

Preferred males are not always good providers: female choice and male investment in tree crickets

Luc F. Bussière, Hassaan Abdul Basit, and Darryl T. Gwynne

Biology Group, University of Toronto at Mississauga, Mississauga, Ontario, L5L 1C6, Canada

Female tree crickets (*Oecanthus nigricornis*) prefer large males but do not receive larger glandular courtship gifts from these males. This finding is puzzling from both the male and female perspectives, because females should prefer males providing more direct benefits, and because males who provide larger gifts achieve higher insemination success. We tested for differences in the quality of male secretions and found that larger males provided more proteinaceous food gifts than did rivals, which could explain why they are preferred by females. The preference in turn could cause depletion of food gift reserves in favored males, because natural remating rates are high and because even a single feeding bout negatively affects glandular stores. Most intriguingly, we showed that preferred males can adaptively decrease the size of courtship food-gifts provided (in order to conserve gifts for future mating events) when they perceive that the probability of multiple future mating opportunities is high. Thus, the elevated mating rates of preferred males (both before and after a focal mating event) could account for the small size of their courtship food-gifts. *Key words:* courtship-feeding, direct benefits, Gryllidae, mate choice, multiple mating, operational sex ratio. [*Behav Ecol*]

When males provide material (direct) benefits to their mates, females are expected to choose males providing the most or best materials (Trivers, 1972). In fact, recent theory suggests that direct benefits such as food gifts or parental care should be more important to choosy females than are indirect (genetic) benefits (Kirkpatrick, 1996; Kirkpatrick and Barton, 1997, but see also Houle and Kondrashov, 2002; Møller and Jennions, 2001). Nevertheless, in many species, the preferred males do not provide more or better direct benefits (Møller and Thornhill, 1998). This apparent paradox has been examined by using several approaches both for systems featuring parental care (Kokko, 1998; Møller and Thornhill, 1998) and for systems in which males feed their mates (Bussière, 2002), but these theoretical arguments remain largely untested. Here, we use a courtship-feeding insect as a model system to examine several hypotheses that might explain why high-quality preferred males do not provide more material benefits to their mates than do rivals.

The food gift provided by male black-horned tree crickets (*Oecanthus nigricornis*) comes from specialized dorsal (metanotal) glands (Fulton, 1915). A female is attracted to the calling song of a male, mounts him, and stimulates secretions from the dorsal glands by palpating hairs on the gland's surface during courtship. Shortly after the onset of courtship feeding, the male transfers a small spermatophore to the female. Unlike some other Orthoptera (Vahed, 1998), the spermatophores of tree crickets are not physically attached to specialized nutritious substances and consist merely of the ejaculate enclosed in an ampulla. After copulation, the female continues to feed from the metanotal gland while sperm pass from the spermatophore to her sperm storage organ, the

spermatheca. Shortly after she stops feeding on the gland, the female reaches back, removes the spermatophore, and eats it, thus preventing the transfer of any remaining sperm. Thus, the provision of large gifts directly improves a male's fitness by increasing his insemination success (Brown, 1997a). Despite this, some food gifts are insufficient to ensure the complete sperm transfer from the spermatophore to the spermatheca (Brown, 1997a).

The courtship meal is important to female fitness as it increases her lifespan (Brown, 1997a), and females discriminate against males whose gift-giving ability has been experimentally diminished (Bussière et al., 2004). However, the most well documented target of female choice in tree crickets, male calling behavior (Brown et al., 1996), is a poor predictor of the size of gift that is offered. This is because although calling frequency (pitch) does indicate body size, body size is itself poorly related to gift size (Brown, 1994; Bussière, 2003). Direct correlations between calling frequency and gift size are also poor (Bussière, 2003).

Because the behavior of both male and female tree crickets influence the final amount of glandular secretions transferred (Brown and Kuns, 2000), the apparent mismatch between male size and gift quantity needs explaining from the perspective of both sexes. There are at least four nonmutually exclusive hypotheses that account not only for the female preference but also for the male allocation of food gift reserves. We will discuss each one briefly before testing the predictions of three of these alternatives.

Hypothesis 1: preferred males provide high-quality gifts at the expense of large gift size

Females may prefer large males because they provide higher-quality gifts than do rivals, even though the quantity of gift is no larger (Brown, 1997a, 1999). If gift quality trades off with total gift reserves, this could also explain the mismatch between male size and gift size from the male perspective: large males may provide high-quality gifts at the expense of providing larger gifts. To test this hypothesis, we examine

Address correspondence to L.F. Bussière, who is now at the School of Biological, Earth, and Environmental Sciences, University of New South Wales, Sydney, NSW, 2052, Australia. E-mail: luc.bussiere@unsw.edu.au.

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differences in gift quality by testing for differences in the protein content and composition of the metanotal glands between males of different sizes.

Hypothesis 2: preferred males mate more often and become depleted of gift reserves

If preferred males mate more often as a consequence of female choice, they could become depleted of their gifts more quickly than are rivals and thus provide smaller gifts than expected for their size. Brown and Kuns (2000) demonstrated that mating frequency affects gift size, but two lines of evidence are lacking to demonstrate that remating accounts for small gifts in large males. First, the effect of remating on gift size (Brown and Kuns, 2000) could be the result of changes in male age or in male perception of mate availability as well as the depletion of gift reserves. We measured the levels of glandular protein in recently mated males to determine whether mating directly causes depletion in protein levels. Second, natural male remating frequencies may never attain the levels imposed in Brown and Kuns' study. We conservatively estimate remating rates in the field to determine whether large males mate often enough to cause the effect observed by Brown and Kuns (2000).

Hypothesis 3: preferred males conserve gift reserves for anticipated future matings

Attractive males may respond to female preferences by adjusting allocation to food gifts in order to optimize the return on their mating investments (Bussière, 2002). Male choice in the form of allocation adjustments is known for other gift-giving insects (Bonduriansky, 2001). There is as yet no evidence that males of any species actively diminish current courtship gift investment in anticipation of future mating opportunities, despite several studies of the effect of the sex ratio on the size of the subsequent gift a male transfers (Kvarnemo and Simmons, 1998; Shelly and Bailey, 1992). Preferred males may anticipate higher mating rates if they encounter more females (because more females approach their attractive calls) and adaptively tailor the size of their courtship gifts to conserve gift resources for future mating opportunities (Bussière, 2002). In addition, low-quality males encountering relatively few females may perceive a more male-biased sex ratio and adaptively increase their gift sizes in response to the greater perceived risk of sperm competition (see Gage and Barnard, 1996). This hypothesis is subtly different from the previous hypothesis in that this decrease in gift size is a result of changes in male perceptions about their biotic environment rather than physiological depletion of courtship gift reserves. To test this alternative, we manipulate experimental males' perceptions of the sex ratio and compare the size of gifts produced by these males.

Hypothesis 4: preferred males anticipate postcopulatory female choice that compensates for small gifts

Finally, preferred males may anticipate cryptic (postcopulatory) female choice (e.g., sperm choice, sensu Pitnick and Brown, 2000) in their favor that compensates for the decreased sperm transfer resulting from small courtship gifts. Large males may therefore donate smaller gifts in order to conserve resources for future matings (Bussière, 2002). Testing this hypothesis requires detailed knowledge of the mechanisms by which female tree crickets might influence fertilization success (Brown, 1999), as well as measures of the influence of male genetic quality on offspring fitness, and is the subject of ongoing work that is not discussed here.

METHODS

Before any of the hypotheses were tested, we first established that the lack of a strong positive correlation between courtship gift size and male size was not simply a problem of statistical power. If power were the only explanation, then a sufficiently powerful test should reveal a significant correlation (although it would not address why the correlation is weak). We improved the power of our test by combining data from several studies by using a meta-analytic technique (Arnqvist and Wooster, 1995; Gurevitch and Hedges, 1993). This approach is superior to simply combining data from several studies in a single correlation because it allows us to calculate the overall effect without assuming that the populations within each study are identical, and because it allows a formal test for heterogeneity among effects.

One of the reported correlations is from Brown (1994), whereas the other eight data sets were collected in the context of our other studies. The following information applies to our own samples. The animals were collected from an old field on campus at the University of Toronto at Mississauga (collection dates, rearing conditions, and mating status of the males are included in Table 1). All cricket husbandry took place in an environmental room maintained at 25°C, a 12-h light/12-h dark cycle, and 80% relative humidity. Crickets were individually housed in cylindrical fiberglass cages (10-cm diameter × 10-cm height) or rectangular plastic cages (8.8 × 8.8 × 16.1 cm., AMAC Corp., stock no. M103). All cages were lined with black fiberglass mesh for substrate, and all crickets received an ad libitum diet of peeled apple slices and cricket feed (Fluker Laboratories) three times a week. We sprayed distilled water into the cages twice daily.

We estimated gift size by observing mating interactions in a controlled laboratory setting. Before and after mating, we weighed all males by using a Mettler AE50 Balance (accurate to 0.1 mg) to estimate weight loss during mating. We staged all mating interactions by placing two cylindrical housing chambers lined with fiberglass mesh (each containing one of the members of the pair) end to end. We recorded the time at which the pair first made antennal contact, the time of the first mounting, the duration of all courtship-feeding bouts, the time of all copulation attempts, the time of spermatophore transfer, and the time of spermatophore removal and consumption by the female.

For most of the samples, we combined several morphometric indices of body size by using principal components analysis (PCA). To obtain these indices, we killed males (by freezing them) after they had mated and measured them by using the National Institutes of Health Image (version 1.62) software on a Macintosh computer connected to a dissecting microscope. We recorded pronotum length, mean hind femur length, mean forewing length, and mean hindwing length for each individual. We then performed a PCA based on the correlation matrix to extract unrotated components of body size for each study. The first principal component (PC1) for all analyses always loaded positively on all morphological measurements and explained more than 60% of the variance in the data. We therefore interpreted these PC1 scores as overall body size. For samples for which we did not measure all individuals, male postmating mass is used as an index of body size. Except where otherwise noted, we also used PCAs to reduce the two indices of gift size, mass lost during mating and duration of courtship feeding, to a single index of gift size. These two indices are subject to different kinds of error, and combining them produces the best overall index of food transfer. PC1 in these analyses always loaded positively on both indices of gift size and always explained more than 70% of the variance in the data. Figure 1 shows the strong correlation

Table 1**A summary of data used to compute the correlation between male body size and courtship-gift size in *O. nigricornis***

Source	Date specimens collected	N	Rearing conditions	Mating status	Index of body size	Index of gift size	Body size: gift size correlation coefficient
Brown, 1994	31 Aug–27 Sep, 1992 18 Aug–8 Oct, 1993	36	Field	Mix of virgin and unknown	Forewing width	Male weight loss	−0.134 (partial r of a multiple regression)
Collected specifically for meta-analysis	April 1997 (Collected as eggs)	17	Lab	Virgin	Male mass after mating	PCA of male weight loss and feeding duration	−0.194
Collected specifically for meta-analysis	April 1997 (Collected as eggs)	15	Lab	Once-mated	Male mass after mating	PCA of male weight loss and feeding duration	−0.222
Collected specifically for meta-analysis	16 Aug–28 Sep, 1999	22	Field	Unknown (assumed to be non-virgin)	PCA of pronotum length, wing lengths and hind femur lengths	PCA of male weight loss and feeding duration	0.197
Collected specifically for meta-analysis	F1 progeny of matings in Bussière, 2003, Ch. 6 (Parents collected as eggs in 04/99)	25	Lab	Virgin	Male mass after mating	PCA of male weight loss and feeding duration	−0.189
Hypothesis 1, Current study	Late August, 2000	22	Field	Unknown (assumed to be non-virgin)	PCA of pronotum length, wing lengths and hind femur lengths	PCA of male weight loss and feeding duration	−0.138
Hypothesis 3, 1998 Current study	5–14 Sep 1998	19	Field	Unknown (assumed to be non-virgin)	PCA of pronotum length, wing lengths and hind femur lengths	PCA of male weight loss and feeding duration	0.103
Hypothesis 3, 2002 Current study	23–29 Aug, 2002	32	Field	Unknown (assumed to be non-virgin)	Male mass after mating	PCA of male weight loss and feeding duration	0.079
Bussière et al., 2004 (Experiment 2)	28 Aug–8 Sep, 1998	32	Field	Unknown (assumed to be non-virgin)	PCA of pronotum length, wing lengths and hind femur lengths	Duration of courtship-feeding	0.167

between feeding duration and male weight loss during mating that was typical for all studies.

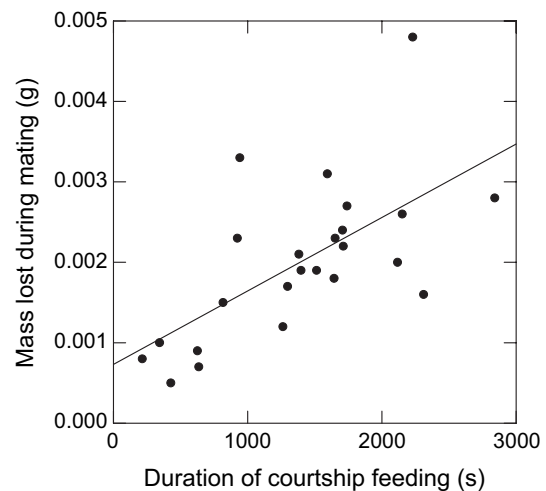
To analyze the overall correlation across studies, we used meta-analysis software (Schwarzer, 1991) to calculate the overall effect size based on the Schmidt-Hunter method (Hunter et al., 1982) and to test for homogeneity of the effect between experiments.

Hypothesis 1: preferred males provide high-quality gifts at the expense of large gift size

Because the courtship gift in tree crickets is consumed gradually as it is secreted, we were unable to analyze the composition of individual courtship gifts. However, we reasoned that an analysis of the gland itself could provide a reasonable index of the food gifts provided by males. We collected male crickets in late August 1999 and again in September 2002 and dissected them by making a dorsal incision from the abdominal terminus to the pronotum. The subcuticular metanotal gland was clearly visible as four distinct tubules that all opened into the metanotal depression on the dorsal surface just posterior to the forewings (Figure 2). All four tubules were cut from their ventral to the dorsal end, and excessive tissue was cleaned off the gland by using saline solution (NaCl 150 mM, KCl 10 mM, CaCl₂ 4 mM, MgCl₂ 2 mM, NaHCO₃ 4 mM, HEPES 5 mM at pH 7.2, sucrose 90 mM, trehalose 5 mM).

Our preliminary ($n = 5$) analyses of lipid content (see Mayer and Candy, 1969) revealed that lipids accounted for a small

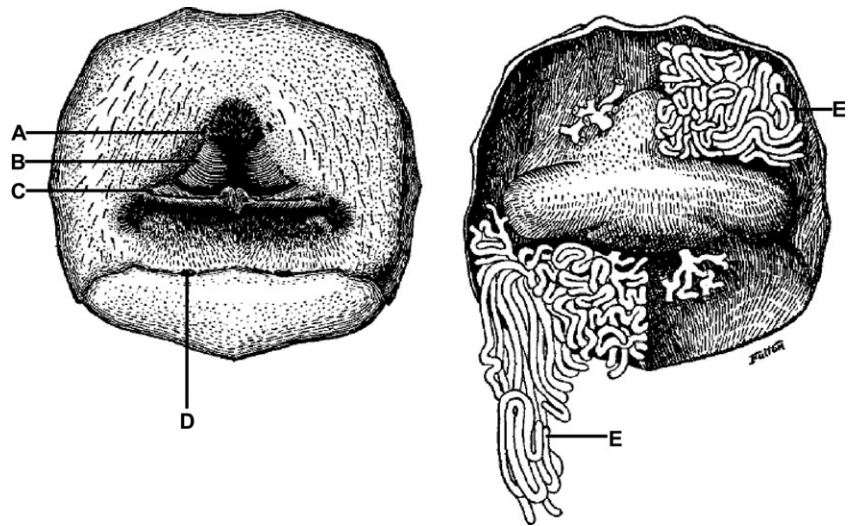
fraction of the metanotal gland products (i.e., 0.42% of fresh gland mass for lipids versus more than 6.0% for proteins). Because previous analyses of courtship gifts in other Orthoptera have also shown that proteins are the largest components

**Figure 1**

A scatterplot showing the correlation between the duration of courtship-feeding and male mass lost during mating. The smoothing line is included only to emphasize the relationship between the two variables.

Figure 2

Dorsal (left) and ventral (right) views of the metanotal tergite of a tree cricket. (A) Opening of anterior gland. (B) Anterior tuft of gland hairs (C) Posterior tuft of gland hairs. (D) Opening of posterior gland. (E) Glandular tubule. The glandular tubules contain the fluid extruded by males and consumed by females during courtship feeding. In *O. nigricornis*, each metanotal tubule is thicker than in the species illustrated. Reproduced from Fulton, 1915: Figure. 8.



of the gifts (Heller et al., 1998; Wedell, 1994), we focused our subsequent analyses on proteins. For a subset of samples in 1999 ($n = 11$), we analyzed each of the four tubules separately to determine if each subunit was responsible for a different protein fraction. This subsample revealed no evidence that different subunits secreted different protein fractions, so for the remaining samples, the four subunits were processed together to avoid the accidental loss of glandular material that sometimes occurred during the separation of tubules. We placed the samples in 50 μL saline, added 12.5 μL of 2 \times SDS buffer, and kept them on ice. We then sonicated (using a Branson Sonifier 250) the sample at a 20% duty cycle until the gland was completely dissolved, and heated the solution for 4 min in a water bath at 95°C to denature the proteins and allow the SDS to bind. The solution was centrifuged (Hermle Z233 MK centrifuge) for 20 min at 4°C and 1100 rpm, and the supernatant was extracted for the protein assay.

We estimated the total quantity of protein in the gland by using the BioRad color photometric method (Bradford, 1976). We added 5000 μL water and 1200 μL protein dye (BioRad Inc.) to each sample. We then vortexed the samples for 30 s and allowed 5 min for the dye to bind to the protein. We determined the concentration of protein by using a spectrophotometer (Bausch and Lomb Spectronic 88) at 595 nm and a bovine serum albumin standard curve. We analyzed two samples for each gland and used the average absorption values for our analysis.

To determine whether males of different size provided different concentrations of specific proteins, we separated the proteins based on size for 15 males by using polyacrylamide gel electrophoresis. We electrophoresed 50 μg protein (based on the colorimetric quantification, above) from each of the samples (along with a prestained protein standard, Invitrogen Canada Inc.) in a 10% acrylamide gel (overlain with a 4% stacking gel) at 180 V for 45 min. We stained the gels in 0.1% Coomassie blue for 1 h and destained them in a 40% methanol and 10% acetic acid solution overnight. Finally, we scanned the gels (Figure 3a) and analyzed the digital images by using ImageQuant (Molecular Dynamics Inc.) to measure the intensities of different protein bands. This software generates objective measures of each band density by computing an area under each band peak. A priori, we had no reason to expect differences in the concentrations of specific protein bands (because we have not characterized any of the proteins, we could not predict which of them might be more costly to produce or more valuable to females). Rather

than examining all possible pairwise comparisons of band density for the 17 bands (which would have required 153 tests), we chose four bands that represented a large fraction of protein (and thus might reasonably be expected to be more important to choosy females) and could be relatively easily distinguished from the background across all individuals (Figure 3a). For each band, we correlated its relative importance (the proportion of the total density for the lane that was accounted for by that individual peak) (Figure 3b) with male body size PC1. We predicted that if there were qualitative differences between large and small males in the fractions of different proteins produced in glands, then we would find correlations between male body size and the proportion of total density accounted for by individual bands.

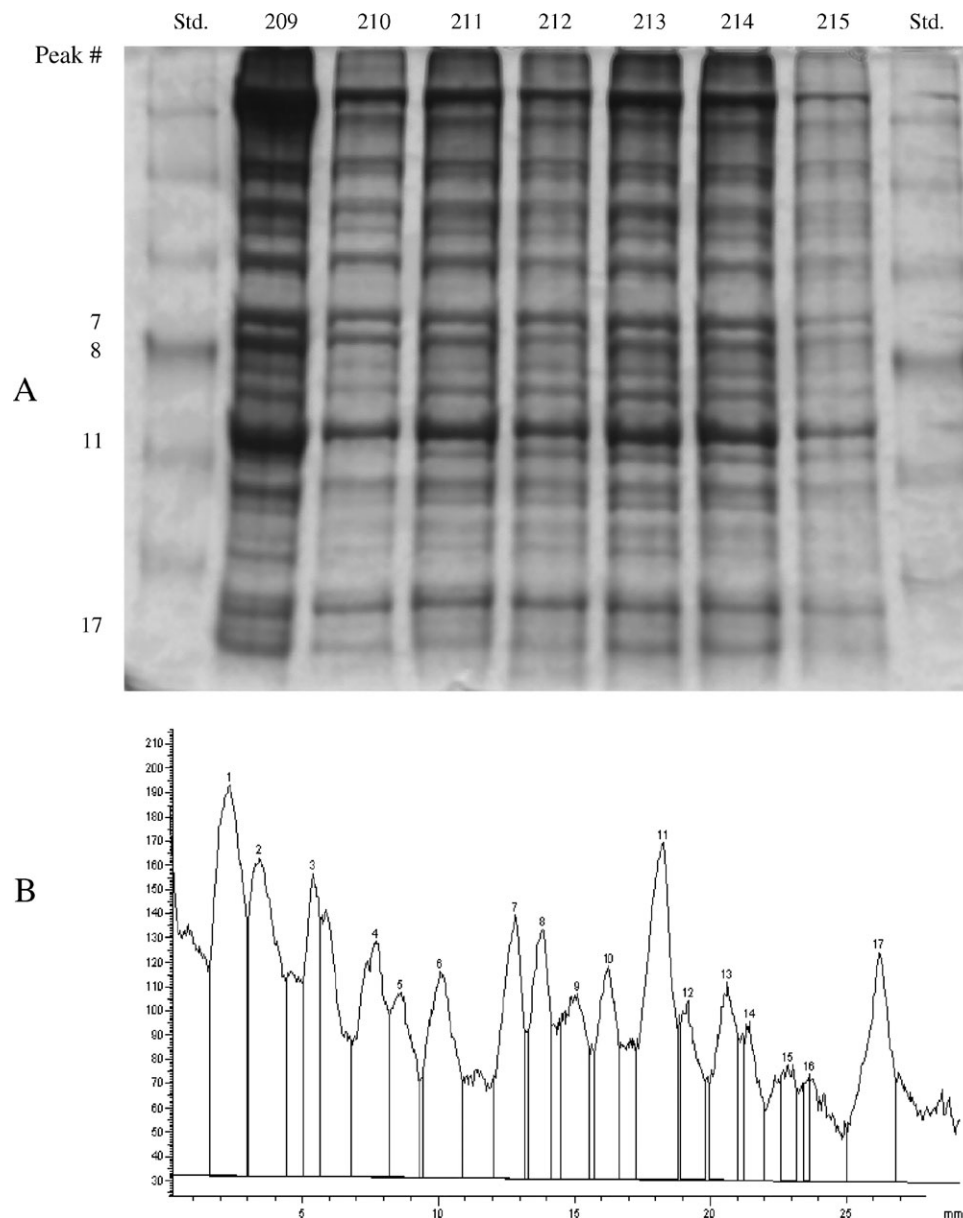
Hypothesis 2: preferred males mate more often and become depleted of gift reserves

How does mating affect protein reserves in metanotal glands?

To test whether mating depletes protein reserves in male metanotal glands, we analyzed the protein content (as described for hypothesis 1, above) of the metanotal glands of males that successfully mated in our test of hypothesis 3, below. After ascertaining that the treatments did not differ in the relationship between PC1 for gift size and postmating glandular protein (interaction term in ANCOVA, $F = 1.38$, $p = .25$), we correlated PC1 for gift size in the previous mating (see Methods) with the glandular protein remaining after mating. Our prediction was that males providing the largest gifts should have the least total protein remaining in their metanotal glands.

How often do male tree crickets mate in nature?

To estimate natural remating rates, we monitored a number of marked individuals that had been translocated to a patch of typical tree cricket habitat. To determine the appropriate density to use in our translocation experiment, we used the triple catch method (Begon, 1979) to estimate the density of tree crickets in a small section of the old field on campus at the University of Toronto at Mississauga, which annually supports a large population of tree crickets. We sampled a rectangular area measuring 244 m^2 from 27–29 August 1998, coinciding with the beginning of adult eclosions in 1998. We marked captured individuals by clipping the right mesotarsus on day 1 and the left mesotarsus on day 2. Because none of the individuals sampled on day 1 had either mesotarsus

**Figure 3**

(A) An electrophoretic gel showing the separation of metanotal gland proteins by size. Male identification is indicated above each lane, and lanes 1 and 9 contain protein standard. Band numbers refer to peaks indicated in part B of the figure. (B) A trace of band density for male no. 204, with distance of band migration (in millimeters) on the x-axis and pixel count (density) on the y-axis. The density of each band is measured by calculating the area underlying its peak in the density plot. For our analysis, we measured the areas under bands 7, 8, 11, and 17.

missing, and none of the individuals sampled on day 2 had the left mesotarsus missing, we are confident that natural injuries did not bias our estimate. Furthermore, individuals with clipped mesotarsi survived well in the laboratory and did not appear to suffer higher mortality than that of unclipped crickets (no mortality after 7 days, $n = 10$).

To conservatively estimate remaining in the field, we translocated 50 individually marked crickets to a previously uninhabited 25-m² plot in D.T.G.'s backyard garden near Glen Williams, Halton County, Ontario, Canada. This density corresponded to the lower end of our confidence interval for natural density and thus was unlikely to result in artificially high encounter rates between crickets. The garden contained native vegetation dominated by *Solidago canadensis* and *Rubus* spp. that had replaced the original vegetation, a lawn, 5 years

previously. The area was previously uninhabited by tree crickets and sufficiently far from any other populations of tree crickets to prevent significant migration to and from the study site (no unmarked individuals were found for the duration of the study). The crickets had all been marked with small spots of fluorescent paint on the pronota and hind femora so that they could be more easily spotted at night by using a portable ultraviolet lamp (BioQuip Products, Inc.). Despite these markings, only a fraction of crickets (mean \pm SE = 23.9 \pm 1.7%) could be found during every sampling session.

We sampled the population at 1-h intervals for five 12–13-h blocks between 6 and 13 September 1998. The sampling periods coincided with the most active period of calling activity for this species (1100–0100 h, Bussière LF, personal observation). Using a 1-h sampling interval ensured that single mating

events (the courtship-feeding portions of which last up to 46 min in the lab, Brown, 1994) were not sampled twice. We may have missed some copulations as a result, but because our aim was to conservatively estimate remating rates, this was preferable to sampling individual matings twice. We identified mating events by counting the number of pairs engaged in courtship-feeding. In the laboratory, sometimes a courtship-feeding pair will fail to mate, but because our estimate was conservative in every other respect, it is probably not greatly inflated by these mating failures (which tend in any case to be brief). Unfortunately, our efforts to mark crickets uniquely (in order to estimate variance in remating rates within the population) were unsuccessful: some crickets lost some of the spots of paint applied to them. Rather than risk misidentifying individuals, we assessed a population mean mating rate, as calculated by dividing the total number of matings by the number of cricket hours sampled throughout the experiment.

Hypothesis 3: preferred males conserve gift reserves for anticipated future matings

We conducted 24 trials for this experiment in September 1998 and another 52 trials in August 2002. All crickets were collected from the old field at the University of Toronto at Mississauga. In 1998, we collected experimental adult males at most 2 days before the start of the trials, whereas in 2002 all males began trials the day of collection. To increase the likelihood of sexual receptivity, females used for mating at the end of trials were isolated in the laboratory for a minimum of 3 days before mating. All conspecific encounters occurred in a clear plastic cylindrical arena lined with fiberglass mesh, as described above.

Experimental males were randomly assigned to either the male-bias or female-bias treatment. In the male-bias treatment, the subjects were paired with a series of 12 different conspecific males over 3 days. In the female-bias treatment, experimental males interacted with a series of 12 different females. We distinguished the "encountered" animals from the focal males by uniquely marking the pronota of encountered males and females with fluorescent paint.

Several previous studies of male encounter rates or sex ratios (see Kvarnemo and Simmons, 1999; Shelly and Bailey, 1992) have controlled for differences in mating history across treatments by using unreceptive females, but this was not practical with *O. nigricornis* because females do not have a consistent refractory period after mating (Bussière LF, personal observation). Moreover, using nonreceptive individuals to manipulate the encounter rate is inappropriate if males can detect sexual receptivity (because nonreceptive individuals would not be perceived as potential mates). Therefore, to prevent mating history from confounding the treatment of interest, we disrupted encounters with a grass leaf as soon as the female mounted the male (windblown leaves and stems sometimes interrupt mating events in nature; Bussière LF, personal observation). To control for this disturbance, subjects in simultaneously conducted male-biased trials were also disrupted by using grass blades in the encounter stage of the experiment.

The total time spent engaged in encounters did not differ between the treatments, because we simultaneously staged encounters for the male-biased treatment with encounters for the female biased treatment. Thus, when a female mounted a male, we terminated both the male-female encounter in question and its paired male-male encounter in the other treatment. In some cases, the control female did not mount the focal male; these interactions (and the paired male-male interactions) were interrupted after 8 min. Because sometimes one of two experimental males whose encounters

occurred simultaneously (for the purpose of controlling encounter time across treatments) failed to mate, we used an unpaired statistical analysis rather than discarding the data collected from the other member of the pair.

After encountering conspecifics over 3 days, each experimental male was paired with an unmarked female and allowed to interact and mate without interruption. If no mating occurred within 30 min, the crickets were separated and excluded from the analysis. An error in calibrating the balance we used caused some of the postmating masses of the crickets in 2002 to be measured incorrectly. To maximize statistical power for the main analysis of the effect of treatment on gift size, we used feeding duration (known to correlate highly with mass lost during mating; Brown, 1997b; Brown and Kuns, 2000) (Figure 1) as an index of gift size in this study rather than a PCA of feeding duration and mass lost during mating.

Statistical analyses

We used Lillifors tests to confirm the normality of distributions analyzed using parametric statistics (Lillifors two-tailed $p > .2$ for all relevant distributions). When sampling was conducted over more than one season, we always tested for a year effect (all $p > .3$) before grouping the data from multiple years to increase the power for subsequent analyses. Except where otherwise noted, all statistical analyses were computed by using Systat software (Wilkinson, 1999).

RESULTS

The correlation between body size and gift size in tree crickets

There was no evidence of heterogeneity between the effects reported by the nine data sets of the correlation between gift size and body size ($\chi^2 = 5.37$, $df = 8$, $p = .72$). When combined, the data described in Table 1 revealed an overall weighted mean r value of -0.023 ($p = .37$), indicating that there is no evidence from the combined data for a positive correlation between body size and gift size. Because the total sample for this analysis is 220, the weakness of this correlation is very unlikely to be a result of low power.

Hypothesis 1: preferred males provide high-quality gifts at the expense of large gift size

To determine if body size influenced gland quality, we computed the residuals of a model 1 regression of glandular protein mass on total gland mass (the regression was highly significant; $F_{1,36} = 33.1$, $p < .001$, $r^2 = .479$), such that positive residuals would indicate that the subject had more protein than expected based on glandular mass alone. We found a significant positive correlation between residual protein mass and PC1 body size ($r = .344$, $n = 38$, $p = .037$) (Figure 4), supporting the hypothesis that larger males have more protein per unit of gland material than do smaller males. However, we found no evidence that the high quality gifts provided by larger males compromise total gift reserves. Total gland mass ($r = .449$, $n = 37$, $p < .001$) and protein mass within the gland ($r = .558$, $n = 37$, $p < .001$) were significantly and positively correlated with body size PC1, suggesting that large males have larger total gift reserves regardless of the quality of the gifts. Finally, males of different sizes did not provide different kinds of protein as detected by electrophoretic separation. There were no significant correlations between male body size (PC1) and the relative density of the four distinct bands in our analyses, even before sequential Bonferroni correction (all $p > .2$, $n = 15$; Rice, 1989).

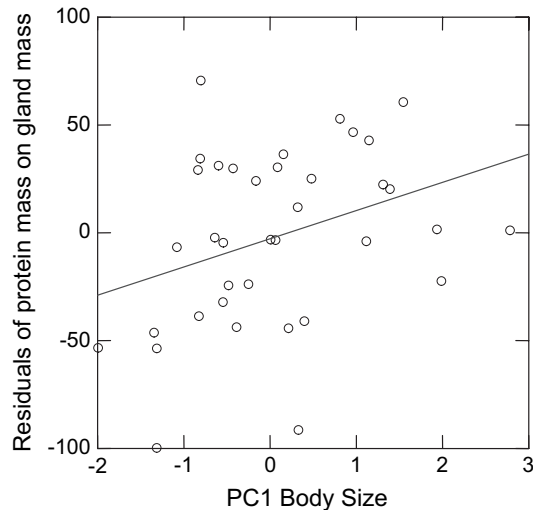


Figure 4
A scatterplot showing the effect of male body size (the first principal component of a PCA on several morphometric traits) on residuals of the regression of metanotal protein mass on total metanotal gland mass. Positive residuals indicate more protein per unit gland mass than expected based on the regression. The smoothing line is included merely to indicate the trend.

Hypothesis 2: preferred males mate more often and become depleted of gift reserves

A single bout of courtship-feeding depletes protein reserves in males. Gift size PC1 was negatively correlated with the total residual protein in the gland after mating, although this correlation was marginally nonsignificant ($r = -0.352$, $n = 31$, $p = .052$) (Figure 5). Note that the power of this test to detect moderate effects is low and that variation in total protein is high.

Natural *O. nigricornis* populations are sufficiently dense that potential encounter rates between mates are high. We estimated a total population of 780 ± 163 (mean \pm SE) individuals in the area sampled, which is equivalent to a 95% confidence interval for density of 1.8–4.8 crickets/m². There was no sex bias in our sample (124 males, 122 females; $\chi^2 = 0.016$, $df = 1$, $p = .90$). Natural remating rates for *O. nigricornis* are high, and remating rates used in Brown and Kuns' study (2000) are within this natural range. We observed males on 374 occasions and females on 342 occasions ($\chi^2 = 1.43$, $df = 1$, $p = .232$) in the course of the five sampling days, and during this time observed 30 matings. Dividing the total number of matings into the number of male cricket hours sampled indicates that each male mated once every 12.5 h, on average. Because we concentrated our observations of mating on the daily period during which most calling occurred, a conservative estimate of remating rate for the average male cricket is approximately once daily. Among males ($n = 9$) whose markings permitted individual identification in several early samples, we observed three males mating twice and one male three times in a single 12-h period.

Hypothesis 3: preferred males conserve gift reserves for anticipated future matings

Four males in 1998 and 14 males in 2002 failed to mate within 30 min of being paired with a female after experimental manipulation, and were excluded from the analysis. There was no significant difference in mating success between treatments ($\chi^2 = 2.62$, $df = 1$, $p = .11$). As predicted, crickets in the

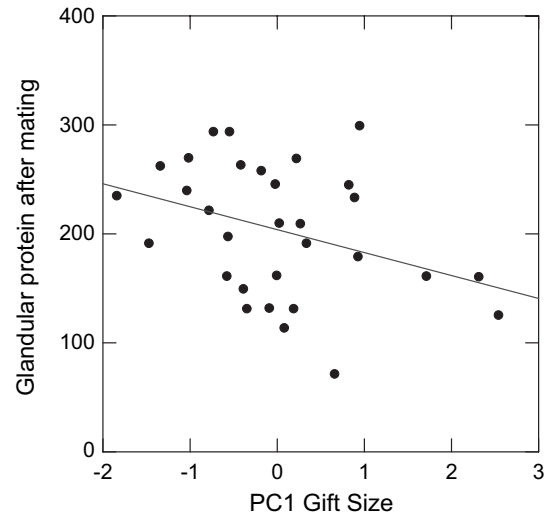


Figure 5
The influence of the size of a previously donated food gift (as measured by PC1 gift size, see Methods) on the amount of protein remaining in a male's metanotal glands after mating.

male-bias treatment donated significantly larger gifts (mean \pm SE = 1085 ± 83 s) than did those in the female bias treatment (774 ± 100 s; $t = 2.398$, $df = 56$, $p = .026$), supporting the hypothesis that males adaptively adjust their allocation to gifts based on their expected mating opportunities or their expected risk of sperm competition. Because within each treatment males only mated once, this result cannot be a consequence of glandular depletion by males in the female-biased treatment.

An alternative explanation for the larger gifts provided by males in the male-biased treatment is that males exposed to many females call more, thus expending energy reserves so that fewer total resources are available for future glandular gifts. We tested for this alternative in 2002 by weighing males before and after the treatment to determine if being exposed to females caused a greater weight loss (or lesser weight gain) than being exposed to males. Contrary to this prediction, we found that although males in the two treatments did not differ in mean weight before the treatment ($t = 1.613$, $df = 50$, $p = .11$), subjects in the male-biased treatment actually lost more weight than those in the female-biased treatment ($t = 2.782$, $df = 44$, $p = .01$; note that the postmating weights for six males were discarded because of an error in calibrating the balance). Thus, differences in energetic reserves cannot explain the difference in gift size across treatments.

DISCUSSION

Where males provide females with valuable material resources in the context of mating, these signals are naturally expected to reflect the quality and/or quantity of a given male's material donation (Thornhill, 1976). The fact that large males provide higher-quality gifts may explain why females prefer these males despite the fact that their gifts are on average no larger than those of rivals. Brown (1997a) noted that females mated with large males and females maintained on diets containing more protein laid more eggs after mating than did females mated with small males or maintained on low-protein diets. Our finding that larger male tree crickets produce glandular material with more protein provides an appealing mechanism (an increase in protein ingested by females) for the similarity in oviposition patterns between these two

experiments. An alternative (though not mutually exclusive) explanation is that the increase in oviposition rate observed after mating with large males is a form of cryptic female choice. Only a thorough test of the genetic benefits acquired from large males and the response of females to such males when protein quality is controlled can distinguish between these alternatives.

The elevated protein levels in gifts provided by larger males may explain why females prefer larger males, but it does not explain the mismatch between male size and gift size from the male perspective because larger males have larger total reserves of glandular protein. Optimality modeling suggests that because of the direct fitness benefits to males of donating larger gifts (i.e., increased insemination), males with larger gift-giving reserves should provide a larger number of larger gifts (Bussière, 2002). Courtship-feeding negatively affects the remaining reserves in courtship-feeding glands even after a single mating event (a result that was marginally non-significant), which is consistent with studies of other courtship-feeding insect species in which the production of nuptial gifts can impose limits on male reproduction (Gwynne, 2001). This result, along with Brown and Kuns (2000) suggests that depletion of gift reserves could occur in males that mate often. Because females prefer to approach larger males (Brown et al., 1996), it follows that these males are the most likely to become depleted of courtship gifts, which could explain the mismatch between male size and gift size observed in *O. nigricornis*.

Perceptions of the sex ratio also affect male investment in courtship gifts. Males exposed to relatively high female encounter rates provided smaller gifts than do rivals exposed to high male encounter rates. Because larger males are likely to attract more females (Brown et al., 1996), these males may provide smaller gifts than expected because they are adaptively conserving resources for future matings. Conserving resources may be particularly important because females discriminate against males depleted of courtship gift reserves (Bussière et al., 2004). Conversely, males experiencing a low encounter rate with receptive females should not conserve resources for unlikely future matings but instead should transfer larger meals as an adaptive response to the relatively high risk of sperm competition from abundant rivals (Parker, 1998). In our experiment, we manipulated the encounter rate, but males in the field could use other cues. For example, males making allocation decisions might acoustically assess not only the abundance of the rivals they hear but also their own quality relative to these rivals.

Adaptive plasticity in male choice is known for other courtship-feeding insects. For example, male *Gryllodes sigillatus* produce larger spermatophylaxes when exposed to rivals, as expected in order to increase sperm transfer under higher levels of sperm competition (Gage and Barnard, 1996; Mallard and Barnard, 2003). Shelly and Bailey (1992) and Kvamemo and Simmons (1999) showed that male *Kavanaughphila nartee* experiencing low female encounter rates were more likely to accept mates than were those experiencing high female encounter rates. Simmons et al. (1994) demonstrated that male *Requena verticalis* provided older females with a smaller spermatophylax. Simmons et al. (1992) found that male *R. verticalis* adjusted the size of the spermatophylax in response to a reduced remating interval; males experiencing longer remating intervals provided larger meals. However, this study did not refute nonadaptive resource accumulation as an alternative explanation for this result. In contrast to the conclusion of some investigators (see Alexander et al., 1997), these results show that insects are capable of complex modifications in behavior as a result of social interactions.

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