comparative studies that report positive associations between sperm competition and testes weight (Møller 1988; Gage 1994; Harcourt et al. 1995; Hosken 1997; Stockley et al. 1997; Byrne et al. 2002). Sperm competition games also predict that, in general, sperm should remain small and numerous, although under restrictive sets of conditions sperm competition could favor increased, decreased, or no change in sperm size (Parker 1982, 1993, 1998; Parker and Begon 1993). Comparative studies have reported positive (Gage 1994; Briskie et al. 1997; Gomendio and Roldan 1991; Balshine et al. 2001; Anderson and Dixon 2002), negative (Stockley et al. 1997) and no association (Hosken 1997) between sperm competition and sperm size.

Implicit in Parker’s sperm competition game models is the existence of genetic variation in ejaculate traits. Without genetic variation, selection will be unable to move evolving populations to evolutionarily stable equilibrium. With some notable exceptions, there are surprisingly few detailed quantitative genetic studies of traits thought to be important in sperm competition. Beatty (1970) reported high heritabilities for sperm head characteristics in mice and rabbits, Ward (2000) reported a high heritability for sperm length in yellow dung flies, Scatophaga stercoraria, and a genetic basis for sperm length and/or testis size has been revealed using hybrid crosses of tettigonoids (Reinhold 1994) and Drosophila (Joly et al. 1995). Microevolutionary manipulations have induced reductions in testis size and sperm production following the removal of selection from sperm competition by enforced monogamy (Hosken and Ward 2001; Pitnick et al. 2001), implying a genetic basis to these traits. Finally, artificial selection for increased or decreased sperm length was successful in the field cricket Gryllus bimaculatus (Morrow and Gage 2001b), and selection for increased and decreased testis length was successful in Drosophila hydei, with correlated responses seen in sperm length (Pitnick and Møller 2000).

The relative paucity of information on genetic variation in traits thought important in sperm competition is unfortunate, because patterns of phenotypic and genotypic variation can be revealing of the patterns of selection currently operating (Houle 1992). For example, in his review of more than 200 quantitative genetic studies, Houle (1992) found that traits closely related to fitness had higher coefficients of additive genetic variation (CV_A) than traits under weak selection. Likewise, Pomiankowski and Møller (1995) found that traits subject to directional sexual selection had far greater CV_A than nonssexual traits or traits subject to stabilizing selection. Estimates of CV_A are also useful because they provide some indication of the relative evolvability of traits (Houle 1992).

The phenotypic expression of traits subject to sexual selection can evolve to be condition dependent, thus providing...
honest signals for females of the underlying genetic quality of potential mates (Andersson 1994; Johnstone 1995; Rowe and Houle 1996; Wilkinson and Taper 1999). It has been suggested that ejaculate or copulatory traits that function in sperm competition could also evolve under selection via postcopulatory cryptic female choice (Eberhard 1996). Thus, females may select males as genetic sires of their offspring based on features of the ejaculate received, such as its chemical composition (Cordero 1995, 1996, 1998; Eberhard and Cordero 1995) or the length of sperm (Pitnick et al. 1999), which convey reliable information related to the male’s underlying genetic quality (Yasui 1997). Although empirical evidence suggests that ejaculates are often costly for males to produce (reviewed in Simmons 2001), indirect models of sexual selection require that variation in ejaculate features become genetically correlated with variation in general male condition for them to act as reliable signals for postcopulatory female choice (Andersson 1994; Rowe and Houle 1996; Simmons 2001). Whereas some studies have examined phenotypic variation in testis size, sperm production, sperm length, and ejaculate quality under developmental constraints such as dietary restriction or viral infection (Vawda and Mandwana 1990; Gage and Cook 1994; Wedell 1996; Sait et al. 1998; Birkhead et al. 1999), a genetic approach to examining condition dependence in ejaculate features has, to our knowledge, never been attempted.

We used the dung beetle Onthophagus taurus as a model system in which to explore the quantitative genetics of traits thought to affect sperm competition. Sperm competition is known to be an important selective agent in this system. Male onthophagines exhibit dimorphic morphology in which large, major males develop horn heads and small, minor males remain hornless (Hunt and Simmons 1998a). The dimorphism is associated with behavioral differences in mate acquisition; major males defend tunnels beneath the dung pad and may assist females in building brood masses (Hunt and Simmons 1998b, 2000). Minor males adopt an alternative tactic whereby they enter a tunnel that is guarded by a major male and sneak copulations with guarded females (Emlen 1997; Moczek and Emlen 2000). Thus, minor males are always subject to sperm competition, whereas major males are subject to a risk of sperm competition that depends on the frequency of sneaks in the population (Simmons et al. 1999).

Sperm competition in O. taurus conforms to a fair raffle (Tomkins and Simmons 2000). After two males have copulated, each male obtains, on average, 50% of fertilizations (Tomkins and Simmons 2000). Nevertheless, there is considerable variation in the proportion of fertilizations obtained by individual males. There is also phenotypic variation in copula duration, testis size, ejaculate volume, and sperm length (Simmons et al. 1999; Tomkins and Simmons 2000). Copula duration has implications for sperm competition, both through its effects on sperm transfer and fertilization success and through its role in physically preventing females from remating (for a review, see Simmons 2001). A positive association between testis size and the number and quality of sperm produced has been reported across a variety of taxa (Møller 1988, 1989; Hosken and Ward 2001). Ejaculates contain both sperm and seminal fluids, the volume of which can influence female remating behavior and thus serve to avoid sperm competition from future males (Parker and Simmons 1989; Eberhard and Cordero 1995; Simmons 2001). Sperm length can influence the flagella forces that facilitate sperm movement (Katz and Drobis 1990; Bressac et al. 1991; Gomendio and Roldan 1991) and sperm length may also be associated with sperm longevity (Stockley et al. 1997). Thus, copula duration, testis size, ejaculate volume, and sperm length, collectively referred to as sperm competition traits, could potentially contribute to variation in fertilization success, and be subject to directional selection. In this study, we use a half-sibling analysis to estimate patterns of phenotypic and genotypic variation to gain insight into the nature of selection currently acting on these sperm competition traits. If they are subject to strong directional selection under sperm competition, they should have relatively high CVAs (Houle 1992; Pomiankowski and Møller 1995).

We have found that female O. taurus exercise precopulatory mate choice, based on the rate of courtship delivered by males. Moreover, there is significant additive genetic variance in courtship rate that is genetically correlated with variation in male condition (Kotiaho et al. 2001). Thus, females have the potential to gain indirect benefits for their offspring through their choice of mates. Here we examine the patterns of genotypic covariance between sperm competition traits and male condition. If ejaculates evolve as honest signals of male quality (Cordero 1995, 1996, 1998; Eberhard and Cordero 1995), we would expect to find significant genetic correlations between ejaculate features and heritable male condition.

**Materials and Methods**

**Breeding Design**

We used a standard half-sibling breeding design (Falconer and Mackay 1996; Roff 1997). Beetles were collected from fresh cattle dung from a paddock in Margaret River, southwest Western Australia. Beetles were maintained in culture for one week and then females were established in individual breeding chambers: 30-cm long, 9-cm diameter sections of PVC piping, three-quarters filled with moist sand topped with 250 ml of fresh cow dung. Chambers were left at 25°C for one week before being sieved and brood masses collected. Brood masses were incubated for another three weeks and emerging adult beetles maintained in single sexed cultures. The unmated adult female beetles were used as the dams in our breeding design. They were provided constant access to fresh dung for two weeks prior to experiments.

We collected 12 adult male beetles from the field and provided each with four of the laboratory reared unmated females. Each sire was housed with his four dams for five days in a small plastic container (7 cm × 7 cm × 5 cm), half filled with moist sand and topped with fresh dung. Females were then established in individual breeding chambers that were sieved every seven days. Brood masses were removed and incubated. Emerging adult male offspring were housed individually in small plastic containers (7 cm × 7 cm × 5 cm), two-thirds filled with moist sand and topped with fresh dung. Offspring were thus maintained for 2–3 weeks prior to the assessment of sperm competition traits.
Mating Trials and Trait Measurement

We performed mating trials in artificial tunnels constructed from clear, rectangular plastic vials measuring 60 mm × 36 mm × 13 mm. Vials were half filled with plaster to create a 60-mm long, 13-mm wide, and 17-mm high tunnel. The plaster floors of tunnels were smeared with cow dung and dried. Before each trial, tunnels were lightly moistened with fresh water. One virgin female, randomly selected from stock culture, was placed into an artificial tunnel. Within five minutes, one experimental male was introduced and the pair observed until mating. A male courts by tapping the female’s back with his forelegs and head (Kotiaho et al. 2001). The female will open her genital tergite to allow intromission, upon which the pair becomes quiescent. We recorded the duration of copulation as the time from intromission until the male withdrew his aedeagus and dismantioned.

Immediately after copulation, females were dissected and the spermaphore was located within the bursa copulatrix. Ejaculate volume was estimated from linear measurements of the height (h) and diameter (d) of the spermaphore matrix. The spermaphore is ovoid in shape, and ejaculate volume was estimated as $4/3\pi(d/2)^2(h/2)$. This method of estimating ejaculate volume is highly repeatable (see Simmons et al. 1999). The spermaphore was then removed from the bursa copulatrix, placed onto a clean dry microscope slide, and ruptured in 20 μl of distilled water. The ejaculate was smeared across the slide and air-dried. We measured the length of 10 sperm using the measurement explorer function of the Optimas Image Analysis package (Media Cybernetics, Silver Spring, MD). Sperm were viewed at ×100 magnification under light field. Sperm were selected at random for measurement, subject to the criteria that they showed no signs of damage. The Optimas package provides a highly repeatable means of assessing sperm length (see Simmons et al. 1999). Moreover, preliminary investigations revealed that assessing 10 sperm per individual provided an accurate and repeatable estimate of between individual variation in sperm length; in this study, variation between subjects was significantly greater than within subjects ($F_{14,146} = 9.65$, $P < 0.001$; repeatability estimate 0.896). The average sperm length was calculated for each individual and used in quantitative genetic analyses.

Males were processed immediately after females. They were weighed to an accuracy of 0.01 mg and the width of their pronotum, measured with digital callipers to an accuracy of 0.01 mm, was taken as a measure of body size. Males were dissected and their testes removed and weighed.

Male Condition

Male condition was estimated as the weight of soma after controlling for body size. Rowe and Houle (1996) refer to condition as the pool of resources available for use, corresponding to the residual reproductive value or state in life-history models. A direct empirical measure of condition thus defined is not possible. Nevertheless, residual analysis has been used widely as a proxy for condition (Jakob et al. 1996; Kotiaho 1999) and is likely to be correlated with condition as defined in life-history models. Intuitively, residual weight should approximate well the stored resources that an individual has available for body maintenance and reproduction; individuals with positive residuals should have more reserves than would be expected for their body size, whereas individuals with negative residuals should have less reserves than would be expected. Indeed, a recent analysis using field crickets has confirmed this expectation (Gray and Eckhardt 2001). Nevertheless, recent critiques have pointed out that simply using residuals from a regression analysis can be problematic. Thus, for our genetic analyses we entered pronotum width as a covariate as recommended by García-Berthou (2001) and Darlington and Smulders (2001). Elsewhere (Kotiaho et al. 2001) we used total body weight in our analysis of condition. However, here we are interested in how condition is associated with testis weight. Thus, we subtract the weight of the testes to provide a measure of soma weight and condition that is independent of the testes. For the statistical analysis of condition, both soma weight and pronotum width were log transformed.

Genetic Analyses

To ensure our design remained balanced, for each sire we selected the three dams that produced the greatest number of sons. We obtained measures of phenotype from four to six sons for each of these dams. We calculated observational components of variance and estimates of narrow-sense heritability following Becker (1984), adopting his correction factors for the slight variation in numbers of offspring per dam and sire. Mixed-model nested analyses of variance with dams nested within sires as a random effect were used to test statistically for sire and dam effects and to estimate observational components of variance. Genetic correlations were calculated from multivariate nested analyses of covariance (Becker 1984). Coefficients of variation were calculated as $100\sqrt{V/V\bar{X}}$ (where $V$ refers to either phenotypic, additive genetic, or residual variance) using untransformed values as recommended by Houle (1992). In the case of condition, the mean value was taken as the average of the least square mean values of soma weight across sires, after controlling for the covariate pronotum width.

Results

Patterns of Phenotypic and Genotypic Variation

We found significant sire effects for all traits measured (Table 1). Mothers made no significant contribution to variation in testis size, ejaculate volume, or copula duration, although there was a significant dam effect on sperm length (Table 1). In contrast, there were no sire effects on soma weight or pronotum width. Dam effects on these measures of body size were highly significant. This is perhaps not surprising given that body size is strongly influenced by environmental conditions provided by the mother for larval development (Hunt and Simmons 1997, 2000) and that dam components include these common environment or maternal effects (Falconer and Mackay 1996). Testis weight and sperm length showed very high heritabilities (Table 2). In the case of sperm length the heritability estimate exceeded 1.0, suggesting a Y-linked contribution to sperm length. Examination of the CVs show that for both testis weight and sperm length...
TABLE 1. Sibling analyses: mixed model nested analyses of variance for morphological traits, sperm competition traits, and condition (estimated as the weight of soma after controlling for body size).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis weight</td>
<td>sire</td>
<td>8.33</td>
<td>11</td>
<td>0.76</td>
<td>6.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>dam(sire)</td>
<td>2.91</td>
<td>24</td>
<td>0.12</td>
<td>0.78</td>
<td>0.754</td>
</tr>
<tr>
<td></td>
<td>residual</td>
<td>20.77</td>
<td>134</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ejaculate volume</td>
<td>sire</td>
<td>12.83</td>
<td>11</td>
<td>1.17</td>
<td>2.60</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>dam(sire)</td>
<td>10.75</td>
<td>24</td>
<td>0.45</td>
<td>0.97</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>residual</td>
<td>61.62</td>
<td>134</td>
<td>0.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm length</td>
<td>sire</td>
<td>0.0379</td>
<td>11</td>
<td>0.0035</td>
<td>4.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>dam(sire)</td>
<td>0.0170</td>
<td>24</td>
<td>0.0007</td>
<td>1.87</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>residual</td>
<td>0.0461</td>
<td>122</td>
<td>0.0004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copula duration</td>
<td>sire</td>
<td>41664</td>
<td>11</td>
<td>3788</td>
<td>3.17</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>dam(sire)</td>
<td>28752</td>
<td>24</td>
<td>1198</td>
<td>1.29</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>residual</td>
<td>124712</td>
<td>134</td>
<td>931</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soma weight</td>
<td>sire</td>
<td>5165</td>
<td>11</td>
<td>469.58</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>dam(sire)</td>
<td>8348</td>
<td>24</td>
<td>347.82</td>
<td>3.59</td>
<td>0.254</td>
</tr>
<tr>
<td></td>
<td>residual</td>
<td>12973</td>
<td>134</td>
<td>96.82</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Pronotum width</td>
<td>sire</td>
<td>1.004</td>
<td>11</td>
<td>0.0913</td>
<td>0.54</td>
<td>0.858</td>
</tr>
<tr>
<td></td>
<td>dam(sire)</td>
<td>4.096</td>
<td>24</td>
<td>0.1707</td>
<td>3.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>residual</td>
<td>7.034</td>
<td>134</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condition</td>
<td>pronotum</td>
<td>0.269</td>
<td>1</td>
<td>0.2693</td>
<td>237.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>sire</td>
<td>0.072</td>
<td>11</td>
<td>0.0065</td>
<td>4.20</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>dam(sire)</td>
<td>0.0373</td>
<td>24</td>
<td>0.0016</td>
<td>1.37</td>
<td>0.133</td>
</tr>
<tr>
<td></td>
<td>residual</td>
<td>0.151</td>
<td>133</td>
<td>0.0011</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 To account for unequal sample sizes of offspring within sires, Satterthwaite’s approximation of the error term was calculated as 0.9919 × dam(sire) + 0.0081 × residual for testes size, ejaculate volume, and copula duration; 0.9991 × dam(sire) + 0.0099 × residual for sperm length; and 0.9993 × dam(sire) + 0.0037 × residual for condition.

almost all of the variation was additive genetic due to sires, with little or no (in the case of sperm length) residual variation. Assuming complete Y-linkage, the appropriate estimate of heritability would be the ratio of twice the variance due to sires to total phenotypic variance (Falconer and McKay 1996; Roff 1997). These values of heritability, equal to 0.49 ± 0.23 SE for testis weight and 0.57 ± 0.31 SE for sperm length, represent minimum estimates of heritability because Y-linkage may not be complete (see also Houde 1992).

Phenotypic and genotypic correlations between traits are shown in Table 3. Not surprisingly, there were phenotypic correlations between measures of body size and testis weight that were significant with Bonferroni adjustment for the number of phenotypic correlations performed. There were also negative phenotypic correlations between copula duration and testis weight and between soma weight and both copula duration and sperm length. However, only the correlation between soma weight and copula duration was significant at the Bonferroni adjusted critical value of $P \leq 0.0026$. Genetic correlations were moderate to low with typically high error. None were significant (Table 3).

Condition Dependence

There were significant sire effects on condition (soma weight after controlling for body size; Table 1), which yielded a high estimate of heritability (Table 2). There was no dam effect on condition. There were phenotypic correlations between condition and sperm length, testis weight and copula duration (Table 3). Genetic correlations due to sires were strong and significant between condition and testis weight, and between condition and sperm length. Greater male condition was genetically correlated with larger testes and the production of shorter sperm (Fig. 1). There were no significant genetic correlations between condition and ejaculate volume or condition and copula duration (Table 3).

TABLE 2. Observational coefficients of variation and narrow-sense heritabilities of morphological traits, sperm competition traits, and condition (estimated as the weight of soma after controlling for body size).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>SD</th>
<th>CV$_{p}$</th>
<th>CV$_{a}$</th>
<th>CV$_{s}$</th>
<th>$h^2$</th>
<th>SE $h^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testis weight (mg)</td>
<td>2.75</td>
<td>0.44</td>
<td>15.84</td>
<td>15.59</td>
<td>2.77</td>
<td>0.97</td>
<td>0.45</td>
</tr>
<tr>
<td>Ejaculate volume (mm$^3$)</td>
<td>3.97</td>
<td>0.17</td>
<td>35.56</td>
<td>22.67</td>
<td>24.40</td>
<td>0.41</td>
<td>0.26</td>
</tr>
<tr>
<td>Sperm length (mm)</td>
<td>0.99</td>
<td>0.03</td>
<td>2.64</td>
<td>2.83</td>
<td>0.00</td>
<td>1.14</td>
<td>0.61</td>
</tr>
<tr>
<td>Copula duration (sec)</td>
<td>160.41</td>
<td>33.92</td>
<td>21.54</td>
<td>16.47</td>
<td>13.89</td>
<td>0.58</td>
<td>0.36</td>
</tr>
<tr>
<td>Soma weight (mg)</td>
<td>82.38</td>
<td>12.62</td>
<td>16.34</td>
<td>2.10</td>
<td>16.21</td>
<td>0.02</td>
<td>0.32</td>
</tr>
<tr>
<td>Pronotum width (mm)</td>
<td>5.34</td>
<td>0.27</td>
<td>5.38</td>
<td>0.00</td>
<td>5.38</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Condition (mg)</td>
<td>82.37</td>
<td>4.01</td>
<td>8.95</td>
<td>8.14</td>
<td>3.70</td>
<td>0.84</td>
<td>0.46</td>
</tr>
</tbody>
</table>

1 Standard errors are approximations only, based on the calculations suggested by Becker (1984). Therefore, the sibling analyses presented in Table 1 provide more reliable testing of the significance of variances due to sires.
TABLE 3. Correlation matrix of morphological traits, sperm competition traits, and condition. Phenotypic correlations are given above the diagonal and genetic correlations (SE estimated according to Becker 1984) are given below the diagonal. All phenotypic correlations are Pearson’s r, except those involving condition that are partial correlations between soma weight and the variable of interest, controlling for pronotum width (*P < 0.05, **P < 0.01, ***P < 0.001, † significant at P ≤ 0.05 with Bonferroni adjustment for the 19 phenotypic correlations performed). Genetic correlations due to sires were not calculated for traits with no additive genetic variance due to sires.

<table>
<thead>
<tr>
<th></th>
<th>Sperm length</th>
<th>Ejaculate volume</th>
<th>Testis weight</th>
<th>Copula duration</th>
<th>Soma weight</th>
<th>Pronotum width</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm length</td>
<td>0.084</td>
<td>-0.042</td>
<td>0.062</td>
<td>-0.014</td>
<td>-0.175*</td>
<td>0.072</td>
<td>-0.200**</td>
</tr>
<tr>
<td>Ejaculate volume</td>
<td>(0.386)</td>
<td></td>
<td>0.143</td>
<td></td>
<td>0.036</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>Testis weight</td>
<td>0.121</td>
<td>0.090</td>
<td>(0.354)</td>
<td></td>
<td>-0.194**</td>
<td>0.465***†</td>
<td>0.236**†</td>
</tr>
<tr>
<td></td>
<td>(0.343)</td>
<td></td>
<td>(0.354)</td>
<td></td>
<td></td>
<td>0.492***†</td>
<td></td>
</tr>
<tr>
<td>Copula duration</td>
<td>-0.036</td>
<td>0.467</td>
<td>-0.348</td>
<td></td>
<td>-0.297***†</td>
<td>-0.138</td>
<td>-0.328***†</td>
</tr>
<tr>
<td></td>
<td>(0.405)</td>
<td>(0.419)</td>
<td>(0.371)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soma weight</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td>—</td>
<td>—</td>
<td>0.825***†</td>
</tr>
<tr>
<td>Pronotum width</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Condition</td>
<td>-0.897</td>
<td>-0.070</td>
<td>0.524</td>
<td></td>
<td>-0.0216</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>(0.255)**†</td>
<td>(0.368)</td>
<td>(0.251)*</td>
<td></td>
<td>(0.368)</td>
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</table>

DISCUSSION

Testis Weight and Sperm Length

Our estimate of heritability in testis weight was high compared with estimates from Drosophila (Pitnick and Miller 2000) and Gryllus (Simons and Roff 1994). However, our data suggest Y-linked inheritance of this trait in O. taurus; almost all of the phenotypic variation in testis weight was additive genetic due to sires, and there were no significant dam effects. Assuming Y-linked inheritance yielded a heritability estimate in the region of 0.50, in line with previous quantitative genetic studies of testis weight (Coulter et al. 1976; Islam et al. 1976; Simons and Roff 1994; Pitnick and Miller 2000).

Our estimate of the heritability in sperm length was also exceedingly high and consistent with Y-linked inheritance. Interestingly, estimates of heritability for sperm traits, including total length, are universally high (reviewed in Morrow and Gage 2001b), and it appears that complex sex-linked patterns of inheritance in sperm length are emerging as the norm. Hybrid crosses between Drosophila simulans and D. sechellia have implicated Y-linkage in the genetic control of sperm length, which may be widespread in the genus (Joly et al. 1995 and references therein). In contrast, Ward’s (2000) study of S. stercoraria implicated sex-linkage to the X chromosome because there was a significant relationship between a male’s sperm length and that of his maternal grandfather. Likewise, X-linkage seems to be the case in G. bimaculatus, where selection for increased sperm length was only successful when the maternal line was incorporated into selection (Morrow and Gage 2001b). In the case of G. bimaculatus, sex-linkage must be via the X chromosome because the species has an XO/XX system of sex determination (Morrow and Gage 2001b). Interestingly, Wang et al. (2001) have recently identified 25 genes that are expressed in the spermatogonia of mice. Amongst these, 10 genes are X-linked and three genes are Y-linked.

Studies of Drosophila have suggested that testis size and sperm length are largely, although not exclusively in the case of D. hydei (see below), under independent genetic control (Joly et al. 1995; Pitnick and Miller 2000). The lack of any genetic correlation between testis weight and sperm length in our study of O. taurus similarly indicates independent inheritance of these traits. Although Pitnick and Miller (2000) found a correlated response in sperm length to selection on testis length, changes in sperm length were minuscule compared with changes in testis length. Pitnick and Miller (2000) suggested that their inability to generate substantial changes in sperm length implied either a lack of genetic variation in this trait or that some uncontrolled selection pressure maintained the wild-type sperm length. Our data for O. taurus show that, despite the high heritability of sperm length, the magnitude of genetic variation in this trait is rather low compared with that of testis weight. Thus, the evolvability of sperm length is likely to be lower than that of testis weight (Houle 1992). Of course, the differences in dimensionality may account for some of the differences in CV_As for testis weight and sperm length (Lande 1977; Houle 1992). Dividing the CV_As by their dimensionalities (Houle 1992) still yields a CV_A in testis weight that is twice that for sperm length. As such, sexual selection due to sperm competition has the potential to move populations to an evolutionarily stable ejaculate expenditure via changes in testis size as predicted by Parker’s sperm competition game models (Parker 1998), and indeed the relatively high CV_A in testis weight is consistent with directional selection on this trait (Pomiankowski and Moller 1995). In contrast, selection under sperm competition is unlikely to have much impact on sperm length. Rather, the relatively low CV_A for this trait is consistent with stabilizing selection (Houle 1992; Pomiankowski and Moller 1995), as predicted from Parker’s game theory analysis of sperm size (Parker 1993). Consistent with these patterns of genetic variation, microevolutionary manipulations in which selection under sperm competition has been relaxed through enforced monogamy have resulted in decreased sperm production and testis size but have revealed no consistent changes in sperm length (Hosken and Ward 2001; Hosken et al. 2001; Pitnick et al. 2001). The accumulating data thus suggest that sperm length may not play a direct role in sperm competition, at least for internal fertilizers. Indeed, a recent study of G. bimaculatus found that sperm length had no impact on fertilization success, and thus was not subject to directional selection (Morrow and Gage 2001a). Although studies of mites (Radwan 1996) and nematodes (LaMunyon and Ward 1998)
have reported positive associations between sperm size and fertilization success, these taxa are unusual in having amoeboid-like sperm.

The physical characteristics of female sperm storage organs may impose stabilizing selection on sperm length, for example, if short or long sperm were unable to gain access to or remain within the storage organs. Stabilizing selection of this nature could explain the frequently observed covariation between sperm storage organ length and sperm length (Dybas and Dybas 1981; Briskie and Montgomerie 1992; Pitnick et al. 1999; Presgraves et al. 1999; Morrow and Gage 2000). Sperm storage tubule length appears to be associated with sperm competition risk across birds, and sperm length has tracked changes in sperm storage tubule length (Briskie et al. 1997). Pitnick et al. (1999) noted that across species of *Drosophila* changes in sperm length were rather slow, compared with changes in female reproductive tract dimensions, as might be predicted if, in general, sperm length exhibits low levels of additive genetic variance. Like others, we can only speculate on the selective pressures acting on female reproductive tract morphology, but a popular hypothesis is that females benefit from greater control over the fertilization process (Eberhard 1996; Hellriegel and Ward 1998; Pitnick et al. 1999).

**Ejaculate Volume**

Our data revealed significant heritable variation in ejaculate volume. The CV_\text{A} for ejaculate volume was greater than any other trait measured and was of a magnitude equivalent to fitness-related traits (Houle 1992) and traits subject to directional sexual selection (Pomiankowski and Möller 1995). Previous studies of ejaculate volume in the bean weevil, *Callosobruchus maculatus*, revealed significant variation in ejaculate volume attributable to dams but not to sires. Assuming no maternal effects and X-linked inheritance, Savalli and Fox (1998) estimated heritability of ejaculate volume to be in the region of 0.45, consistent with our estimate in *O. taurus*. It is important to note, however, that maternal effects cannot be ruled out in Savalli and Fox’s study. The only other study to examine the quantitative genetics of ejaculate volume is that of Sakaluk and Smith (1988). They found significant heritable variation in one component of the ejaculate, the weight of the spermatophyllax (a product of the accessory glands through which females feed during insemination) relative to body weight, but not the weight of the sperm containing ampulla of the spermatophore. Their estimate of heritability (0.49) was again consistent with that found in *O. taurus*.

Ejaculate volume is likely to be subject to directional selection because of its positive effects on female reproduction. Elsewhere (J. S. Kotiaho, L. W. Simmons, J. Hunt, and J. L. Tomkins, unpubl. ms.) we have found a significant effect of males on female life span and both the number and weight of broods females produce during their life span. Such paternal effects have also been reported in *C. maculatus*, where ejaculate weight contributes directly to female life span and fecundity (Fox 1993; Fox et al. 1995a,b) and are widespread in insect taxa (Simmons 2001). In the case of *C. maculatus*, females receiving large ejaculates do not remate as readily, so that males producing large ejaculates also benefit via the avoidance of sperm competition from future males (Savalli and Fox 1999). Like those of Savalli and Fox (1998), our results suggest that ejaculate volume in *O. taurus* has the potential to respond readily to selection.

In *Drosophila* and some other Diptera, seminal fluids have been shown to have negative impacts on female fitness due to accessory gland products involved in sperm displacement and incapacitation, inhibition of female receptivity, and stimulation of oviposition (Wolffner 1997; Simmons 2001). Genetic studies have revealed considerable variation in alleles coding for specific seminal peptides contained within the ejaculate (Clark et al. 1995). It seems that much of this var-
Copula Duration

We found that 58% of the variance in copula duration was additive genetic variance due to sires and that there was no significant variance due to dams. Genotypic variation in copula duration has been examined for a number of species. Although heritabilities were not assessed, Carroll and Corneli (1995) found that population differences in copula duration in soapberry bugs, Iadera haematoloma, persisted in common-garden experiments, implying genetic divergence of this trait, and Krebs (1991) found that variation in copula duration was inherited additively from both sexes and was autosomal in origin for D. mojavensis. Mühlhäuser et al. (1996) reported a heritability of 0.39 for copula duration of S. stercoraria, whereas Gromko and his colleagues have reported heritabilities for copula duration ranging from 0.23 to 0.46 for D. melanogaster (Gromko 1987; Gromko et al. 1991). Finally, Savalli and Fox (1998) estimated heritability to be between 0.25 and 0.35 among female C. maculatus but found no heritable variation in copula duration among males, suggesting female control over copula duration in this species.

In D. melanogaster, copula duration is positively correlated, both phenotypically and genotypically, with male fertility (number of offspring produced by once-mated females), illustrating its importance for the numbers of sperm transferred and thus its significance for sperm competition success (Gromko et al. 1984; Gilchrist and Partridge 2000). Like Savalli and Fox (1998), however, we found no correlation between copula duration and ejaculate volume and only a weak phenotypic correlation between copula duration and testis weight that was not robust to correction for a-error. Moreover, sperm competition experiments suggest that copula duration does not directly influence fertilization success in O. taurus (Tomkins and Simmons 2000). Thus, as with C. maculatus (Savalli and Fox 1998), the evolutionary significance of genotypic variation in copula duration is unclear. In J. haematoloma, copula duration serves as a means of postcopulatory mate guarding, with insemination occurring very early in the mating association (Carroll 1993; Carroll and Corneli 1995). However, copulation in O. taurus lasts for just 2–3 min compared with some 10 min to 11 days in J. haematoloma. Moreover, male O. taurus have a reduced copula duration in the presence of rival males (Moczek 1999) rather than the increased copula duration predicted by the mate guarding hypothesis (for a review, see Simmons 2001). Thus, copula duration is unlikely to serve a mate guarding function in O. taurus. Males have been found to copulate for longer with previously unmated females than with once-mated females (Tomkins and Simmons 2000), suggesting that observed copula duration may represent a manifestation of male mate choice. In this study we found that males in poor condition copulated for longer. Nevertheless, how male fitness is influenced by variation in copula duration is yet to be revealed. The CV_A in copula duration is certainly of an order of magnitude equivalent to traits known to be of importance to fitness (Houle 1992; Pomiankowski and Moller 1995).

Condition Dependence

Consistent with our previous analysis (Kotiaho et al. 2001), we found strong and significant sire effects on condition. The estimate of heritability was high at 0.86 with little residual variance and no significant variance due to dams. As with testis weight and sperm length, this pattern of genotypic variation is consistent with some Y-linkage of alleles that contribute to condition. Our estimate of CV_A is lower than the 27.05 reported in Kotiaho et al. (2001). There we used standardized residuals from a regression of log(body weight) on log(pronotum width). The present analysis differs in two respects. First, we use soma weight, rather than body weight, which excludes the weight of the testes. Most importantly, however, here we used raw values of soma and pronotum and the unstandardized residuals after controlling for the covariate, pronotum width, to calculate CV_A. The use of untransformed values allows a more accurate comparison between CVs when comparisons of patterns of selection are of interest (Houle 1992). Thus calculated, the CV_A for condition (8.14) was more in line with CV_A's reported for the life-history traits longevity (9.89) and fecundity (11.9) of D. melanogaster (Houle 1992).

Surprisingly, we found significant genotypic correlations between condition and testis weight and between condition and sperm length. Males in good condition had larger testes and produced shorter sperm. Manipulations of phenotypic condition in meal moths, Plodia interpunctella, have shown that sperm production is reduced by stress induced through nutrient limitation but that stress has no impact on sperm length (Gage and Cook 1994). However, ours is the first study to reveal a genetic basis to condition dependence in testis size and the first to indicate condition dependence in sperm morphology. Recently, Morrow and Gage (2001c) revealed taxonomically widespread intraspecific variation in sperm length for which there seemed little explanation. Our analysis suggests that, contrary to established dogma, the production of numerous short sperm may be costly, so that males may vary in their ability to produce numerous short sperm depending on their condition. Parker’s (1982) theoretical analysis predicts that male fitness should be maximized by the production of numerous small sperm, and our findings predict that males in good condition should have a higher fertilization success than males in poor condition.

The genotypic variation in ejaculate quality found here could hold important implications for the evolution of multiple mating by females. Yasui (1997) proposed a good-sperm model for the evolution of polyandry, suggesting that by mating multiply, females could ensure their eggs were fertilized by males with competitively superior ejaculates and, to the extent that ejaculate quality and offspring quality are correlated, obtain indirect fitness benefits for their offspring. The genetic correlations between male condition and testis weight and between male condition and sperm length provide the necessary link to facilitate the acquisition of indirect genetic benefits from the incitation of sperm competition. Interestingly, females show a premating preference for male
courtship rate that is itself genetically correlated with male condition (rA = 0.709 ± 0.132 SE; for genetic analysis see Kotiaho et al. 2001) and testis weight (rA = 0.859 ± 0.201 SE), although not sperm length (rA = 0.266 ± 0.367 SE). Thus, male condition in O. taurus may be subject to both precopulatory and postcopulatory sexual selection, with females benefiting from mate choice and polyandry via improved offspring condition. The next step will be to confirm whether males in good condition have a higher fertilization success when subject to sperm competition and whether polyandrous females produce offspring of higher condition.

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LITERATURE CITED


Houde, A. E. 1992. Sex-linked heritability of a sexually selected


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