Cryptic female choice predicated on wing dimorphism in decorated crickets

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Male decorated crickets, Gryllodes sigillatus, normally lack hind wings and are incapable of flight (short-winged males), but occasionally exhibit fully developed hind wings that make rudimentary flight possible (long-winged males). Long-winged males bear a cost of flight in the form of decreased insemination success, which arises as a consequence of two interrelated factors: (1) long-winged males exhibit a lower reproductive investment relative to short-winged males, as measured by the mass of a male's spermatophore and reproductive organs and (2) the postcopulatory behavior of females favors males that maximize their reproductive investment. Of particular importance to male mating success is the spermatophylax, a large gelatinous mass forming part of the spermatophore and consumed by the female after mating. Consumption of the spermatophylax keeps the female preoccupied while sperm are discharged from the remaining portion of the spermatophore (sperm ampulla) into her reproductive tract. The spermatophylax of long-winged males is significantly smaller than that of short-winged males and consequently requires less time to consume. As a result, the sperm ampulla of long-winged males is frequently removed before its complete evacuation and significantly sooner than that of short-winged males. Because the spermatophore-removal behavior of females mediates the relative insemination success of short-winged and long-winged males, it can be considered a form of cryptic female choice. Key words: crickets, Gryllodes sigillatus, mate choice, nuptial food gift, sexual selection, spermatophore, wing dimorphism.

In many insect species, females retain control of sperm transfer and usage and can thereby influence the paternity of their offspring (e.g., Sakaluk, 1984; Thornhill, 1976; Ward, 1993). The intrinsically greater female control of the reproductive process means that females are capable of exerting mating preferences even after copulation has occurred (review in Eberhard, 1996). Males offer an ideal model with which to identify male traits that are subject to female postcopulatory preferences: the ejaculate of a male typically remains outside the female's genital opening after mating in the form of an externally attached spermatophore (Alexander and Otte, 1967), and females are thus well positioned to determine the fate of their mates' gametes through the judicious removal of spermatophores (Sakaluk, 1984; Simmons, 1986). In decorated crickets, Gryllodes sigillatus, the spermatophore includes a large gelatinous mass, the spermatophylax, which the female detaches and feeds on after mating (Sakaluk, 1984, 1985, 1987). While the female consumes this nuptial food gift, sperm are evacuated from the remaining portion of the spermatophore (sperm ampulla) into her reproductive tract. Smaller spermatophylaxes require less time to consume, and males providing small gifts are penalized in the form of premature ampulla removal and reduced sperm transfer (Sakaluk, 1984, 1985, 1987). Females can greatly influence the paternity of their offspring through their ampulla-removal behavior (Sakaluk, 1986; Sakaluk and Eggert, 1996), and hence this behavior constitutes a powerful mechanism of postcopulatory female choice.

Gryllodes sigillatus is a wing-dimorphic species dominated by the common short-winged form (Ghouri and McFarlane, 1958; Toms, 1993). Males normally lack hind wings and are incapable of flight (short-winged form), but occasionally exhibit fully developed hind wings (long-winged form) that make rudimentary flight possible (Sakaluk SK, personal observation); long-winged G. sigillatus occur both in nature and in laboratory colonies (Toms, 1993; Sakaluk, personal observation). Wing polymorphisms are common in insects and appear to reflect a fundamental trade-off between energy devoted to dispersal and energy devoted to reproduction (Dingle, 1985; Harrison, 1980). Numerous studies of wing-polymorphic species have shown that long-winged, flighted females typically have a lower fecundity and reproduce at a significantly later age than short-winged, flightless females (review in Roff and Fairbairn, 1993). Comparisons of different wing morphs with respect to reproductive parameters have rarely involved males, however, perhaps because males typically have a reduced gametic investment relative to females and are thus less likely to reveal costs of flight through a reduction in reproductive output. In support of this proposition, Roff and Fairbairn (1993) found no difference in the mass of testes of short-winged and long-winged male sand crickets, Gryllus firmus, nor were there any differences between males of the two morphs in their ability to sire offspring when pairs of males were permanently confined with single females. Similarly, Holtmeier and Zera (1995) found no difference in the ability of short-winged and long-winged male Gryllus rubens to sire offspring when males of the two morphs were paired in similar competitive interactions.

In most cricket species, the male's spermatophore does not include a spermatophylax, but consists solely of a small sperm ampulla (Alexander and Otte, 1967). When the ampulla is augmented with a spermatophylax, as is the case in G. sigillatus, a sixfold increase in the total mass of the spermatophore accrues (Sakaluk and Smith, 1988). As a consequence of their investment in a food gift (spermatophylax), minimum refractory periods of male G. sigillatus are about an order of magnitude longer than those of non-gift-giving cricket species (Sakaluk, 1985). Given that the synthesis of food gifts is costly, costs of flight are more likely to be manifest in reduced reproductive performance of long-winged male G. sigillatus than has proved to be the case for long-winged males of other cricket species (e.g., Holtmeier and Zera, 1993; Roff and Fairbairn, 1993). Specifically, we might expect long-winged male...
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G. sigillatus to be compromised in their ability to synthesize food gifts and hence to be at a disadvantage with respect to the postcopulatory preferences of females. I tested this prediction by comparing the mass of reproductive organs of short-winged and long-winged male G. sigillatus, the mass of their ejaculates, the size of their food gifts, and their insemination success in mating trials with virgin females.

METHODS
Reproductive investment
Male G. sigillatus were obtained from a stock colony that was initiated from approximately 60 adults collected at Tucson, Arizona, USA, in May 1987 and maintained according to standard procedures (see Sakahik, 1991). Measurements and observations on short-winged and long-winged individuals extended over 2.5 years and several generations (F2-F5) because long-winged individuals appeared in lab colonies only intermittently, and it was not always possible to measure long-winged males each time they became available. Whenever a long-winged male was removed for observation and measurement, a short-winged male was randomly selected from the same rearing cage to control for any effects of local rearing environment and to ensure that equal numbers of long-winged and short-winged males were measured each generation. Because breeding cages were stocked with nymphs of the same colony, short-winged and long-winged males taken from the same colony were of similar, albeit unknown, age.

I obtained a spermatophore from each experimental male by manually removing it from the male’s spermatophoric pouch (Sakahik and Smith, 1988); the two components of the spermatophore were easily separated and weighed to the nearest 0.01 mg using a Fisher XA analytical balance. I weighed males after their spermatophores were removed. One day after these initial measurements, experimental males were used in mating trials (see below). After mating trials, males were killed and their reproductive organs were dissected. For each male, I determined the mass of the testes and that of the accessory glands; spermatophore components and reproductive organs were dried to constant weight at 60°C in a drying oven for 24 h before being reweighed.

RESULTS
Reproductive investment
Univariate comparisons of reproductive investment by long-winged and short-winged males are shown in Table 1. The accessory glands and testes of short-winged males were of significantly higher mass than those of long-winged males. The spermatophores of short-winged males were also significantly larger than those of long-winged males, but this difference was due entirely to the spermatophylax portion of the spermatophore; spermatophylaxes of short-winged males were significantly larger than those of long-winged males, but there was no difference in ampulla mass between the two groups.

Pearson correlations between male body mass and the mass of spermatophore components and reproductive organs are shown in Table 2; correlational patterns were similar across wet and dry measurements, so for convenience, only correlations for wet measurements are given. Correlational patterns
were similar across the two wing morphs. Correlations between male body mass and mass of spermatophore components and reproductive organs were all positive, but not all of them were significant. In both wing morphs, spermatophylax mass was highly correlated with ampulla mass. The mass of the testes and the mass of the accessory glands were not correlated for either wing morph. Masses of reproductive organs also were not correlated with masses of spermatophore components.

I examined variation in the mass of reproductive organs and spermatophore components using principal components analysis, a multivariate technique in which a series of independent variables (principal components) is derived from the original measurements. The advantage of this kind of analysis is that it reduces the number of original variables to a smaller set of components that are uncorrelated, and hence it measures different dimensions of the data set (Manly, 1986). The first principal component emerging from an analysis of all wet and dry measurements (excluding male body mass) accounted for 56% of the total variance in the data set and had weak to moderate positive loadings on all of the original variables (Table 3). Essentially, the first principal component (PCI) appears to measure a male's overall reproductive investment. PCI score was positively correlated with male body mass in both short-winged ($r = .40, p = .024$) and long-winged males ($r = .74, p < .001$). Hence, I used an ANCOVA to compare PCI scores of short-winged and long-winged males, with male body mass entered as the covariate. The least-squares mean PCI score (± SE) of short-winged males ($0.62 ± 0.33$) was significantly higher than that of long-winged males ($-0.64 ± 0.34, p = .012$), suggesting that short-winged males make a greater reproductive investment than do long-winged males, even after accounting for variation due to body mass.

The second (PC2) and third (PC3) principal components accounted for an additional 17% and 14% of the variation in the data set, respectively; collectively, the first three components explained about 86% of the variation. PC2 had strong positive loadings on wet and dry testes mass, whereas the third principal component had strong positive loadings on wet and dry accessory gland mass. There was no significant correlation between PC2 and PC3 scores and male body mass for either wing morph. PC2 scores of short-winged males ($0.54 ± 0.19$) were significantly higher than those of long-winged males ($-0.55 ± 1.26, t = 3.68, p < .001$); however, there was no significant difference between the wing morphs with respect to PC3 scores ($t = 1.61, p = .11$).

### Table 2
Principal components (PC) analysis of wet (W) and dry (D) weights of spermatophore components and reproductive organs of male decorated crickets

<table>
<thead>
<tr>
<th>Component</th>
<th>Eigenvalue</th>
<th>Proportion of variation explained</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCI</td>
<td>5.584</td>
<td>0.558</td>
<td>0.558</td>
</tr>
<tr>
<td>PC2</td>
<td>1.664</td>
<td>0.166</td>
<td>0.725</td>
</tr>
<tr>
<td>PC3</td>
<td>1.563</td>
<td>0.156</td>
<td>0.881</td>
</tr>
<tr>
<td>PC4</td>
<td>0.541</td>
<td>0.054</td>
<td>0.915</td>
</tr>
</tbody>
</table>

### Table 3
Pearson correlations between body mass and mass of spermatophore components and reproductive organs (mg; wet measurements only) of male decorated crickets

<table>
<thead>
<tr>
<th>Component</th>
<th>Short-winged</th>
<th>Long-winged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body mass</td>
<td>Body mass</td>
</tr>
<tr>
<td>Spermatophylax</td>
<td>0.37*</td>
<td>0.42*</td>
</tr>
<tr>
<td>Ampulla</td>
<td>0.11</td>
<td>0.43*</td>
</tr>
<tr>
<td>Accessory glands</td>
<td>0.24</td>
<td>0.55**</td>
</tr>
<tr>
<td>Tests</td>
<td>0.12</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>0.34</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* p < .05; ** p < .01; *** p < .001.

Male mating success

Females that were mated to long-winged males took significantly less time to consume the spermatophylax (28.9 ± 2.6 min) than did females mated to short-winged males (39.1 ± 3.9 min; $t$ test (approximated for unequal variances), $t = 2.13, df = 43.2, p = .034$). Females that were mated to long-winged males removed the sperm ampulla significantly sooner after copulation (38.2 ± 2.8 min) than did females mated to short-winged males (51.0 ± 4.4 min; $t$ test (approximated for unequal variances), $t = 2.42, df = 42.5, p = .020$).

The time taken by the female to consume the spermatophylax was regressed against the mass of the spermatophylax that had been removed from her mate the previous day (the mass of a male's spermatophylax remains relatively constant across successive matings; see Sackhuk and Smith, 1988). The analysis revealed that as the size of the food gifts provided by males increases, so does the duration of courtship feeding (Figure 1A, $b = 4.08, r^2 = 0.21, df = 48, p < .001$). The time after mating at which the female removed and consumed the sperm ampulla also increased significantly with the mass of her mate's previous spermatophylax (Figure 1B, $b = 3.67, r^2 = 0.14, df = 48, p = .007$). An increase in male reproductive investment, as measured by PCI score, also resulted in longer durations of courtship feeding (Figure 2A, $b = 3.45, r^2 = 0.16, df = 48, p = .005$).

**DISCUSSION**

Long-winged male *G. sigillatus* bear a cost of flight in the form of decreased mating success, which arises as a consequence of two interrelated factors: long-winged males exhibit a lower reproductive investment relative to short-winged males, and the post-copulatory behavior of females favors sperm transfer by males who maximize their reproductive investment. Of particular importance to male insemination success is the spermatophylax, the large gelatinous mass forming part of the spermatophore and consumed by the female after...
(A) The time required for the complete consumption of the spermatophylax and (B) the time after mating at which the sperm ampulla was removed by the female after copulation plotted as a function of the mass of the previous spermatophylax produced by a male. Data pooled for short-winged and long-winged males. Both regressions are statistically significant ($p < .001$ and $p = .007$, respectively).

Mating. Spermatophylax feeding lasts anywhere from 5 min to 2 h, and typically within a few minutes of the complete consumption of the spermatophylax, the female removes and eats the sperm ampulla. The smaller spermatophylaxes of long-winged males require less time to consume, and males providing such gifts are penalized in the form of premature ampulla removal and reduced sperm transfer (Sakaluk, 1984, 1985, 1987). The amount of sperm transferred is vital to male fitness because it is the principal determinant of a male's fertilization success, particularly when his ejaculate must compete with those of other mating partners of the female (Sakaluk, 1986; Sakaluk and Eggert, 1996).
Thornhill (1983) described as "cryptic" those forms of female choice that occur only after copulation has been initiated and that lead to certain males enjoying an advantage over others in achieving fertilizations (review in Eberhard, 1996; Eberhard and Cordero, 1995). For example, female scorpionflies (*Harpobittacus nigriceps*) that mate with males that provide them with inadequate nuptial prey are more likely to remate sooner and delay oviposition until after remating relative to females that receive larger prey items at mating; such behavior almost certainly reduces the fertilization success of those males offering meager food gifts (Thornhill, 1983). Similarly, in *G. sigillatus*, a male that provides a female with a small spermatophylax at mating is more apt to have his sperm ampulla removed prematurely and, as a result, transfer fewer sperm (Sakahuk, 1984, 1985). The behavioral rule of thumb that leads females to terminate sperm transfer after consumption of the nuptial food gift is perhaps one of the better understood mechanisms of cryptic female choice (Eberhard, 1996). In *G. sigillatus*, such choice likely imposes significant sexual selection on at least two phenotypic characteristics of males, body size and wing condition, as both of these traits influence the size of the spermatophylax that a male is able to synthesize; large males and those of the short-winged morph produce larger food gifts on average and are therefore at an advantage with respect to female postcopulatory preferences.

Roff and Fairbairn (1993) noted that the incidence of macroptery in males relative to females is generally much lower in Orthoptera than in other insect taxa. They proposed that the particular reliance of male orthopterans on costly long-range acoustic signals to attract females could exacerbate the costs of being winged for males of this group, a suggestion borne out by Crnkrok and Roff's (1995) study of differences in the calling behavior of the two wing morphs of male sand
crickets, *Gryllus firmus*. Given that the costs of flight are more readily apparent in male *G. sigillatus* relative to other cricket species, at least as manifest by morph-specific differences in reproductive investment (cf. Holderegger and Zera, 1995; Roff and Fairbairn, 1993), we might expect a lower incidence of macroptery in males relative to females in keeping with the general orthopteran trend. In *G. sigillatus*, however, the incidence of macroptery is the same or only slightly higher in females (Mathad and McFarlane, 1968), suggesting that benefits to dispersal, where they exist, are similar across the sexes.

Although spermatophylaxes of short-winged male *G. sigillatus* were significantly larger than those of long-winged males, there was no difference in ampulla mass between the two groups. These results suggest that although males of the two morphs provide different-sized nuptial food gifts to females, they manufacture ejaculates of similar magnitude. Within both wing morphs, there was a significant positive correlation between the mass of the spermatophylax and the mass of the ampulla, a result which, superficially at least, appears to be inconsistent with the observed difference in spermatophylax mass and the absence of any such difference in ampulla mass between the two morphs. One possibility is that a biological difference in ampulla mass exists, but the statistical test lacked sufficient power to detect it. Even were this the case, however, it appears that any difference in ampulla mass between the two morphs is far too trivial (mean difference in ampulla mass = 0.03 mg).

The correlation between spermatophylax mass and ampulla mass observed in *G. sigillatus* has been established for the spermatophores of numerous bushcricket species (Vahed and Gilbert, 1996; Wedell, 1995). Such a correlation has been taken as evidence that males adjust the size of their food gifts in accordance with the size of their ejaculates; the rationale is that fewer sperm require less time to transfer, and hence a smaller food gift suffices to ensure the complete evacuation of a smaller ampulla. Whether the mass of the ampulla is related to the time required for its complete evacuation remains to be established for *G. sigillatus*, but recent work suggests that male crickets are capable of fine-grained adjustments in the number of sperm packaged in their ejaculates (Gage and Barnard, 1996). Notwithstanding this capability, long-winged male *G. sigillatus* do not appear to reduce the size of their ejaculates in a manner commensurate with the smaller food gift, a result which, superficially at least, appears to be inconsistent with the observed difference in spermatophylax mass and the absence of any such difference in ampulla mass between the two morphs. One possibility is that a biological difference in ampulla mass exists, but the statistical test lacked sufficient power to detect it. Even were this the case, however, it appears that any difference in ampulla mass between the two morphs is far too trivial (mean difference in ampulla mass = 0.03 mg).

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