

EXPERIMENTAL EVIDENCE THAT SEXUAL CONFLICT INFLUENCES THE OPPORTUNITY, FORM AND INTENSITY OF SEXUAL SELECTION

Matthew D. Hall,^{1,2} Luc F. Bussière,^{3,4,5} John Hunt,^{6,7} and Robert Brooks^{1,8}

¹*Evolution & Ecology Research Centre and School of Biological, Earth & Environmental Sciences, University of New South Wales, Sydney, New South Wales 2052, Australia*

²*E-mail: m.hall@student.unsw.edu.au*

³*Zoologisches Museum der Universität Zürich, Winterthurerstrasse 190, CH 8057 Zürich, Switzerland*

⁴*School of Biological and Environmental Sciences, University of Stirling, Stirling, FK9 4LA Scotland, United Kingdom*

⁵*E-mail: luc.bussiere@stir.ac.uk*

⁶*Centre for Ecology & Conservation, University of Exeter in Cornwall, Penryn, TR10 9EZ, United Kingdom*

⁷*E-mail: j.hunt@exeter.ac.uk*

⁸*E-mail: rob.brooks@unsw.edu.au*

Received February 21, 2008

Accepted May 6, 2008

Sexual interactions are often rife with conflict. Conflict between members of the same sex over opportunities to mate has long been understood to effect evolution via sexual selection. Although conflict between males and females is now understood to be widespread, such conflict is seldom considered in the same light as a general agent of sexual selection. Any interaction between males or females that generates variation in fitness, whether due to conflict, competition or mate choice, can potentially influence sexual selection acting on a range of male traits. Here we seek to address a lack of direct experimental evidence for how sexual conflict influences sexual selection more broadly. We manipulate a major source of sexual conflict in the black field cricket, *Teleogryllus commodus*, and quantify the resulting changes in the nature of sexual selection using formal selection analysis to statistically compare multivariate fitness surfaces. In *T. commodus*, sexual conflict occurs over the attachment time of an external spermatophore. By experimentally manipulating the ability of males and females to influence spermatophore attachment, we found that sexual conflict significantly influences the opportunity, form, and intensity of sexual selection on male courtship call and body size. When males were able to harass females, the opportunity for selection was smaller, the form of selection changed, and sexual selection was weaker. We discuss the broader evolutionary implications of these findings, including the contributions of sexual conflict to fluctuating sexual selection and the maintenance of additive genetic variation.

KEY WORDS: Cryptic female choice, fitness surface, Gryllidae, postcopulatory choice, selection analysis, *Teleogryllus commodus*.

Sexual conflict occurs whenever an interaction between males and females results in sex-specific fitness optima that cannot be simultaneously resolved (Parker 1979; Arnqvist and Rowe 2005). Under one form of sexual conflict, interlocus conflict (sensu

Arnqvist and Rowe 2005), differences in the evolutionary interests of males and females can select for traits that manipulate the outcome of the reproductive interaction. Both empirical and theoretical studies have demonstrated the importance of sexual

conflict in terms of the coevolution of female resistance and male manipulation (Holland and Rice 1998, 1999), as well as in driving speciation (Parker and Partridge 1998; Arnqvist et al. 2000), influencing the risk of extinction (Kokko and Brooks 2003; Le Galliard et al. 2005) and the evolution of senescence (Promislow 2003; Maklakov et al. 2007). However, these studies have focused primarily on the evolutionary outcomes of sexual conflict rather than the selection that occurs within a single generation and is the basis of these eventual evolutionary outcomes. A recent quantitative genetic model of sexual conflict by Moore and Pizzari (2005) highlighted the importance of measuring the variation in fitness that results from sexual conflict and separating estimates of selection from the estimates of evolutionary change.

Although Parker (1979) drew attention to the many implications of sexual conflict almost 30 years ago, the broader implications of sexual conflict for sexual selection have not received as much attention as one might expect. Sexual selection is a consequence of competition between members of the same sex to successfully mate with individuals of the opposite sex (Andersson 1994). Both direct conflict between males (or, less commonly, between females) and mate choice in one sex among competing members of the other sex are well-established agents of sexual selection. However, because sexual conflict often leads to the evolution of male and female traits that manipulate mating success, it is also a potentially potent and general cause of sexual selection (Parker 2006). Moreover, it is not only the traits directly associated with the interactions involved in the conflict that are under selection, but rather the entire male phenotype. There is currently, however, a lack of direct evidence of how sexual conflict modifies the process and outcome of sexual selection.

A fundamental prediction of sexual conflict theory is that the antagonistic selection that males and females impose on one another will differ from other patterns of selection such as the sexual selection generated by female mate choice. Contrasting patterns of sexual selection have previously been shown between female choice and male competition (Moore and Moore 1999), as well as between pre- and postcopulatory mate choice (Arnqvist and Danielsson 1999; Pizzari et al. 2002). Empirical studies of interlocus sexual conflict have, however, largely only addressed how it causes antagonistic coevolutionary selection on males and females (interlocus sexual conflict, Rice and Holland 1997). In addition to the importance of testing whether sexual conflict results in sexual selection that differs from that generated by other sexually selected factors, quantifying the effect of an interaction on fitness functions may be a good means to assess the extent to which that interaction is a manifestation of sexual conflict (Arnqvist and Rowe 2005).

We can quantify the process of sexual selection by formally estimating a number of parameters derived in evolutionary theory. The opportunity for selection (I), describes the variation in rela-

tive fitness and the maximum potential strength of selection that may act on any given trait (Crow 1958; Arnold and Wade 1984). The opportunity for selection does not indicate, however, whether selection is occurring or which, if any, combinations of traits are favored. Instead, the form of selection can be described using multivariate selection analysis (Lande and Arnold 1983; Phillips and Arnold 1989) and differences in selection then quantified using a sequential model building approach (Draper and John 1988; Chenoweth and Blows 2003). Finally, the overall strength of selection can be summarized by calculating the total selection intensity (V), based on the variation in predicted fitness values from the selection analysis (Schluter 1988). Rather than characterizing a particular form of selection on an individual trait, the total selection intensity summarizes the overall selection on the combination of traits in a selection analysis, incorporating both linear and nonlinear forms of selection.

Here we present an experiment in which we manipulated the presence/absence of an important source of sexual conflict and characterized the resulting changes in sexual selection in the Australian black field cricket, *Teleogryllus commodus*. In this species, sexual conflict occurs over the removal of an externally attached spermatophore (Bussière et al. 2006), with males and females trying to manipulate attachment times in their favor. For females, the timing of removal is a form of postcopulatory female choice that biases sperm transfer toward attractive males (Bussière et al. 2006) and potentially reduces the amount of prostaglandin precursors that are transferred by way of the spermatophore and act to increase costly egg laying (Loher et al. 1981; Stanley-Samuelson et al. 1986, 1987). Immediately following copulation, however, males aggressively oppose spermatophore removal by the female (Loher and Rence 1978; Evans 1988). In many species of field cricket, males guard the female following copulation (Alcock 1994) and act aggressively toward rival males to prevent remating or to secure additional matings (Simmons 1986; Sakaluk 1991; Bateman and MacFadyen 1999; Wynn and Vahed 2004). In *T. commodus*, however, males actively direct their aggression toward the female to constrain the female's ability to exercise postcopulatory choice (Bussière et al. 2006). By vigorously harassing females, via antennal flagellation, aggressive chirping, and biting, males are able to manipulate their own insemination success by increasing the attachment time of their spermatophore and their success in fertilizing the females' eggs.

It has previously been suggested that females may use the intensity of male harassment as an indicator of the physiological quality of the male (Thornhill and Alcock 1983; Zuk 1987; Wiley and Poston 1996). It is often, however, the poor quality males who harass females the most intensely. For example, those that take the longest time to attract a mate (Bussière et al. 2006) or have the greatest level of parasite infection (Simmons 1990). Accordingly, Bussière et al. (2006) suggest that because it is the

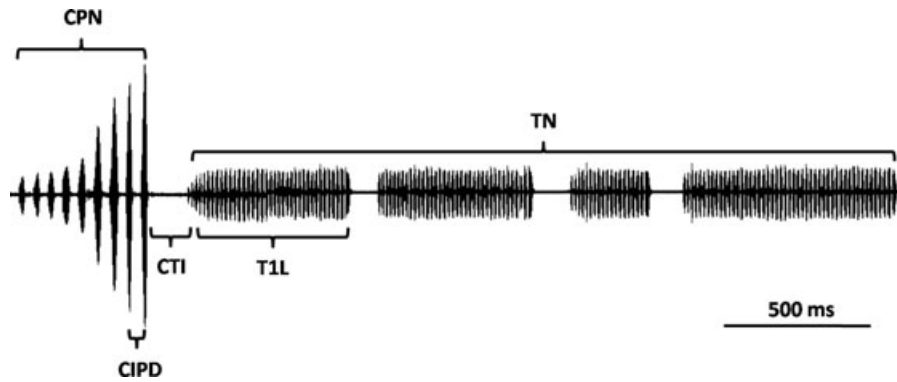


Figure 1. The courtship call of *Teleogryllus commodus*. The waveform diagram shows a single amplitude-modulated chirp sequence followed by a longer trill sequence that is consistent in intensity. We measured the number of pulses in the chirp (CPN), the duration of the interval between the last pulses in the chirp (CIPD), the interval between the chirp and trill (CTI), the length of the first trill sequence (T1L), the number of trills (TN), and the dominant frequency (DF, not shown).

unattractive males who benefit the most from the harassment behavior, the outcome of sexual conflict and spermatophore removal may oppose sexual selection via female choice. We therefore predict that the sexual conflict over spermatophore attachment times will have important consequences for which combination of male sexual traits are favored by sexual selection. If male harassment does constrain the opportunity for females to exercise mating preferences, we would expect both a change in the form and an overall weakening in the intensity of sexual selection. If on the other hand harassment reinforces mate choice, we would expect it to increase the opportunity for and strength of selection.

Using a series of behavioral trials, we created two artificial situations that manipulated both the ability of females to remove spermatophores and the ability of males to harass females. We then quantified the changes in sexual selection acting upon male traits closely related to mate choice, spermatophore removal, and harassment. In *T. commodus*, males have two distinct types of calls (Loher and Rence 1978): a long-distance advertisement call to attract females and a short-distance courtship call to entice mating. Females prefer males based on the structural properties and calling effort of the advertisement call (Brooks et al. 2005; Bentsen et al. 2006). How the courtship call relates to female choice in this species is unknown, however, in the closely related *T. oceanicus* the temporal properties of the courtship call are essential to induce mating (Balakrishnan and Pollack 1996) and may be used by females as a signal of male immune function (Tregenza et al. 2006).

In many cricket species, male body size is also an important component in determining spermatophore removal and harassment behavior. In *Gryllus bimaculatus*, for example, Simmons (1986) demonstrated that females remove the spermatophore of smaller males earlier than those of larger males. Similarly, in *T. commodus* male body size is related to aggressiveness and

fighting ability (Shackleton et al. 2005), and is also likely to be important in the ability of males to harass females. In this experiment, therefore, we wished to estimate how sexual conflict over spermatophore attachment influences the opportunity, form, and intensity of sexual selection on male courtship call properties (Fig. 1) and body size.

Methods

Animals for this experiment originated from a large, out-bred laboratory culture of *T. commodus* collected in 2004 and 2006 from Smith's Lake (32°22'S, 152°30'E), New South Wales, Australia. Initially we isolated 1500 nymphs in individual plastic containers (7 × 7 × 5 cm) provided with cat food (Friskies Go-Cat Senior, Nestlé Australia, Sydney), vials of water stoppered with cotton wool, and a piece of egg carton for shelter. We kept the crickets in a constant temperature room (28°C, 14:10 h light:dark regime) and replaced food and water weekly. Every two days we checked for eclosures, weighed new adults, and measured their pronotum width. From these two measures, we extracted a single principle component that described 99% of variation in overall cricket body size.

EXPERIMENTAL BEHAVIOR TRIALS

Using a series of behavioral trials, we created two experimental treatments that manipulated the balance of sexual conflict. In the female only treatment, females could exercise postcopulatory spermatophore removal uninhibited, whereas in the male present treatment males could freely harass the females. In total, we used 230 focal males and 460 females during the behavioral trials. The trials occurred over two consecutive nights every four days, using crickets that were between 10 and 12 days posteclosion. First, on the night before a trial we mated all available females to a random stock male and confined them to a small plastic tube

(5 × 1 cm) for 60 min to prevent spermatophore removal. This ensures that the females have a large store of sperm and are less likely to leave subsequent spermatophores attached due to sperm limitation (Wynn and Vahed 2004; Bussière et al. 2006). Bussière et al. (2006) have shown that the attractiveness of the first random male has no influence on the spermatophore attachment time of the second focal male.

To minimize observer disturbance, which could influence both male and female mating behaviors, we conducted the trials in a room dimly lit by red incandescent lights (40 watt, Philips Lighting, Eindhoven, The Netherlands). During the behavioral trials, we placed each mating pair in an arena consisting of a medium-sized plastic container (17 × 12 × 8 cm), lined with a damp paper towel. A mating arena of this size makes it possible to remove males without disturbing the female, and allows greater opportunity for females to escape harassment as the ability to escape can be unnaturally low in smaller containers (Simmons 1991).

We then conducted the behavioral trials during the first hour after dark. On the first night, we mated each focal male to a female. In half the trials, we allowed the female to mate and removed the male immediately following copulation (female only treatment). Each female in this treatment therefore controlled spermatophore removal freely. In contrast, in the other half of the trials we allowed the male to remain in the mating arena following copulation (male present treatment). In this case, each male was able to restrict a female's ability to remove the spermatophore through postcopulatory harassment. For each trial, we recorded the time of spermatophore attachment until it was removed or the pair began to remate. On the following night, we mated the same focal males again with a new female, this time using the opposite experimental treatment. In subsequent analyses, we only include the 212 males with complete data including estimates of body size, spermatophore attachment times for the two behavioral treatments, and the courtship call measures.

CALL RECORDING AND ANALYSIS

We recorded the courtship call for each focal male at approximately 14 days post eclosion, during the first 4 h of the evening. To induce a male to call, we placed the male together with a

female in a small plastic container (7 × 7 × 5 cm). If the female mounted the male before at least 10 complete courtship calls were recorded, we gently tapped the container to interrupt the mating. Calls were recorded as uncompressed audio using a Sony Hi-MD walkman (MZ-NH700, Sony Corporation, Tokyo, Japan) connected to a condenser microphone (C1163, Dick Smith Electronics, Chullora, NSW, Australia), which was mounted in the lid of the container. A junction unit was used between the microphone and recorder to power the microphone. We analyzed the courtship calls using the Raven sound analysis software (ver. 1.2, Cornell Bioacoustics Research Program, Ithaca, NY). Calls were first filtered to remove ambient noise below 3 kHz and above 6 kHz.

We measured the following call properties (Fig. 1) for five randomly selected calls per male: chirp pulse number; chirp interpulse duration; chirp to trill interval; trill number; first trill length; and dominant frequency. For each call property, we calculated the repeatability based on the variance components derived from an analysis of variance (ANOVA), with equal sample sizes (Becker 1992). In subsequent analyses, we used the averages of the five calls as our estimates for the call properties of each male. The repeatability estimates and averages for the six courtship call properties are shown in Table 1.

SPERMATOPHORE ATTACHMENT AS A PROXY FOR FITNESS

For many cricket species, the duration of spermatophore attachment is the biggest postcopula determinant of male fitness. Increasing the duration of spermatophore attachment typically results in an increase in the number of sperm transferred and in a greater share of paternity for the focal male (Sakaluk 1984; Simmons 1986, 1987; Sakaluk and Eggert 1996; but see Simmons et al. 2003). In *T. commodus*, however, Bussière et al. (2006) showed using once-mated females that there is no significant increase in the number of sperm transferred after an attachment time of 36 min. By using a single mating and quantifying total sperm numbers in the spermatheca, Bussière et al. (2006) demonstrated that there is a finite capacity for each female to store sperm, rather than indicating a diminishing returns relationship between spermatophore attachment time and sperm transfer. Importantly, when multiple mating is allowed we find there is a significant linear

Table 1. Descriptive statistics for the courtship call of the males used in the selection analysis.

	Abbreviation	Repeatability	Mean	SD
Chirp pulse number	CPN	0.567	9.265	1.685
Chirp interpulse duration (ms)	CIPD	0.552	31.190	6.917
Chirp to trill interval (ms)	CTI	0.276	142.905	40.616
Trill 1 Length (\sqrt{s})	T1L	0.245	2.111	0.325
Trill number (\log_e)	TN	0.353	1.160	0.475
Dominant frequency (kHz)	DF	0.595	4.001	0.166

relationship between the attachment time of the spermatophore from a second male to mate with a female and the proportion of sperm in the female's spermathaeca that are his (M. D. Hall, L. R. Bussière, M. Demont, P. I. Ward, and R. Brooks, unpubl. ms.). Therefore, although the total number of sperm stored in the spermathaeca does not change after 36 min, the mix of sperm within the spermathaeca does. Accordingly, we used the unscaled spermatophore attachment times as our proxy for fitness in the subsequent selection analyses.

ESTIMATING AND TESTING FOR DIFFERENCES IN THE OPPORTUNITY FOR SELECTION

We estimated the opportunity for selection (I) separately for each treatment as the variance in relative fitness (Arnold and Wade 1984), specifically the variance in spermatophore attachment times. We performed a randomization test to determine whether the difference in I between the two treatments was significant. Our test statistic was the difference in I between the female only and male present treatments. We first estimated I using the real data, and then recalculated the statistic by shuffling (with replacement) spermatophore attachment times across treatments. We then used the Monte Carlo analysis of PopTools (ver. 2.6.9, CSIRO, www.cse.csiro.au/poptools) to approximate a P -value by calculating the number of times the real test statistic exceeded that of the randomized data in 10,000 randomizations.

CHARACTERIZING SEXUAL SELECTION WITHIN TREATMENTS

To characterize the form of sexual selection imposed on courtship calls and body size in each treatment, we used separate multiple regressions (Lande and Arnold 1983) to estimate the vector of linear selection gradients (β) and the matrix of quadratic and correlational selection gradients (γ). Following the multiple regression, we doubled the quadratic regression coefficients (γ_{ii}), as the estimates obtained from statistical packages must be doubled to obtain the correct quadratic selection gradients (Stinchcombe et al., in press). Before analysis, however, it was necessary to transform TN and TL1 using natural logarithms and square-root transformations, respectively, as both were positively skewed. We also scaled our fitness measure, spermatophore attachment time, to a relative fitness measure, by dividing by the mean within each treatment. Last, we standardized all other traits to a mean of zero and standard deviation of one as recommended by Lande and Arnold (1983).

To determine the significance of linear and nonlinear sexual selection within each treatment we assessed the fit of the respective models. We used the overall significance of the regression model incorporating only the linear (β) terms to evaluate if linear selection was occurring. To evaluate the significance of nonlinear selection, however, we first conducted a canonical analysis

of the separate γ matrices (Phillips and Arnold 1989; Blows and Brooks 2003). The canonical analysis generates new eigenvectors (m_i) that describe the major axes of the nonlinear response surface. Conducting a canonical analysis increases the ability to detect nonlinear selection by decreasing the number of nonlinear selection coefficients in our study from 28 to seven (Blows and Brooks 2003). We then analyzed the nonlinear selection acting on the seven new eigenvectors using a standard second-order polynomial regression. To evaluate the significance of nonlinear sexual selection within each treatment we used partial F -tests (Chenoweth and Blows 2005) to determine if the addition of the seven quadratic terms significantly improved the fit of the model.

DIFFERENCES IN SEXUAL SELECTION BETWEEN TREATMENTS

We used a sequential model building approach to determine if the linear and nonlinear forms of sexual selection differed significantly between the two treatments (Draper and John 1988; see method in appendix A of Chenoweth and Blows 2005). This approach combines quantitative (call properties and body size) and qualitative (experimental treatment) variables and compares the unexplained sum of squares in a reduced model and the model containing the terms of interest using partial F -tests. To determine if linear sexual selection differed between the treatments we evaluated if the addition of linear (β) by treatment interaction terms improved the fit of the linear model. Likewise, to evaluate if nonlinear sexual selection differed between the treatments, we evaluated if the addition of nonlinear (γ_{ii} and γ_{ij}) by treatment interactions improved the fit of the full regression model incorporating linear, quadratic, and correlational selection terms.

ESTIMATING THE INTENSITY OF SELECTION

We compared the differences in overall selection between the female only and male present treatments by calculating the total selection intensity (V) separately for each treatment. We first calculated the expected fitness for each individual using the full regression model incorporating linear, quadratic, and correlational regression coefficients. We then estimated V as the squared coefficient of variance in the expected fitness values (Schluter 1988). As with our estimation of the opportunity for selection, we used a randomization approach to test if any difference in total selection between the two treatments was significant.

VISUALIZING FITNESS SURFACES

To visualize the nonlinear sexual selection occurring within each treatment, we explored the canonical rotations of the separate γ matrices (Phillips and Arnold 1989; Blows and Brooks 2003). The resulting M matrices indicate how the original traits directly relate to the response surface eigenvectors. The strength and form of nonlinear selection acting on each of these eigenvectors is

indicated by their eigenvalues (λ_i). We also tested the significance of selection acting on each new eigenvector using a quadratic regression model (Blows and Brooks 2003). It is important to note that the eigenvectors of each treatment arise from separate canonical rotations and thus m_1 from the male present treatment represents a different axis in multidimensional space than m_1 from the female only treatment. Finally, to visualize the fitness surfaces of each treatment, we used thin-plate splines as estimated by the Tsp function of the fields package in R and plotted the surfaces using R (ver. 2.4.1, R Development Core Team, www.R-project.org).

Results

VARIATION IN FITNESS AND THE OPPORTUNITY FOR SEXUAL SELECTION

Females took nearly twice as long to remove a spermatophore when the male remained following mating (male present, 5185 ± 123.9 s), compared to when the male was immediately removed (female only, 2886 ± 181.4 s). Male harassment of females, therefore, appears to significantly increase the time taken to remove the spermatophore (paired *t*-test, $t_{211} = 10.823$, $P < 0.001$). Although the mean attachment times increased in the male present treatment, the overall variation in spermatophore attachment was significantly reduced (*F*-test, $P < 0.001$). Accordingly, the opportunity for sexual selection was seven times smaller (Randomization test, $P < 0.001$) when males were able to harass (male present, $I = 0.121$, 95% CI = 0.087, 0.158), than when females controlled spermatophore removal in the absence of males (female only, $I = 0.837$, 95% CI = 0.669, 0.996).

CHARACTERIZING SEXUAL SELECTION WITHIN TREATMENTS

The standardized linear, quadratic, and correlational selection gradients estimated separately for each treatment are presented in Table 2. For both treatments, spermatophore removal generated strong sexual selection on male courtship calls and body size. In the male present treatment, although there was no significant linear sexual selection (model significance: $F_{7,204} = 0.903$, $P = 0.505$), the addition of nonlinear sexual selection significantly improved the fit of the model (partial *F*-test: $F_{7,197} = 5.148$, $P < 0.001$). We found a similar pattern in the female only treatment, in which linear sexual selection was not significant but nonlinear selection was (linear selection $F_{7,204} = 0.458$, $P = 0.864$; nonlinear selection: $F_{7,197} = 8.923$, $P < 0.001$).

DIFFERENCES IN SEXUAL SELECTION BETWEEN TREATMENTS

The form of sexual selection on male courtship calls and body size varied significantly between the two treatments. However, linear selection did not contribute to differences in sexual selection, as overall the linear selection by treatment interactions were not significant (partial *F*-test: $F_{7,408} = 0.776$, $P = 0.608$). Instead, the difference in sexual selection between treatments was solely due to nonlinear selection as indicated by the significant addition of the nonlinear selection by treatment interactions (partial *F*-test: $F_{28,358} = 1.677$, $P = 0.019$). Furthermore, the variation between the two treatments in nonlinear selection was greatest for quadratic selection on TN ($F_{1,358} = 8.307$, $P = 0.004$) and CIPD ($F_{1,358} = 4.624$, $P = 0.032$) and correlational selection between TN and T1L ($F_{1,358} = 6.822$, $P = 0.009$), CPN and CIPD ($F_{1,358} = 5.814$,

Table 2. The vector of standardized linear selection gradients (β) and the matrix (γ) of standardized quadratic (γ_{ij}) and correlational (γ_{ij}) selection gradients. Linear and nonlinear selection gradients were estimated in separate regressions (* $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$).

	β	CPN	CIPD	CTI	T1L	TN	DF	Body size
Female only treatment								
CPN	0.128	-0.018						
CIPD	-0.021	-0.238**	-0.264*					
CTI	-0.030	0.096	-0.113	-0.144				
T1L	0.025	0.207	0.142	0.005	-0.250			
TN	-0.011	0.081	0.151	0.091	-0.316*	-0.416**		
DF	-0.031	0.024	0.075	-0.051	-0.058	-0.015	-0.242*	
Body size	0.035	-0.100	0.091	0.041	-0.149	0.144	-0.020	-0.028
Male present treatment								
CPN	-0.018	0.008						
CIPD	-0.019	0.005	0.048					
CTI	-0.020	0.006	-0.104***	0.012				
T1L	0.004	0.022	-0.011	0.047	-0.002			
TN	0.014	-0.029	-0.031	0.061	0.033	0.078		
DF	-0.005	0.002	0.032	-0.011	-0.039	-0.047	-0.070	
Body size	0.030	0.019	0.053	0.030	-0.011	-0.045	-0.013	-0.018

Table 3. The M matrices of the eigenvectors from the canonical analyses of γ . The eigenvalues (λ_i) of each eigenvector (m_i) are shown in the first column (* $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$).

m_i	λ_i	CPN	CIPD	CTI	TIL	TN	DF	Body size
Female only treatment								
m_1	0.324***	0.595	-0.315	0.109	0.427	-0.270	-0.044	-0.526
m_2	0.109	-0.442	0.355	-0.533	0.337	-0.366	0.104	-0.370
m_3	-0.158	0.223	0.463	0.142	0.621	-0.054	-0.159	0.549
m_4	-0.176*	-0.475	-0.197	0.406	0.090	-0.249	-0.704	-0.076
m_5	-0.265**	-0.193	0.389	0.689	0.069	0.162	0.410	-0.371
m_6	-0.336***	-0.204	-0.413	0.186	0.094	-0.566	0.532	0.376
m_7	-0.860***	0.310	0.447	0.103	-0.544	-0.618	-0.124	-0.010
Male present treatment								
m_1	0.212***	0.069	0.539	-0.497	-0.263	-0.562	0.203	0.176
m_2	0.062	-0.509	0.325	-0.404	-0.165	0.568	-0.014	-0.350
m_3	0.036	0.347	0.574	0.033	0.501	0.362	-0.211	0.349
m_4	-0.005	0.516	-0.120	-0.391	0.328	-0.067	0.010	-0.674
m_5	-0.034	-0.588	0.004	-0.056	0.668	-0.413	-0.179	-0.055
m_6	-0.071*	0.059	-0.274	-0.417	-0.190	-0.018	-0.810	0.233
m_7	-0.144***	-0.021	-0.430	-0.509	0.251	0.234	0.475	0.461

$P = 0.016$), T1L and CIPD ($F_{1,358} = 4.606$, $P = 0.033$), and TN and body size ($F_{1,358} = 4.221$, $P = 0.032$).

To visualize the different nonlinear selection patterns in each treatment, we compared fitness surfaces comprising the major axes of the separate canonical rotations (Table 3). In the male present treatment, the two major axes of nonlinear selection, as indicated by the largest significant eigenvalues, displayed both concave (m_1) and convex (m_7) selection. This surface, however, is a relatively flat saddle (Fig. 2). The highest fitness along the

m_7 axis generally represents positive values of DF and body size and negative values of CTI and CIPD, whereas the two local fitness peaks along the m_1 axis contrast CIPD with CTI and TN. In contrast, for the female only treatment, the two major axes displayed only convex selection (m_6 and m_7). Accordingly, a single fitness peak defines the dominant form of selection (Fig. 3). For this surface, the area of highest fitness represents intermediate values of TN and CIPD as these traits have high loadings in both m_6 and m_7 .

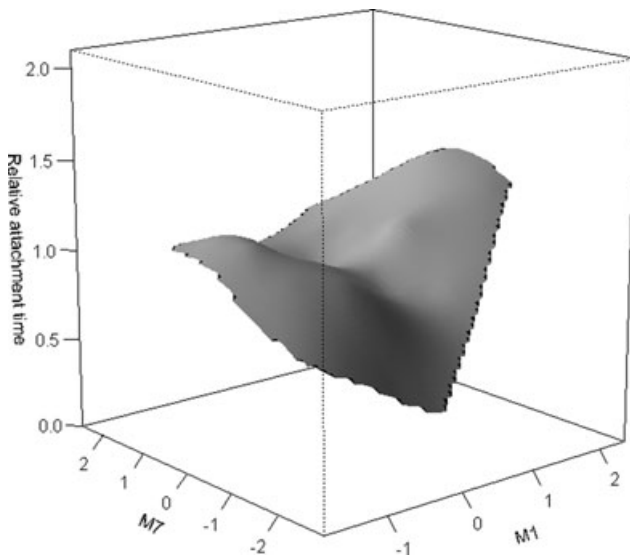


Figure 2. The fitness surface of the two major canonical axes m_1 and m_7 of the male present treatment. The two axes represent the eigenvectors with both the strongest nonlinear selection (highest eigenvalues) and the strongest positive (m_1) and negative (m_7) eigenvalues.

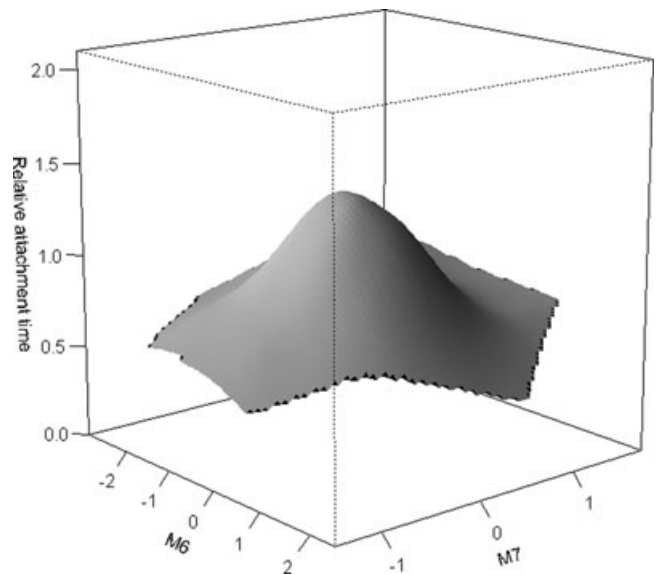


Figure 3. The fitness surface of the major canonical axes m_6 and m_7 of the female only treatment. The two axes represent the eigenvectors with the strongest nonlinear selection (highest eigenvalues).

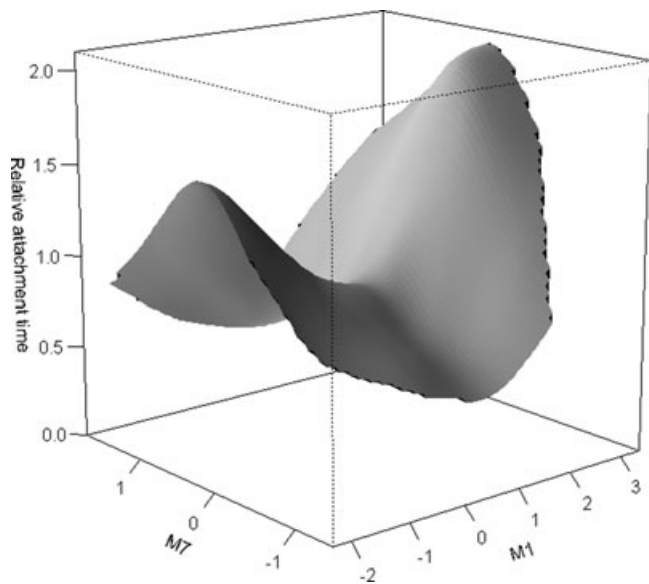


Figure 4. The fitness surface of the major canonical axes m_1 and m_7 of the female only treatment. The two axes represent the eigenvectors with the strongest positive (m_1) and negative (m_7) eigenvalues.

THE INTENSITY OF SEXUAL SELECTION

Finally, the intensity of sexual selection also differed significantly between the two treatments (Randomization test, $P < 0.001$). In the male present treatment, the total selection intensity ($V = 0.020$, 95% CI = 0.015, 0.027) was an order of magnitude lower than in the female only treatment ($V = 0.221$, 95% CI = 0.170, 0.280). The ability of males to harass females therefore appears to significantly reduce the total selection intensity. To visually contrast the differences in selection intensities between treatments we compared the fitness surfaces comprising the two axes with the strongest significant positive and negative eigenvalues from the separate canonical rotations. For the male present treatment, this once again represents Figure 2 (discussed above). In contrast, for the female only treatment, Figure 4 (which comprises the m_1 and m_7 eigenvectors of the female only treatment rotation, Table 3) illustrates two local fitness peaks occurring along the m_1 axis contrasting CPN and T1L with body size. The surface from the male present treatment (Fig. 2) is clearly much flatter than the very strong saddle surface of the female only treatment (Fig. 4).

Discussion

We have shown that in *T. commodus*, sexual conflict over spermatophore attachment can significantly influence the nature of sexual selection. By experimentally manipulating the balance of the conflict to favor either males (male present treatment) or females (female only treatment) we observed significant differences in the opportunity, form, and intensity of sexual selection on com-

ponents of the male courtship call and body size. When we allowed males to harass females, there was less opportunity for selection, a change in the form of selection and a weaker intensity of selection relative to when females express choice without male harassment.

The evolutionary implications of the interaction between sexual conflict and sexual selection have been the target of an increasing number of empirical studies (reviewed in Arnqvist and Rowe 2005). Studies of experimental evolution in various *Drosophila* species, for example, have examined if sexual selection generates female resistance and male manipulation (Holland and Rice 1999; Pitnick et al. 2001; Wigby and Chapman 2004; Crudginton et al. 2005). In water striders, comparative studies have demonstrated the repeated coevolution of male grasping and female antigrasping morphology (Arnqvist and Rowe 2002). Similarly, both phylogenetic and comparative analysis has shown that sexual conflict and postmating sexual selection drive speciation across diverse groups of insects (Arnqvist et al. 2000). All the aforementioned studies have addressed an important, but specific outcome of sexual conflict: the antagonistic coevolution between males and females. Sexual conflict, however, is characterized by reproductive interactions that generate variation in fitness and modify the process of sexual selection in general (Parker 1979, 2006). Our findings demonstrate that there are broader implications for sexual conflict and the evolution of male sexual traits than the coevolution between male and female manipulative behaviors.

How sexual conflict influences the opportunity for sexual selection will determine the overall constraints placed on the evolution of male sexual traits (Crow 1958; Arnold and Wade 1984). Consistent with previous studies (Loher and Rence 1978; Bussière et al. 2006), we found that male harassment delays the premature removal of spermatophores by female black field crickets, increasing individual male fitness, but reducing the overall variation in relative fitness. Accordingly, the opportunity for selection was significantly greater when females alone controlled spermatophore removal, compared to when males were able to harass females. By reducing the opportunity for sexual selection, male harassment limits the maximum strength of both linear and nonlinear sexual selection (Arnold and Wade 1984; Arnold 1986) for any trait that covaries with spermatophore attachment, including traits unmeasured in this study such as advertisement call properties, morphology, or contact pheromones.

Although the opportunity for selection indicates a maximum potential strength of sexual selection, it does not convey information about the selection that is actually occurring on a suite of traits (Arnold and Wade 1984). Using multivariate selection analysis (Lande and Arnold 1983; Phillips and Arnold 1989; Blows and Brooks 2003), however, we characterized directly the form and intensity of sexual selection. In both treatments, we found significant nonlinear selection for male courtship call components

and body size. When females exert postcopulatory choice uninhibited, for example, the dominant form of selection (Fig. 3) is a single peaked surface. In contrast, when males are able to harass females, the dominant form (Fig. 2) changes to a saddle-shaped surface. Male harassment, however, not only changes the focus of sexual selection on male courtship calls and body size, but also weakens its intensity (Fig. 2 cf. Fig. 4). Our findings demonstrate that the very nature of sexual selection, in terms of form and intensity, changes significantly depending on how sexual conflict over spermatophore removal is resolved between the sexes.

Our findings suggest that the sexual selection generated by sexual conflict could be changed fundamentally by simple changes in the environment that allow females to escape male harassment, such as microhabitat structure or the availability of alternative mates. In *Gryllobates sigillatus*, for example, male efficiency in preventing spermatophore removal decreases under competition from several male rivals or when alternate mates are available (Bateman and MacFadyen 1999). Accordingly, any spatial and temporal variability will cause sexual selection to favor different combinations of male traits in *T. commodus* at different times or in different places. Classical sexual selection models (Sasaki and Ellner 1997; Reinhold 2000) have suggested that such fluctuating selection can maintain additive genetic variation in traits under selection. Genetic polymorphism in the color patterns of guppies, for example, are in part explained by fluctuating selection due to variation in predation risk (Endler 1987, 1991) or ambient light (Gamble et al. 2003). Our findings put the evolutionary relationship between environmental variation and sexual conflict on the same logical footing. We suggest that fluctuating sexual selection due to changes in the nature of sexual conflict is another important process that can maintain additive genetic variation in traits that influence fitness.

There has been some dispute regarding the extent to which some behaviors, including spermatophore removal in Gryllid crickets, represent sexual conflict (Eberhard 1996; Arnqvist and Rowe 2005). Three lines of evidence suggest that female spermatophore removal and male harassment are a manifestation of conflict. First, the obvious fact that when harassment is prevented, females remove the spermatophore sooner. It has been suggested, however, that male harassment of female crickets may be an indicator of the benefits of mating with the male (Thornhill and Alcock 1983; Zuk 1987; Simmons 1990; Hockham and Vahed 1997). If harassment is indeed an indicator of male vigor, one might expect shorter spermatophore attachment when harassment is prevented. The second line of evidence, in direct contradiction of this prediction, is that poor-quality males harass more intensely (Simmons 1990; Bussière et al. 2006). The third line is our finding that the presence of harassment significantly weakens and opposes, rather than reinforces, the selection females exert in the absence of harassment. Our results, therefore, support the recent view that

harassment is a consequence of sexual conflict over insemination success (Bussière et al. 2006). More generally, our approach of comparing fitness surfaces in two or more treatments that manipulate the presence or intensity of sexual conflict, might be useful in resolving the controversial question of whether females are benefiting via indirect selection from mating with males that harass intensely (Cameron et al. 2003; Cordero and Eberhard 2003).

In conclusion, by examining the influence of sexual conflict on the evolution of male overall phenotype, we have highlighted the broader evolutionary implications of sexual conflict. We have demonstrated that in *T. commodus*, how sexual conflict over spermatophore attachment favors males or females will influence the opportunity, form, and intensity of sexual selection on the courtship call and body size. Our findings suggest that any environmental influences that cause variation in the outcome of sexual conflict will have important consequences for the combinations of male sexual traits favored by sexual selection and the maintenance of the genetic variation underlying these traits. Combining our results with estimates of the underlying multivariate genetic variation in courtship call and body size (Blows et al. 2004; Blows and Hoffmann 2005; Blows 2007) may further demonstrate how sexual conflict can enhance or inhibit evolutionary change in a range of sexual traits.

ACKNOWLEDGMENTS

This project was funded by an ARC Discovery grant to RB. LFB was supported by the University of Zurich and the University of Stirling. JH was supported by a NERC Fellowship. We would like to thank R. Bonduriansky, H. Kokko, and two anonymous referees for comments on the manuscript and L.W. Simmons for access to an unpublished manuscript.

LITERATURE CITED

- Alcock, J. 1994. Postinsemination associations between males and females in insects: the mate-guarding hypothesis. *Annu. Rev. Entomol.* 39:1–21.
- Andersson, M. 1994. *Sexual selection*. Princeton Univ. Press, Princeton, NJ.
- Arnold, S. J. 1986. Limits on stabilizing, disruptive, and correlational selection set by the opportunity for selection. *Am. Nat.* 128:143–146.
- Arnold, S. J., and M. J. Wade. 1984. On the measurement of natural and sexual selection: theory. *Evolution* 38:709–719.
- Arnqvist, G., and I. Danielsson. 1999. Postmating sexual selection: the effects of male body size and recovery period on paternity and egg production rate in a water strider. *Behav. Ecol.* 10:358–365.
- Arnqvist, G., and L. Rowe. 2002. Correlated evolution of male and female morphologies in water striders. *Evolution* 56:936–947.
- . 2005. *Sexual conflict*. Princeton Univ. Press, Princeton, NJ.
- Arnqvist, G., M. Edvardsson, U. Friberg, and T. Nilsson. 2000. Sexual conflict promotes speciation in insects. *Proc. Natl. Acad. Sci. USA* 97:10460–10464.
- Balakrishnan, R., and G. S. Pollack. 1996. Recognition of courtship song in the field cricket, *Teleogryllus oceanicus*. *Anim. Behav.* 51:353–366.
- Bateman, P. W., and D. N. MacFadyen. 1999. Mate guarding in the cricket *Gryllobates sigillatus*: influence of multiple potential partners. *Ethology* 105:949–957.

- Becker, W. A. 1992. *Manual of quantitative genetics*. Academic Enterprises, Pullman, WA.
- Bentsen, C. L., J. Hunt, M. D. Jennions, and R. Brooks. 2006. Complex multivariate sexual selection on male acoustic signaling in a wild population of *Teleogryllus commodus*. *Am. Nat.* 167:E102–E116.
- Blows, M. W. 2007. A tale of two matrices: multivariate approaches in evolutionary biology. *J. Evol. Biol.* 20:1–8.
- Blows, M. W., and R. Brooks. 2003. Measuring nonlinear selection. *Am. Nat.* 162:815–820.
- Blows, M. W., and A. A. Hoffmann. 2005. A reassessment of genetic limits to evolutionary change. *Ecology* 86:1371–1384.
- Blows, M. W., S. F. Chenoweth, and E. Hine. 2004. Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. *Am. Nat.* 163:329–340.
- Brooks, R., J. Hunt, M. W. Blows, M. J. Smith, L. F. Bussière, and M. D. Jennions. 2005. Experimental evidence for multivariate stabilizing sexual selection. *Evolution* 59:871–880.
- Bussière, L. F., J. Hunt, M. D. Jennions, and R. Brooks. 2006. Sexual conflict and cryptic female choice in the black field cricket, *Teleogryllus commodus*. *Evolution* 60:792–800.
- Cameron, E., T. Day, and L. Rowe. 2003. Sexual conflict and indirect benefits. *J. Evol. Biol.* 16:1055–1060.
- Chenoweth, S. F., and M. W. Blows. 2003. Signal trait sexual dimorphism and mutual sexual selection in *Drosophila serrata*. *Evolution* 57:2326–2334.
- . 2005. Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. *Am. Nat.* 165:281–289.
- Cordero, C., and W. G. Eberhard. 2003. Female choice of sexually antagonistic male adaptations: a critical review of some current research. *J. Evol. Biol.* 16:1–6.
- Crow, J. F. 1958. Some possibilities for measuring selection intensities in man. *Hum. Biol.* 30:1–13.
- Crudgington, H. S., A. P. Beckerman, L. Brustle, K. Green, and R. R. Snook. 2005. Experimental removal and elevation of sexual selection: does sexual selection generate manipulative males and resistant females? *Am. Nat.* 165:S72–S87.
- Draper, N. R., and J. A. John. 1988. Response-surface designs for quantitative and qualitative variables. *Technometrics* 30:423–428.
- Eberhard, W. G. 1996. *Female control: sexual selection by cryptic female choice*. Princeton Univ. Press, Princeton, NJ.
- Endler, J. A. 1987. Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Anim. Behav.* 35:1376–1385.
- . 1991. Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Res.* 31:587–608.
- Evans, A. R. 1988. Mating systems and reproductive strategies in 3 Australian gryllid crickets: *Bobilla victoriae* Otte, *Balamara gidya* Otte and *Teleogryllus commodus* Walker (Orthoptera, Gryllidae: Nemobiinae; Trigonidiinae; Gryllinae). *Ethology* 78:21–52.
- Gamble, S., A. K. Lindholm, J. A. Endler, and R. Brooks. 2003. Environmental variation and the maintenance of polymorphism: the effect of ambient light spectrum on mating behaviour and sexual selection in guppies. *Ecol. Lett.* 6:463–472.
- Hockham, L. R., and K. Vahed. 1997. The function of mate guarding in a field cricket (Orthoptera: Gryllidae; *Teleogryllus natalensis* Otte and Cade). *J. Insect. Behav.* 10:247–256.
- Holland, B., and W. R. Rice. 1998. Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution* 52:1–7.
- . 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proc. Natl. Acad. Sci. USA* 96:5083–5088.
- Kokko, H., and R. Brooks. 2003. Sexy to die for? Sexual selection and the risk of extinction. *Ann. Zool. Fenn.* 40:207–219.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Le Galliard, J.-F., P. S. Fitze, R. Ferriere, and J. Clobert. 2005. Sex ratio bias, male aggression, and population collapse in lizards. *Proc. Natl. Acad. Sci. USA* 102:18231–18236.
- Loher, W., and B. Rence. 1978. The mating behavior of *Teleogryllus commodus* (Walker) and its central and peripheral control. *Z. Tierpsychol.* 46:225–259.
- Loher, W., I. Ganjian, I. Kubo, D. Stanley-Samuelson, and S. S. Tobe. 1981. Prostaglandins: their role in egg-laying of the cricket *Teleogryllus commodus*. *Proc. Natl. Acad. Sci. USA* 78:7835–7838.
- Maklakov, A. A., C. Fricke, and G. Arnqvist. 2007. Sexual selection affects lifespan and aging in the seed beetle. *Aging Cell* 6:739–744.
- Moore, A. J., and P. J. Moore. 1999. Balancing sexual selection through opposing mate choice and male competition. *Proc. R. Soc. Lond. B* 266:711–716.
- Moore, A. J., and T. Pizzari. 2005. Quantitative genetic models of sexual conflict based on interacting phenotypes. *Am. Nat.* 165:S88–S97.
- Parker, G. A. 1979. Sexual selection and sexual conflict. Pp. 123–166 in M. S. Blum and N. A. Blum, eds. *Sexual selection and reproductive competition in insects*. Academic Press, New York.
- . 2006. Sexual conflict over mating and fertilization: an overview. *Philos. Trans. R. Soc. Lond. B* 361:235–259.
- Parker, G. A., and L. Partridge. 1998. Sexual conflict and speciation. *Philos. Trans. R. Soc. Lond. B* 353:261–274.
- Phillips, P. C., and S. J. Arnold. 1989. Visualizing multivariate selection. *Evolution* 43:1209–1222.
- Pitnick, S., W. D. Brown, and G. T. Miller. 2001. Evolution of female remating behaviour following experimental removal of sexual selection. *Proc. R. Soc. Lond. B* 268:557–563.
- Pizzari, T., D. P. Froman, and T. R. Birkhead. 2002. Pre- and post-insemination episodes of sexual selection in the fowl, *Gallus g. domesticus*. *Heredity* 88:112–116.
- Promislow, D. 2003. Mate choice, sexual conflict, and evolution of senescence. *Behav. Genet.* 33:191–201.
- Reinhold, K. 2000. Maintenance of a genetic polymorphism by fluctuating selection on sex-limited traits. *J. Evol. Biol.* 13:1009–1014.
- Rice, W. R., and B. Holland. 1997. The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behav. Ecol. Sociobiol.* 41:1–10.
- Sakaluk, S. K. 1984. Male crickets feed females to ensure complete sperm transfer. *Science* 223:609–610.
- . 1991. Postcopulatory mate guarding in decorated crickets. *Anim. Behav.* 41:207–216.
- Sakaluk, S. K., and A.-K. Eggert. 1996. Female control of sperm transfer and intraspecific variation in sperm precedence: antecedents to the evolution of a courtship food gift. *Evolution* 50:694–703.
- Sasaki, A., and S. Ellner. 1997. Quantitative genetic variance maintained by fluctuating selection with overlapping generations: variance components and covariances. *Evolution* 51:682–696.
- Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution* 42:849–861.
- Shackleton, M. A., M. D. Jennions, and J. Hunt. 2005. Fighting success and attractiveness as predictors of male mating success in the black field cricket, *Teleogryllus commodus*: the effectiveness of no-choice tests. *Behav. Ecol. Sociobiol.* 58:1–8.
- Simmons, L. W. 1986. Female choice in the field cricket *Gryllus bimaculatus* (De Geer). *Anim. Behav.* 34:1463–1470.

- . 1987. Sperm competition as a mechanism of female choice in the field cricket, *Gryllus bimaculatus*. *Behav. Ecol. Sociobiol.* 21:197–202.
- . 1990. Postcopulatory guarding, female choice and the levels of gregarine infections in the field cricket, *Gryllus bimaculatus*. *Behav. Ecol. Sociobiol.* 26:403–407.
- . 1991. On the post-copulatory guarding behaviour of male field crickets. *Anim. Behav.* 42:504–505.
- Simmons, L. W., J. Wernham, F. Garcia-Gonzalez, and D. Kamien. 2003. Variation in paternity in the field cricket *Teleogryllus oceanicus*: no detectable influence of sperm numbers or sperm length. *Behav. Ecol.* 14:539–545.
- Stanley-Samuelson, D. W., R. A. Jurenka, G. J. Blomquist, and W. Loher. 1987. Sexual transfer of prostaglandin precursor in the field cricket *Teleogryllus commodus*. *Physiol. Entomol.* 12:347–354.
- Stanley-Samuelson, D. W., J. J. Peloquin, and W. Loher. 1986. Egg-Laying in response to prostaglandin injections in the Australian field cricket *Teleogryllus commodus*. *Physiol. Entomol.* 11:213–220.
- Stinchcombe, J. R., A. F. Agrawal, P. A. Hohenlohe, S. J. Arnold, and M. W. Blows. in press. Estimating non-linear selection gradients using quadratic regression coefficients: double or nothing? *Evolution*.
- Thornhill, R., and J. Alcock. 1983. *The evolution of insect mating systems*. Harvard Univ. Press, Cambridge, MA.
- Tregenza, T., L. W. Simmons, N. Wedell, and M. Zuk. 2006. Female preference for male courtship song and its role as a signal of immune function and condition. *Anim. Behav.* 72:809–818.
- Wigby, S., and T. Chapman. 2004. Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution* 58:1028–1037.
- Wiley, R. H., and J. Poston. 1996. Perspective: indirect mate choice, competition for mates, and coevolution of the sexes. *Evolution* 50:1371–1381.
- Wynn, H., and K. Vahed. 2004. Male *Gryllus bimaculatus* guard females to delay them from mating with rival males and to obtain repeated copulations. *J. Insect. Behav.* 17:53–66.
- Zuk, M. 1987. The effects of gregarine parasites, body size, and time of day on spermatophore production and sexual selection in field crickets. *Behav. Ecol. Sociobiol.* 21:65–72.

Associate Editor: H. Kokko