Comparative evidence for a cost to males of manipulating females in bushcrickets

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Recent theoretical and empirical research on sexual conflict has tended to focus on the costs to females of being manipulated by males. The costs to males associated with the production of manipulative traits have received relatively little attention. In numerous insects, including bushcrickets (Orthoptera: Tettigoniidae), males are known to transfer substances in the ejaculate that inhibit the receptivity of females to further matings in a dose-dependent manner. The aim of this study was to test the prediction that, across bushcricket taxa, larger ejaculates and nuptial gifts will be associated with, on the one hand, longer sexual refractory periods in females and, on the other hand, longer sexual refractory periods in males. Data on the duration of the sexual refractory period in both males and females, together with ejaculate mass, spermatophylax mass, and male body mass, were obtained for 23 species of bushcricket. Both comparative analysis by independent contrasts and species regression revealed a positive relationship, across taxa, between the duration of the female’s sexual refractory period and both relative ejaculate mass and relative nuptial gift mass. Positive relationships were also found between the duration of the male’s sexual refractory period and both relative ejaculate mass and relative nuptial gift mass, indicating that there is a trade-off between resources spent on spermatophore size and the male’s potential mating rate. This appears to be the first comparative evidence that there is a cost to males associated with manipulating the remating behavior of their mates. **Key words:** mating costs, nuptial feeding, sexual conflict, sexual refractory period, sexually antagonistic coevolution. [Behav Ecol 18:499–506 (2007)]

**INTRODUCTION**

It is becoming increasingly clear that mating and fertilization often involve a conflict of interest between the sexes because male and female fitness optima may differ over, for example, female mating rate (reviewed by Arnqvist and Rowe 2005; Hosken and Snook 2005; Tregonza et al. 2006). The recent proliferation of published research on sexual conflict in relation to mating has tended to focus on the costs to females of being manipulated by males, yet it is recognized that coevolutionary arms races between the sexes could lead to substantial fitness costs for both sexes (Gavrilets et al. 2001; recent proliferation of published research on sexual conflict 2005; Hosken and Snook 2005; Tregenza et al. 2006). The example, female mating rate (reviewed by Arnqvist and Rowe 2005). Recent comparative evidence suggests that...
There are, however, costs incurred by males producing large ejaculates and nuptial gifts. Both models of ejaculate expenditure (e.g., Parker and Ball 2005) and models of nuptial gift allocation (e.g., Kondoh 2001) assume that there will be a trade-off in males between resources spent on current reproduction and lifetime mating rate. This trade-off has profound implications in terms of the evolution of male mate choice (reviewed by Clutton-Brock and Parker 1992; Bonduriansky 2001; Gwynne 2001; Simmons and Kvarnemo 2006) and strategic ejaculate allocation (reviewed by Wedell et al. 2002; see also Parker and Ball 2005). However, few empirical studies have tested the assumption that such a trade-off does occur. Energetic costs to males of producing large ejaculates and nuptial gifts are often inferred from the observation that males of insect species with relatively large ejaculates and nuptial gifts have long sexual refractory periods (e.g., Simmons 1990, 1993; Heller and von Helversen 1991; Hayashi 1993; Sakaluk et al. 2004), during which time the male appears to be in a state where he is no longer able to mate, to replenish the testes, to secrete the spermatophore and the nuptial gift (Davies and Dadour 1989; Simmons 1990, 1993, 1995; Heller and von Helversen 1991; Simmons et al. 1992, 1999; Hayashi 1993; Reinhold and von Helversen 1997; Lehmann GUC and Lehmann AW 2000b). As yet, however, there has been no formal comparative analysis of the effects of relative ejaculate size and nuptial gift size on the duration of the sexual refractory period in males, other than Wedell (1994), who found that bushcricket species with male refractory periods greater than 2 days in length tend also to have a relatively high protein content in the spermatophylax. The aims of the present study were 1) to examine a cost to males of producing large ejaculates in bushcrickets, that is, to test the prediction that bushcricket species with relatively larger nuptial gifts and ejaculates will have longer sexual refractory periods in males and 2) to examine, for the same set of bushcricket species, a benefit of producing large ejaculates and nuptial gifts, that is, to test the prediction that relatively larger ejaculates and nuptial gifts are associated with the induction of longer sexual refractory periods in females across species. The latter prediction has previously been tested in tettigonids by Wedell (1993b), who found positive relationships across genera between the duration of the females’ sexual refractory periods and the absolute mass of both the nuptial gift and ejaculate. It has been proposed, however, that the results of Wedell (1993b) may have been confounded by phylogeny because this factor was not controlled for (Gwynne 2001).

MATERIALS AND METHODS

A range of bushcricket species (Table 1) were collected from the field, as adults, from a variety of European locations (primarily France, Spain, and UK; for details, see Vahed and Gilbert 1996; Vahed 1998b, 2006). Sexes were separated and maintained under laboratory conditions as outlined by Hartley and Dean (1974). Data on the duration of the sexual refractory periods for males and females were obtained both from wild-caught individuals and from offspring subsequently reared in the laboratory (for further details of the treatment of the egg stage, see Hartley 1990). For each species, individual adults were uniquely marked using enamel paint. Matings were set up in black nylon mesh observation cages (measuring 10 × 10 × 10 cm), one pair per cage.

In order to determine the duration of the sexual refractory period for the males, immediately after mating, the male was left in the observation cage and was presented with a fresh, sexually receptive female (i.e., one that had been showing signs of phonotaxis to the male’s call or exhibiting a response song where present). The pairs were then observed for at least 5 h. If the male did not show renewed sexual activity, he was returned to the rear ing cage before being presented with a fresh female on each subsequent day (for a period of at least 5 h) until sexual activity by the male was resumed. These pairings were set up at a time of day corresponding to the peak of sexual activity for the given species. This varied between species. For example, some species were more active after sunset (e.g., Uromenus rugosicollis and Ruspolia nitidula), whereas others were more active during midmorning to mid-afternoon (e.g., Anonconotus alpinus and Steropleurus spp). Although the resumption of tegminal stridulation (calling) by the male could be used as an indicator that the male is ready to mate, observations suggested that this may not always be a reliable sign. In the tettigonine bushcrickets observed, males would often give brief bursts of stridulation within 5 min of mating, even though, in most cases, the males were not ready to mate again. Similarly, in Leptophyes latiscutula, males would engage in “aggressive stridulation” (see Hartley 1991) before they were ready to remate. Therefore, males were only recorded as having regained sexual activity when they adopted the precopulatory position in the presence of a female. In this behavior, the male turns his abdomen to face the female’s head, bending his abdomen downward to expose the secretory glands situated on his dorsal tergites which are used to entice the female to copulate (see Engelhardt 1915). To determine the refractory periods of the females, a similar method was employed. The newly mated female was presented with a fresh, sexually receptive male for at least 5 h after mating at a time of day corresponding to the peak of mating activity for the species. If mating did not occur in this period, the female was returned to the rear ing cage before being presented with a different sexually receptive male (and observed for at least 5 h) on each subsequent day until copulation occurred.

Novel data on the sexual refractory periods of males were collected for 12 species of tettigonid, and data for a further 11 were obtained from the literature (Table 1). Novel data on the sexual refractory periods of females were obtained for 9 species, whereas data for a further 14 were taken from the literature. Data on mean ampulla mass, nuptial gift mass, and male mass for all 23 species were taken from the literature (Table 1; NB: there appear to be 22 species in this table because data for Metrioptera roeselii and Metrioptera bicolor were combined. This was done because the data on the duration of the female refractory period taken from Wedell [1993b] were based on the mean of the mean values for both species). An assumption of this study is that differences in ampulla mass between species reflect differences in ejaculate mass or volume. Although this is likely to be the case, it is conceivable that some of the variation in ampulla mass across species is due to differences in the thickness of the ampulla wall, rather than ejaculate volume. Data were analyzed using both independent contrasts to control for phylogeny (for details, see Harvey and Pagel 1991; Purvis and Rambaut 1995) and species regression. Data were log10 transformed to meet the assumptions of parametric linear regression. The phylogeny used in the analysis by independent contrasts was taken primarily from Naskrecki (2000). The phylogenetic relationships of species within the genus Poecilimon were taken from Keller (1984, 2006), whereas the relationships between the different genera in the subfamily Tettigoniinae were taken from Rentz and Colless (1990) (this is because some of the genera from this subfamily in the present study were not included in Naskrecki’s [2000] phylogeny). Because information on branch lengths was not available, the contrasts were not scaled. To control for allometry, analyses were performed on
Table 1
Mean duration of the sexual refractory period in males and in females (under normal, as opposed to food limited, conditions), together with male body mass, ampulla mass, and spermatophylax (sp’lax) mass for the different tettigoniid species (sample sizes are in parentheses)

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Male refractory period (h)</th>
<th>Female refractory period (h)</th>
<th>Male mass (mg)</th>
<th>Ampulla mass (mg)</th>
<th>Sp’lax mass (mg)</th>
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<td>Phaneropterinae</td>
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<td>1. Phaneroptera nana</td>
<td>3.15 (3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.0 (5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>289.0 (7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.24 (7)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.16 (7)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2. Leptophyes punctatissima</td>
<td>1.17 (7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.09 (7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>175.0 (15)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.05 (10)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.97 (14)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>3. Leptophyes lativentra</td>
<td>54.0 (12)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>165.6 (15)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>478.0 (17)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.28 (17)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>103.65 (17)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>4. Poecilimon schmidtii</td>
<td>26.42 (7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.6 (11)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>525.0 (8)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.17 (6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.39 (6)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>5. Poecilimon volucianus</td>
<td>72.0 (64)&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>72.0 (249)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>630.0 (34)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>31.3 (13)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>118.6 (13)&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>6. Poecilimon affinis</td>
<td>48.0 (25)&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>48.0 (860)&lt;sup&gt;c,s&lt;/sup&gt;</td>
<td>1328.0 (4)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.89 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>170.27 (4)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>7. Poecilimon mariannae</td>
<td>48.0 (21)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>165.6 (10)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>584.4 (21)&lt;sup&gt;h&lt;/sup&gt;</td>
<td>24.0 (21)&lt;sup&gt;h&lt;/sup&gt;</td>
<td>109.0 (21)&lt;sup&gt;h&lt;/sup&gt;</td>
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<td>Tettigoniinae</td>
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<td>8. Tettigonia viridissima</td>
<td>104.0 (3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>172.8 (27)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>1805.0 (27)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>61.8 (27)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>188.5 (27)&lt;sup&gt;j&lt;/sup&gt;</td>
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<td>9. Dicticus verrucivorus</td>
<td>24.0 (20)&lt;sup&gt;k&lt;/sup&gt;</td>
<td>110.4 (263)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>1618.0 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.09 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>123.42 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>10. Platycles albopunctata</td>
<td>1.0 (3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.0 (3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>479.0 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.2 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.37 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>11. Anonconotus alpinus</td>
<td>0.2 (12)&lt;sup&gt;l&lt;/sup&gt;</td>
<td>1.07 (5)&lt;sup&gt;l&lt;/sup&gt;</td>
<td>604.0 (6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.94 (6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.71 (6)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>12. Metrioptera spp (bicolor + roeselii)</td>
<td>1.38 (2 + 2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.8 (27 + 31)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>391.5 (3 + 3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.48 (3 + 3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.0 (3 + 3)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Conocephalinae</td>
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<td>13. Conocephalus discolor</td>
<td>1.03 (9)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.0 (5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150.0 (2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.38 (2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.2 (2)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>14. Ruspolia nitidula</td>
<td>0.04 (7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.6 (10)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>556.0 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.21 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.59 (3)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Hetrodinae</td>
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<td>15. Acanthoplus discoidalis</td>
<td>72.0 (40)&lt;sup&gt;m&lt;/sup&gt;</td>
<td>171.84 (22)&lt;sup&gt;m&lt;/sup&gt;</td>
<td>4650.0 (14)&lt;sup&gt;m&lt;/sup&gt;</td>
<td>280.0 (14)&lt;sup&gt;m&lt;/sup&gt;</td>
<td>650.0 (14)&lt;sup&gt;m&lt;/sup&gt;</td>
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<td>Bradyporinae</td>
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<td>16. Ephippiger ephippiger</td>
<td>105.6 (5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115.2 (23)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>2313.0 (5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>148.97 (6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>468.76 (5)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>17. Stereopleurus stali</td>
<td>72.03 (13)&lt;sup&gt;m&lt;/sup&gt;</td>
<td>115.2 (20)&lt;sup&gt;o&lt;/sup&gt;</td>
<td>1290.0 (51)&lt;sup&gt;p&lt;/sup&gt;</td>
<td>55.35 (17)&lt;sup&gt;p&lt;/sup&gt;</td>
<td>322.0 (17)&lt;sup&gt;p&lt;/sup&gt;</td>
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<td>18. Stereopleurus asturienis</td>
<td>120.0 (6)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.0 (6)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1210.0 (10)&lt;sup&gt;n&lt;/sup&gt;</td>
<td>60.5 (10)&lt;sup&gt;n&lt;/sup&gt;</td>
<td>270.0 (10)&lt;sup&gt;n&lt;/sup&gt;</td>
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<td>19. Uromenus rugosicollis</td>
<td>24.0 (10)&lt;sup&gt;n&lt;/sup&gt;</td>
<td>72.0 (3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1143.0 (9)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.72 (9)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.0 (9)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Listroscelidinae</td>
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<td>20. Requena verticalis</td>
<td>62.4 (8)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>96.0 (24)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>440.0 (8)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>13.07 (39)&lt;sup&gt;s&lt;/sup&gt;</td>
<td>36.2 (39)&lt;sup&gt;s&lt;/sup&gt;</td>
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<td>Zaprochilinae</td>
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<td>21. Kawanaphila nartee</td>
<td>128.88 (16)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>456.0 (11)&lt;sup&gt;n&lt;/sup&gt;</td>
<td>55.0 (10)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>5.95 (1)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>5.76 (1)&lt;sup&gt;y&lt;/sup&gt;</td>
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<td>Phasmodinae</td>
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<td>22. Phasmodes ranatraiformis</td>
<td>24.0 (4)&lt;sup&gt;w&lt;/sup&gt;</td>
<td>43.2 (5)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>234.8 (5)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>9.3 (5)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>~1.0&lt;sup&gt;x&lt;/sup&gt;</td>
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<sup>a</sup> Novel data.
<sup>b</sup> Data from Vahed and Gilbert (1996).
<sup>c</sup> Data from Heller and von Helversen 1991.
<sup>d</sup> Time until >90% of males remated.
<sup>e</sup> Data from Reinhold and Helversen (1997).
<sup>f</sup> Data from Heller et al. (1998).
<sup>g</sup> Median remating interval (mean not given).
<sup>h</sup> Data from Lehmann GUC and Lehmann AW (2000a).
<sup>i</sup> Data from Lehmann GUC and Lehmann AW (2000b).
<sup>j</sup> Data from Wedell (1993b).
<sup>k</sup> Data from Wedell (1993a).
<sup>l</sup> Data from Vahed (2002).
<sup>m</sup> Data from Bateman and Ferguson (2004).
<sup>n</sup> Data from Vahed (1997).
<sup;o</sup> Data from Vahed (1998a).
<sup>p</sup> Data from Bateman (1998).
<sup>q</sup> Data from Gwynne (1990).
<sup>r</sup> Data from Gwynne (1986).
<sup>s</sup> Data from Simmons et al. (1993).
<sup>t</sup> Data from Simmons (1990).
<sup>u</sup> Data from Simmons and Gwynne (1991).
<sup>v</sup> Simmons LW (personal communication).
<sup>w</sup> Bailey and Lebel (1998).
<sup>x</sup> Estimated value (spermatophylax is vestigial, comparable in relative mass to that of Ruspolia according to Gwynne 2001).
residuals from the least-squares regressions of contrasts in log ampulla mass and contrasts in log spermatophylax mass on contrasts in log male body mass. Stepwise regression (backward deletion method) was used to determine the effects of residual contrasts in log ampulla mass and residual contrasts in log spermatophylax mass on 1) contrasts in the duration of the male refractory period and 2) contrasts in the duration of the female refractory period. The analysis using species means, as opposed to contrasts, as independent data points was performed in a similar manner to the above. For the independent contrasts, all regressions were forced through the origin, as recommended by Harvey and Pagel (1991) and Purvis and Rambaut (1995).

RESULTS

The mean duration of the sexual refractory period in males for each species varied from only 2.4 min in R. nitidula and 12 min in A. alpinus to approximately 5 days in Steropleurus asturiensis and Kawanaphila nartee (Table 1, Figure 1). The mean interval between successive copulations for females of each species varied from 1.07 h in A. alpinus and 3.09 h in L. punctatissima to approximately 7 days in Tettigonia viridissima and Acanthoplus discoidalis and 19 days in K. nartee (Table 1, Figure 1).

Comparative analysis by independent contrasts

Contrasts in log spermatophylax mass were positively related to contrasts in log male body mass (slope = 1.12 ± 0.30, $F_{1,21} = 13.77, P < 0.01$), as were contrasts in log ampulla mass (slope = 1.05 ± 0.18, $F_{1,21} = 33.76, P < 0.001$). A positive relationship was found between residual contrasts in spermatophylax mass and residual contrasts ampulla mass (slope = 1.21 ± 0.25, $F_{1,21} = 23.04, P < 0.001$). There was no significant relationship between contrasts in log duration of the male refractory period and contrasts in male body mass ($F_{1,21} = 1.02, P = 0.33$) or between contrasts in log duration of the female refractory period and contrasts in male body mass ($F_{1,21} = 0.23, P = 0.64$).

As predicted, positive relationships were found between contrasts in the duration of the female refractory period and both contrasts in residual ampulla mass (slope = 1.40 ± 0.30, $F_{1,21} = 21.92, P < 0.001$; Figure 2a) and contrasts in residual spermatophylax mass (slope = 0.64 ± 0.22, $F_{1,21} = 8.85, P < 0.01$; Figure 2b). Stepwise regression (backward deletion method) revealed that residual ampulla mass contrasts were the best predictor of contrasts in the duration of the female refractory period. Removal of residual spermatophylax mass contrasts caused no significant change in the fit of the model ($F$ change = 0.05, $P = 0.82$). Positive relationships were also found, as predicted, between contrasts in the duration of the male refractory period and both residual ampulla mass contrasts (slope = 2.11 ± 0.32, $F_{1,21} = 43.80, P < 0.001$; Figure 3a) and residual spermatophylax mass contrasts (slope = 1.12 ± 0.23, $F_{1,21} = 26.95, P < 0.001$; Figure 3b). Stepwise regression (backward deletion method) revealed that variation across taxa in both residual ampulla mass contrasts and residual spermatophylax mass contrasts explained a significant proportion of variation in male refractory period contrasts (contrasts in male refractory period = 1.5 (±0.44) residual ampulla mass contrasts + 0.50 (±0.27) residual spermatophylax mass contrasts, $F_{2,21} = 26.47, P < 0.001$).

Figure 1
The relationship between the duration of the sexual refractory period in males and the duration of the sexual refractory period in females across bushcricket species. Numbers correspond to the species listed in Table 1.

Figure 2
A benefit to males of producing large spermatophores: positive relationships between contrasts in the duration of the sexual refractory period in females and both (a) contrasts in residual ampulla (ejaculate) mass and (b) contrasts in residual nuptial gift mass across bushcricket taxa.
positive relationship was found between residual spermato-

philax mass and log male body mass (log male body mass = 1.13 + 1.37 residual spermatophylax mass; $\hat{r}^2 = 0.47, F_{1,21} = 17.48, P < 0.001$). Stepwise regression (backward deletion method) revealed that interspecific variation in the duration of the male refractory period was explained by both residual ampulla mass and residual spermatophylax mass (log male refractory period = 1.13 + 1.68 residual ampulla mass + 0.68 residual spermatophylax mass; $\hat{r}^2 = 0.67, F_{2,21} = 19.36, P < 0.001$). There was a significant correlation, across species, between the duration of the male refractory period and the duration of the female refractory period (Pearson’s $r = 0.60, n = 22, P < 0.01$; Figure 1).

**DISCUSSION**

Both species regression and comparative analysis by independent contrasts revealed, as predicted, a positive relationship between the duration of the male’s postmating sexual refractory period and both residual ampulla (ejaculate) mass and nuptial gift (spermatophylax) mass. At one extreme, the male’s sexual refractory period lasted only a matter of minutes in *R. nitidula* and *A. alpinus*, species in which the spermatophore (ampulla + spermatophylax) represents only 2%, or less, of male body mass. At the other extreme, the male’s sexual refractory period lasted for 5 days on average in *S. asturiensis* and *K. nartee*, species in which the spermatophore represents more than 20% of male body mass. This study therefore supports the oft-made assumption that there is a trade-off in males between resources spent on current reproduction and potential mating rate (e.g., Kondoh 2001; Parker and Ball 2005). The results also add to the growing body of evidence that males suffer costs as a result of the production of traits that may have been elaborated through a process of sexually antagonistic coevolution. Other examples of costly manipulative or coercive male traits include conspicuous prey-mimicking tail patterns in Goodeiniae fish (Macias-Garcia and Ramirez 2005; Stuart-Fox 2005), sand pillars in fiddler crabs (Backwell et al. 1995), and mate-grasping devices in water striders (Arness 1994; Westlake and Rowe 1999). Such costs associated with the production of manipulative traits or armaments are predicted to limit coevolutionary arms races between the sexes (Harrdill and Smith 2005).

To balance such costs, the production of relatively larger ejaculates was found to benefit the male by delaying the female from remating; as predicted, a positive relationship was found across bushcricket taxa between the duration of the female’s postmating sexual refractory period and the relative mass of both the ampulla and spermatophylax, as previously demonstrated by Wedell (1993b). The existence of such a relationship suggests that bushcricket females appear not to have evolved complete resistance to refractory-inducing substances transferred by males (see also Simmons and Gwynne 1991; Sakaluk et al. 2006). Multiple regression analysis revealed that relative ampulla mass (as opposed to relative spermatophylax mass) was the best predictor of the duration of the female’s sexual refractory period. Vahed (2006) similarly found that relative ampulla mass (as opposed to either relative...
spermatophylax mass or relative sperm number) was the best predictor of the lifetime degree of polyandry across bushcricket taxa. This suggests that it is primarily non-sperm substances in the ejaculate itself, rather than the nuptial gift, that affect the receptivity of females to further matings (but see Sakaluk et al. 2006). This conclusion is supported by laboratory studies of individual bushcricket species in which the duration of ampulla attachment has been manipulated independently of the amount of spermatophylax consumed (Gwynne 1986; Wedell and Arak 1989; Simmons and Gwynne 1991). The precise nature of the ejaculate substances involved in manipulating female remating behavior in bushcrickets and the physiological mechanisms by which they operate, however, have yet to be determined (see Vahed 2006).

The relative duration of the sexual refractory periods for males and females is expected to influence courtship roles (whether males or females are the more choosy or competitive sex when it comes to mating) via its effect on the operational sex ratio (reviewed in Gwynne 2001; see also Kokko and Monaghan 2001; Simmons and Kvarnemo 2006). Courtship role reversal has so far only been documented in bushcricket species with large spermatophores relative to male body mass (reviewed in Gwynne 2001). This may be because the production of relatively large ejaculates has a disproportionately higher cost compared with the production of small ejaculates. In the present study, a 10-fold increase in relative ampulla mass across species was associated with a 194-fold (antilog of 2.29) increase in the duration of the male’s sexual refractory period. Discussions of the role of male mating costs in causing courtship role reversal in bushcrickets have tended to focus on the costs to the male of producing large “nutritious” spermatophylaxes or on the relative “parental” contribution of nutrients to offspring (reviewed in Simmons and Kvarnemo 2006). The present study, however, suggests that the production of the large ejaculate may be relatively more costly for the male than the production of the spermatophylax (the slope of the regression of log male refractory period against log ampulla mass is greater than the slope of log male refractory period against log spermatophylax mass). It should be noted that in the bushcricket K. nartee, which has become a model species for the study of the causes of courtship role reversal (reviewed in Gwynne 2001; Simmons and Kvarnemo 2006), the male produces one of the largest ejaculates, relative to male body mass, of any bushcricket studied so far. The ampulla is similar in mass to the spermatophylax, representing about 10% of male body mass (Table 1). It is possible, therefore, that it is the costs of producing the ejaculate, rather than the nuptial gift, that underlie cases of courtship role reversal in bushcrickets.

Recently, there has been considerable debate over the extent to which females might benefit from mating with males with manipulative traits. Recent discussion has focused on the possible benefits to females in terms of producing sons that are better at manipulating females (Chapman et al. 2003a, 2005b; Cordero and Eberhard 2003, 2005; Eberhard and Cordero 2003; Stewart et al. 2005). The possible “good genes” benefits have received relatively less attention recently (but see Macias-Garcia and Ramirez 2005; Stuart-Fox 2005) but see also Engels and Sauer 2006). Therefore, although the elaboration of spermatophore size through selection on males to delay their matings from remating may have initially represented sexually antagonistic coevolution, in species with relatively large spermatophores, such a process could be seen as representing sexually mutualistic coevolution (see Macias-Garcia and Ramirez 2005; Stuart-Fox 2005). It remains to be determined, however, whether such indirect genetic benefits, together with direct nutritional benefits, associated with large spermatophores (reviewed in Vahed 1998b, 2007; Gwynne 2001) outweigh the costs to bushcricket females of being delayed, or prevented (see Vahed 2006), from engaging in polyandrous matings.

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REFERENCES


Hayashi F. 1993. Male mating costs in two insect species (Protothyme, Megaloptera) that produce large spermatophores. Anim Behav. 45:343–349.


