Love will tear you apart: different components of female choice exert contrasting selection pressures on male field crickets

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Female mate choice is a driving force in the evolution of male secondary sexual characters. It can be dissected into several components: discrimination describes the degree to which females distinguish male trait variation, responsiveness indicates the speed or likelihood of females’ reactions to a mate, and preference functions illustrate how the probability of mating relates to male trait variation. Relatively little is known about how these components interact to produce final mating decisions and influence the strength and direction of sexual selection, so I used field cricket data (Teleogryllus oceanicus) to measure interactions between individual preference functions, discrimination, and 2 measures of responsiveness (number of responses and response effort). Preference function shape varied considerably between individual females. Highly discriminating females showed greater numbers of responses to playbacks and were more likely to have stabilizing preference functions. When I constructed 2 population-level preference functions using either response number or response effort, the first yielded a linear, directional function, whereas the second implied a stabilizing function. The clear differences between the 2 imply that different components of female choice can exert contrasting selection pressure on a single male trait. Overall, both response number and discrimination were mutually reinforcing and likely govern the strength of sexual selection in this population. The direction of selection in a wild setting ultimately depends on the relative importance of response number versus response effort, where exogenous factors such as predation risk or density will determine which component of female choice predominates mating decisions. Key words: choosiness, discrimination, mate choice, preference function, sexual selection, Teleogryllus oceanicus. [Behav Ecol]
also plausible. Choosiness has been demonstrated to be a facultative response to variables such as energetic costs, predation risk, and the chooser’s quality among others, and different strategies for adjusting choosiness may maximize the efficiency of female mate choice under different scenarios. For instance, a female experiencing high predation risk may simply sample fewer mates, as opposed to reducing the amount of time spent evaluating each mate or utilizing more finely tuned mechanisms of discrimination (Hedrick and Dill 1995). Reinhold et al. (2002) found that responsiveness and discrimination were uncorrelated in the grasshopper Chorthippus biguttulus, suggesting that the components operate independently in that species. Thus far, however, few studies have sought to characterize relationships between the components of female preference variation to assess whether they affect the strength and direction of sexual selection in a parallel versus divergent manner.

Here I used the Pacific-distributed field cricket Teleogryllus oceanicus to clarify the interactions between different components of female choice. Mating preferences in T. oceanicus have been well studied, and male song parameters known to influence female choice can be easily manipulated for experimental purposes (Simmons et al. 2001; Simmons 2004). I performed playback experiments using females from a laboratory population derived from a Hawaiian stock. Two-choice mate preference designs make it difficult for the experimenter to evaluate female failures to respond to a given stimulus: the interpretation is biased toward assuming that she makes a positive response to a song each and every time she is presented with a choice (Wagner 1998; Shackleton et al. 2005). I therefore used a no-choice experimental design to measure the components of female choice and construct absolute preference functions for a set of T. oceanicus females. For clarity, I have adapted the terminology of previous authors to describe each of the components I studied: female responsiveness encompasses both response number, or the number of potential mates a female exhibits a positive response toward (RN), and response effort (RE), which I define here as response speed. Discrimination (D) measures the difference in female response to more preferred mates versus less-preferred mates (Jennions and Petrie 1997; Gray and Cade 1999). Measures of discrimination employed by researchers vary widely and are necessarily a best estimate of what is most biologically meaningful in the particular system being studied. Here, I applied the methods of Gray and Cade (1999) and Brooks and Endler (2001) and tested whether the 2 techniques covaried (see Figure 2 for details about each technique). Given the potentially flexible nature of female choice (Widemo and Sæther 1999), I tested whether RN, RE, and D vary independently.

Recent attention has focused on the evolutionary significance of individual preference variation (Ritchie et al. 2005; Klappert et al. 2007). I therefore constructed individual preference functions using response effort as a proxy for mating probability to explore how preference variation interacts with other components of female choice. This allowed me to test whether specific preference function shapes were associated with greater

Figure 1
Hypothetical examples of independent variation in the components of female choice. The x axis represents a continuously distributed male trait under sexual selection, and the y axis represents the likelihood that a female will mate with a male showing the given trait value. (A) Variation in preference function shape independent of discrimination and responsiveness. Here, the female producing the stabilizing function (solid line) and the female producing the open-ended linear function (dashed line) show similar overall responsiveness and discriminate equally between the most and least attractive male trait values, despite having different preference function shapes. (B) Variation in responsiveness independent of preference function shape or discrimination. The shape and relative discrimination of the 2 functions remains constant, whereas that of the more responsive female (dashed line) is shifted vertically. (C) Variation in discrimination independent of preference function shape or responsiveness. The sinusoidal shape of the 2 functions remains constant, as does overall responsiveness or the mean position on the y axis, but the more discriminating female (solid line) shows greater differentiation in responses to male traits than the less discriminating female (dashed line).

Figure 2
Graphical examples of female discrimination and responsiveness derived from 3 crickets used in this study. The same data are used to compare the techniques of Brooks and Endler (2001) and Gray and Cade (1999) for measuring female discrimination (D) in the left and right columns, respectively. Row A: The least discriminating female. Row B: A female that showed intermediate discrimination but high response effort. Row C: The most discriminating female. Brooks and Endler (2001) measure discrimination by calculating the standard deviation of a female’s average response to all male trait values (vertical bars), whereas Gray and Cade (1999) calculate the number of standard deviations between a female’s response to her most preferred male trait value and her average response to all values (vertical bars). A female’s mean response to all song models is her response effort (RN) indicated by the horizontal dashed line. Values along the x axis are continuous and correspond to the 6 different song models used. Response time (speed) was used as a measure of response effort but is inverse transformed for clarity in the figures.
responsiveness or discrimination. Finally, I evaluated whether using response number and response effort as proxies for mating probability produce similar population-wide preference functions. If females do not alter their investment in mate choice by producing correlated responses, then average preference functions may differ in shape and give conflicting signals about the direction of selection on male traits.

MATERIALS AND METHODS

Cricket collection and rearing

A *T. oceanicus* laboratory colony was established using approximately 100 crickets collected from the Hawaiian island of Kauai in May 2006. Crickets were reared at 25 °C on a 12:12 light:dark cycle in 15-L containers and were provided egg cartons for shelter, water, and Fluker’s cricket chow ad libitum. To reduce the likelihood that maternal effects influencing female choice reflected environmental conditions predominating the field at the time of collection, I only used females from the F2 laboratory generation. Females were separated into boxes of controlled densities of 20 females each when sex differences became apparent during development. The boxes were monitored daily, and each female was marked with a unique identifier using enamel paint on the day she eclosed. I only tested virgin females between 5 and 9 days old to avoid age-related variation in female choice.

Song models

*Teleogryllus oceanicus* song contains 2 components: a trill-like long chirp consisting of 3–8 pulses, followed by a sequence of short chirps (Figure 3). Each short chirp consists of a pair of lower amplitude sound pulses. Previous work has shown that *T. oceanicus* females prefer songs containing a greater overall proportion of long chirp (Simmons et al. 2001). Therefore, I used the software package Raven v. 1.2 (Ithaca, NY) to construct song models differing only in the proportion of long chirp, following the technique of Simmons et al. (2001). To maintain all sound pulse lengths and the carrier frequency constant, individual long- and short-chirp pulses were excised from a song recorded in the wild at 25 °C. All parameters of the wild song were comparable to average parameters measured in other Hawaiian *T. oceanicus* populations (Zuk et al. forthcoming). Excised pulses were then concatenated to construct 6 song models that contained 0%, 20%, 40%, 60%, 80%, and 100% long chirp, respectively. The following song parameters were held constant: long-chirp pulse duration (39.5 ms), long-chirp interpulse interval (23.0 ms), the interval between the long chirp and the short chirp (65.0 ms), short-chirp duration (78.0 ms), the interval between short chirps (65.0 ms), the interval between pulses within each short chirp (9.0 ms), and short-chirp pulse duration (34.0 ms). The carrier frequency was 4.81 kHz.

![Oscillogram of a representative *Teleogryllus oceanicus* calling song. The song consists of 2 discrete phrases, the long chirp and the short chirp. Song parameters follow the terminology of Simmons (2004): PD, pulse duration; IPI, interpulse interval; CD, chirp duration; ICI, interchirp interval.](image)

Phonotaxis trials

A total of 566 phonotaxis trials were performed in a 116-cm-long by 30-cm-wide chamber with a speaker behind wire mesh at either end. Twenty females were subjected to a minimum of 24 no-choice trials each, during which each of the 6 song models was played back 4 times. Collecting data in this way required repeated testing of females, which could result in females becoming habituated as the sequence of trials proceeded. I used a general linear model (GLM) to test whether response times increased as trials proceeded, and I randomized the sequence and direction of all songs to reduce the possibility that the results were confounded by systematic habituation effects. All trials for a given cricket were carried out on the same day.

Playbacks were performed using Sony Sport CD players and Sony SRS-A27 computer speakers, and the sound level was calibrated to 70 dB at the release point using a digital sound meter (model 8922). At the start of each trial, I gently placed a female in the center of the chamber and inverted a plastic container 11 cm in diameter over her. She was allowed to rest in silence for 2 min. I began playback of the randomly designated song model and carefully removed the inverted container after 30 s so as not to startle the cricket. Timing commenced when the container was removed, and females were given 300 s to complete each trial plus 30 s of silence between trials. The phonotaxis chamber was cleaned with distilled water after each trial to reduce the possibility that olfactory cues would influence females’ choices.

I recorded a positive response if a female moved toward the playback and touched the screen adjacent to the speaker within the allotted 300 s and a negative response if a female either stayed stationary in the center of the chamber for the entire trial or moved to the screen opposite the playback. In the case of a positive response, I recorded her response time. If a female failed to respond positively to a given song model, I retested her with it only after all 24 randomized song trials were completed to ensure that a full complement of 4 trials for each song model were available for analysis. In all cases, females responding positively displayed typical phonotactic movement patterns; they approached the speaker following a sinusoidal path. Following the trials, I measured each female’s pronotum length as a proxy for body size.

Analysis

I calculated the number of playbacks each female positively responded to during the first 24 randomized trials (*R*), her average response effort (*D*), and her discrimination (*D*). To obtain *R*, I calculated the mean response time over all trials to all songs. Females with a faster average rate of response were assumed to have expended greater effort during their responses (higher *R*). *D* was calculated in 2 ways: adapting the methods of Gray and Cade (1999) and Brooks and Endler (2001). The first method calculates *D* by subtracting a female’s average response to all song models (*R*) in this case) from her response to her most preferred song model and dividing this quantity by the standard deviation of her response to her most preferred song model. The second method calculates *D* by simply taking the standard deviation of the average response to all song models. Figure 2 provides examples of how I calculated *D* using each method.

The correspondence between the 2 methods of calculating *D* was tested using a Pearson product–moment correlation. To examine the relationships among *R*, *R*, and *D*, I considered all possible 2-way comparisons using Pearson product–moment correlations. When comparing *R* and *D*, I only used the method of Gray and Cade (1999) because comparison using the method of Brooks and Endler (2001) would be
confounded by an uncontrolled scale effect (variance of female responses \(D\)) is a priori expected to increase with the mean \(\bar{D}\). Data were log\(_{10}\) transformed prior to analysis to ensure normality. Body size was not correlated with any parameter measured (see Results) and was therefore not included in the analyses.

To test whether responsiveness and discrimination were associated with the shape of female preference functions, I estimated the linear and quadratic components of each female’s preference function using the respective coefficients from a quadratic regression of response effort \(R_E\) on song model (song models represent discrete intervals of a parameter that varies continuously in nature). Strong linear components are indicative of linear preference functions, whereas strong quadratic components are associated with stabilizing or disruptive functions. Positive quadratic regression coefficients indicate stabilizing preference functions in this study because regressions were performed on response time data, for which shorter response times indicate higher \(R_E\). I used Pearson product-moment correlations to test whether preference function shape, that is, the linear and quadratic regression coefficients, covaried with \(R_N\), \(R_E\), and \(D\). I only used the technique of Gray and Cade (1999) for estimating \(D\) in this analysis because both the quadratic regression and the estimate of \(D\) of Brooks and Endler (2001) depend on the variance of all female responses, which would confound the correlation. Estimates of \(D\) using the technique of Gray and Cade (1999) are more likely to be independent of the coefficients obtained from regression analyses because \(D\) in this case does not rely on any properties of the underlying distribution of female responses. I also repeated the analyses using only regression coefficients that were statistically significant.

I constructed 2 average preference functions across all females using either binary response number data or response effort data. The first fitted a nonparametric cubic spline to binary responses \(R_N\) for all individuals tested with the 6 different song models using the program glmsWIN v. 1.0 (Schluter 1988). I used an iterative method to estimate the most appropriate smoothing parameter (lambda = 10) and performed 1000 bootstrap replicates to construct a mean spline with standard errors (Schluter 1988). For the second method, I used a quadratic regression of response effort \(R_E\) on song model to estimate the shape of the preference function. In all figures, time data are inverse transformed so that larger values consistently indicate stronger responses; however, all analyses were performed on raw or log\(_{10}\) transformed data. Analyses were carried out in Minitab v. 12 and SAS v. 9.1.

**RESULTS**

**Covariance between different measures of discrimination**

The techniques employed by Gray and Cade (1999) and Brooks and Endler (2001) produced measurements of discrimination that were highly correlated (Pearson correlation: \(r = 0.702\), \(n = 20\), \(P = 0.001\)) (Figure 4).

**Covariance among \(R_N\), \(R_E\), and \(D\)**

Females responding to a greater number of songs were significantly more discriminating regardless of which method was used to measure discrimination (Pearson correlations: \(r = 0.631\), \(n = 20\), \(P = 0.003\); \(r = 0.661\), \(n = 20\), \(P = 0.004\) for Brooks and Endler [2001] and Gray and Cade [1999] measures of \(D\), respectively) (Figure 5). There was no relationship between average response effort \(R_E\) and response number \(R_N\) (Pearson correlation: \(r = 0.067\), \(n = 20\), \(P = 0.778\)). Similarly, response effort and discrimination were unrelated (Pearson correlation: \(r = 0.169\), \(n = 20\), \(P = 0.475\)). Body size was not associated with any of the female choice parameters measured (Pearson correlations: all \(P > 0.140\)). As trials proceeded, females took longer to respond (GLM: \(F_{1,463} = 64.03\), \(P < 0.001\)), but this habituation occurred equally across all the randomly presented song models (GLM: \(F_{5,463} = 1.84\), \(P = 0.104\)) and explained a minor portion of the overall variance in responsiveness (GLM: model \(r^2 = 0.18\), \(P < 0.001\)).

**Individual female preference functions**

When preference functions were constructed for individual females using response effort to each song model, both linear and quadratic regression coefficients covaried with discrimination (Figure 6). Preference functions from highly discriminating females were more likely to be stabilizing (Pearson correlation: \(r = 0.516\), \(n = 20\), \(P = 0.020\)), whereas linear components predominated functions from less discriminating females (Pearson correlation: \(r = -0.466\), \(n = 20\), \(P = 0.038\)). When I only included significant quadratic regression
coefficients in the analysis, discriminating females were still more likely to have stabilizing preference functions (Pearson correlation: $r = 0.773$, $n = 8$, $P = 0.025$). There were not enough significant linear regression coefficients to similarly restrict the analysis of linear terms. Preference function shape appeared to be unrelated to responsiveness; neither linear nor quadratic regression coefficients covaried with response number or response effort (Pearson correlations: all $P > 0.140$). Linear coefficients ranged from $-4.31$ to $2.32$, and quadratic coefficients ranged from $-0.019$ to $0.042$ (Figure 6).

Average preference functions

Average preference functions across all individuals constructed using a cubic spline and quadratic regression are shown in Figure 7. The function constructed from response numbers ($R_{N}$) indicates a open-ended linear function favoring songs with a greater proportion of trill-like long chirp, whereas the function constructed using response effort ($R_{E}$) is stabilizing and favors intermediate male trait values.

DISCUSSION

Highly influential Fisherian models of sexual selection have identified female choosiness as a key factor influencing the strength of sexual selection and, ultimately, the potential for male secondary sexual trait elaboration (Lande 1981; Arnold 1983). Choosiness, that is, responsiveness and discrimination, reflects female investment in mate choice and can be affected by the direct costs of female behavior (Parker 1983; Real 1990). In addition, variation in the quality of available mates, the quality of the choosing female, her decision strategy (e.g., best-of-n or threshold), external environmental conditions, and the female’s sensitivity to differences in male traits may all affect how she invests in mate choice (Janetos 1980; Gibson and Langen 1996; Widemo and Sæther 1999). Females may adjust responsiveness and discrimination in a number of different ways. Jennions and Petrie (1997) suggested that females control their investment in mate choice by altering the number of mates sampled and by varying the amount of time spent with each mate. More discriminating females are expected to sample a greater number of potential mates and/or expend more effort evaluating them. Here, more discriminating *T. oceanicus* females exhibited a greater number of positive responses to playbacks, but I found little support for the idea that they also expended greater response effort during phonotaxis.

Differences in discrimination imply variation in the motivation or efficiency of females to evaluate less-preferred mates;
for example, more discriminating females may show a greater response to preferred males if an underlying physiological mechanism affords them increased sensitivity to male trait differences or a broader number of traits to evaluate. In the sea-weed fly *Cloeota frigida*, for example, discrimination and responsiveness were shown to be pleiotropically linked effects (Gilburn and Day 1999), and more responsive females have been shown in several species to exhibit stronger mating preferences (Brooks and Endler 2001; McGlothlin et al. 2004; Ritchie et al. 2005; though see Gray 1999). However, the behavioral linkage between responsiveness and discrimination in *T. oceanicus* only applies to the number of positive responses a female makes and not her response effort. It is plausible, therefore, that *T. oceanicus* females that are more discriminating mediate their investment in mate choice by sampling a greater number of males.

In addition to responding more frequently, highly discriminating *T. oceanicus* females tended to have stabilizing—as opposed to linear or disruptive—preference functions. Combined with the variation between individual preference functions uncovered in this study, this poses a question of unitary importance in the study of sexual selection in natural populations: to what extent does individual preference variation influence the direction and strength of sexual selection? If responsiveness or discrimination is associated with certain preference function shapes, then variation in individual preferences might be an important factor influencing the overall direction of selection on males (Brooks and Endler 2001). For example, the more discriminating females in a population might contribute more to the overall direction of sexual selection on males if they consistently exert a particular preference, whereas indiscriminate females do not. Similarly, responsiveness may influence the strength and direction of selection if preference functions of the most responsive females have a characteristic shape. The direction of selection on male traits in other species has been suggested to depend on the preferences of the most responsive females, for example, in the cricket *Gryllus integer* (Hedrick and Weber 1998), the guppy *Poecilia reticulata* (Brooks and Endler 2001), and the fruit fly *Drosophila montana* (Ritchie et al. 2005). In *T. oceanicus*, the greater response numbers (RN) of discriminating females, plus their tendency to have stabilizing preference functions, suggest that the direction of sexual selection on the song parameter I examined is influenced in parallel by females’ inclination to initiate a phonotactic response and by the discrimination that they then exercise.

The shape of population-wide average preference functions for the females studied here depended on whether response number or response effort was used as a proxy for the probability of mating. Clearly, both a female’s willingness to respond to a calling male and her subsequent response effort will contribute to the likelihood that she eventually mates with him. The proportion of long chirp in male *T. oceanicus* calling song has been shown to be important in mate choice (Simmons et al. 2001). The long-chirp element consists of pulses that are greater in amplitude than those of the short chirp, so the greater the proportion of long chirp in a *T. oceanicus* song the greater intensity a female perceives. Greater signal intensity increases the likelihood of female responses (Gerhardt 1994; Hedwig and Poulet 2005), so songs containing more long chirp may be more likely to stimulate females to initiate phonotaxis. Once an initial response threshold is crossed, however, response effort (speed and accuracy) may be mediated by factors other than simply the intensity of the signal, such as the relative proportions of the song components that were varied in this study. The ultimate direction of sexual selection on song therefore depends not only on females’ ability to perceive and integrate multiple simultaneous signals (Hebets and Papaj 2005; Castellano and Rosso 2007) but also on the interaction between signals and the different components of female choice.

Female field cricket responses to male song appear to be multimodal. Variation in one male signal parameter had differing effects on female response number versus response effort, accounting for the 2 distinct preference functions uncovered in *T. oceanicus*. Under this model, the proportion of long chirp in male *T. oceanicus* song mediates how likely a female is to respond phonotactically in the first place and also influences the strength of her subsequent response effort. Intriguingly, these effects do not appear to be synergistic because response number was uncorrelated with average response effort. Knowledge of the underlying neurophysiological mechanisms of cricket phonotaxis supports the idea that the same external cue can release different behavioral outcomes and suggests a proximate explanation for the contrasting preference function shapes. It is well known that a single song pattern may initiate entirely different context-dependent behaviors, for example, male calling song in *T. oceanicus* is sufficient to induce both female phonotaxis and female mounting (Balakrishnan and Pollak 1996). Additionally, Hedwig and Poulet (2004) recently found that *Gryllus bimaculatus* females initiated a rapid turning response toward a single sound pulse before their central nervous systems had time to process and evaluate subsequent pulses involved in pattern recognition. Initial turning responses occurred on a fast timescale and were strongly mediated by signal intensity, whereas pattern recognition and evaluation took place over a slower timescale and were initiated after phonotaxis was well underway (Hedwig and Poulet 2005). This is consistent with the idea that the likelihood a *T. oceanicus* female will respond to a given song and her subsequent response effort during phonotaxis are regulated by separate physiological processes, both of which are differentially influenced by variation in a single song parameter.

The preference functions constructed from each type of *T. oceanicus* response data suggest contrasting directions of sexual selection on males. However, the ultimate direction of selection will clearly depend on the balance between initiating a phonotactic response and the effort or speed with which phonotactic responses are made. The importance of each is expected to be context and condition dependent. For example, female choice is affected by predation risk (Hedrick and Dill 1993) as well as background noise (Schwartz et al. 2001), condition (Cotton et al. 2006), and prior experience (Wagner et al. 2001). Population density is also expected to have a large impact on the extent to which different components of female choice predominate mating decisions. Females may use more finely tuned mechanisms of discrimination when an abundance of males are available to choose from, but they lack the opportunity to employ these mechanisms of mate choice when signaling males are rare (Lehmann 2007). Theoretical arguments also suggest that the intensity of sexual selection may be highest at high population densities but that alterations in female behavior or search strategy might mitigate density-driven effects (Kokko and Mappes 2005; Kokko and Rankin 2006). Such alterations could arise through interactions between the components of female choice. My results provide evidence that such interactions play an important role in shaping both the strength and the direction of sexual selection, especially when organisms must balance the drive to mate with competing exogenous pressures such as predation risk or population density. Investigating both the mechanistic bases and evolutionary outcomes of the interacting components of female choice would provide further insight into the complex factors shaping sexual selection pressure in the wild.
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