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Complex Multivariate Sexual Selection on Male Acoustic Signaling in a Wild Population of *Teleogryllus commodus*

Caroline L. Bentsen,[1,*] John Hunt,[1,2,†] Michael D. Jennions,[3,‡] and Robert Brooks[1,§]

1. School of Biological, Earth and Environmental Sciences, The University of New South Wales, Sydney, New South Wales 2052, Australia;
2. Centre for Ecology and Conservation, University of Exeter in Cornwall, Tremough Campus, Penryn TR10 9EZ, United Kingdom;
3. School of Botany and Zoology, Australian National University, Canberra, Australian Capital Territory 0200, Australia

ABSTRACT: Mate choice may impose both linear (i.e., directional) and nonlinear (i.e., quadratic and correlational) sexual selection on advertisement traits. Traditionally, mate recognition and sensory tuning have been thought to impose stabilizing (i.e., negative quadratic) sexual selection, whereas adaptive mate choice effects directional selection. It has been suggested that adaptive choice may exert positive quadratic and/or correlational sexual selection. Earlier, we showed that five structural components of the advertisement call of male field crickets (*Teleogryllus commodus*) were under multivariate stabilizing selection under laboratory conditions. Here we experimentally estimate selection on these five traits plus a measure of calling activity (the number of repeats in a looped bout of calling) in the field. There was general support for multivariate stabilizing selection on call structure, and calling activity was under strong positive directional selection, as predicted for a signal of genetic quality. There was, however, also appreciable correlational selection, suggesting an interaction between male call structure and calling effort. Interestingly, selection for short interbout durations of silence favored longer intercall durations in the field, in contrast to results from continuous looped call playback in the laboratory. We discuss the general im-

* Present address: AustralAsian Resource Consultants, Suite 5b, 1 Swann Road, Taringa, Queensland 4068, Australia; e-mail: cbentsen@aarc.net.au.

† E-mail: j.hunt@exeter.ac.uk.

‡ E-mail: michael.jennions@anu.edu.au.

§ Corresponding author; e-mail: rob.brooks@unsw.edu.au.

portance of nonlinear selection in the honest signaling of genetic quality.

How mating signals evolve by sexual selection depends on both the shape of the female preference function and its relationship to the underlying phenotypic distribution of the male signal (Ryan and Rand 1993*a*, 2003; Ritchie 1996; Greenfield 2002). Open-ended preference functions will always exert directional selection on male signals (Gerhardt 1991; Ryan and Keddy-Hector 1992; Ryan and Rand 1993*a*; Greenfield 2002), although this is not necessarily linear in form. Preference functions that peak at specific values of the male signal (i.e., unimodal preference functions sensu Shaw and Herlihy 2000) may exert stabilizing and/or directional sexual selection on the signal. When the peak of the female preference function coincides with the mean value of the male signal in the population, sexual selection will be stabilizing (Gerhardt 1991; Ryan and Rand 1993*a*; Greenfield 2002). By contrast, when the peak differs from the population mean but is within the range of male signals expressed in the population, selection will have both directional and stabilizing components (Ryan and Keddy-Hector 1992). Finally, if the preference peak is outside the phenotypic distribution of the male signal, selection will always be directional (Ryan and Rand 1993*a*).

Biologically, the shape of the preference function is determined by the capabilities of the female's sensory organs and nervous system, which are also the products of selection. Unimodal preference functions arise when the receivers' sensory organs are best tuned to detecting signals within a particular range (e.g., auditory tuning frequency in the cricket frog; Ryan and Wilczynski 1988; Ryan et al. 1992) or when filters in the nervous system ensure that only phenotypes in a restricted range are recognized as potential signals (Stumpner and von Helversen 2001). The recognition of appropriate mates (Paterson 1982, 1985;

Butlin et al. 1985; Ryan and Rand 1993b; Hennig and Weber 1997), the transmission properties of the environment (Bennet-Clarke 1970; Patten et al. 2004), and natural selection operating in contexts other than mate choice (Endler and Basolo 1998) have all been shown to bias female sensory capabilities, leading to greater responses to stimuli with a limited range of values.

By contrast, open-ended preference functions commonly exist for signals with greater energetic content (reviewed by Ryan and Keddy-Hector [1992]). In acoustic species, greater signal energy is typically manifested as greater call intensity, higher repeat rate, or longer call duration (Klump and Gerhardt 1987; Gerhardt 1991; Greenfield 2002). At a proximate level, such signals are known to provide a greater stimulus to both the receptor organs and the nerve filters that process the signal (Endler and Basolo 1998). It has been argued that such signals reliably convey information about the signaler's phenotypic or genetic quality because of the energetic costs of increased signal production (Klump and Gerhardt 1987; Ryan and Keddy-Hector 1992; Welch et al. 1998; Gerhardt and Huber 2002; Brandt and Greenfield 2004; Greenfield and Rodriguez 2004; Hunt et al. 2004a). Although honest-signaling theory has predominantly focused on the relative costs of signaling by high- and low-quality males (Zahavi 1975; Grafen 1990; Johnstone 1995), differential benefits may also play a crucial role in handicap signaling (Getty 1998a, 1998b, 2002). In order for signals to honestly advertise genetic quality, the net benefits of a given level of investment in a signal must be greater for males of high quality than they are for males of low quality. Such a relationship could arise if the open-ended preference function was not linear but had a concave (positive quadratic) curvature. Indeed, many theoretical treatments of sexual selection model mating success as an exponentially increasing function of signal expression (e.g., Kokko 2001; Kokko et al. 2002).

We know a great deal about stabilizing and directional selection on acoustic mating signals from phonotaxis trials in which females can respond to experimentally manipulated signals (Klump and Gerhardt 1987; Gerhardt 1991; Ryan and Rand 1993b; Polakow et al. 1995; Ritchie 1996; Murphy and Gerhardt 2000; Shaw and Herlihy 2000; Simmons et al. 2001; Klappert and Reinhold 2003). The majority of studies have, however, considered only the effect of manipulating one call character at a time. In reality, selection seldom operates on a single trait independently of other traits, and combinations of traits could have effects on individual fitness that cannot be predicted from consideration of the effect of varying a single trait in an experimental study (Lande and Arnold 1983). Indeed, nonlinear selection analysis (Lande and Arnold 1983; Phillips and Arnold 1989) has formally shown that combi-

nations of traits can have multiplicative effects on fitness via the action of correlational selection (e.g., Brodie 1992; Blows et al. 2003; LeBas et al. 2003). The resulting pattern of selection operating on a suite of traits can thus be complex (e.g., Blows et al. 2003; Blais et al. 2004; McGlothlin et al. 2005) and impossible to predict from univariate analyses alone. Interestingly, correlational selection, in which two or more signal components influence attractiveness multiplicatively, has been invoked as a possible cause of directional, concave sexual selection (LeBas et al. 2003; McGlothlin et al. 2005).

In the native Australian black field cricket *Teleogryllus commodus*, males broadcast an advertisement call from a simple burrow structure to attract a mate (Loher and Rence 1978; Evans 1988). The advertisement call begins with a single chirp sequence followed by a variable number of trill sequences that have a higher pulse repetition rate (fig. 1; Bentley and Hoy 1972; Hill et al. 1972). Previously, we used two-choice, laboratory-based phonotaxis trials where we simultaneously, yet independently, manipulated five male call properties (see fig. 1; the five are dominant frequency, chirp pulse number, the duration of the interval between the chirps, trill number, and the interval between call phrases; Brooks et al. 2005) and showed that females exert multivariate stabilizing selection on these structural call characters. In the field, however, males call in bouts that may last from a few minutes to more than half a night, and so they may vary greatly in their "time on air." Even though we know that the total amount of time per night that a male spends broadcasting his call (hereafter termed "calling effort") is condition dependent and imposes a large cost to male survival in *T. commodus* (Hunt et al. 2004a), we still know very little about how selection shapes this form of male advertisement and how, or
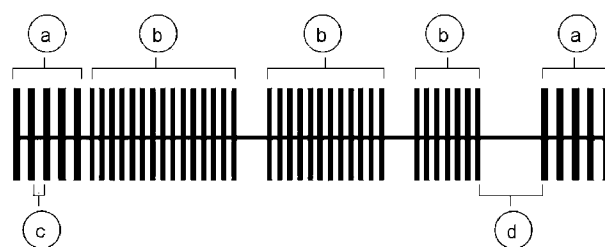
Figure 1: Schematic representation of the advertisement call of male *Teleogryllus commodus*. Each call consists of a chirp (*a*) followed by a series of one or more trills (*b*). We manipulated the number of pulses in the chirp, the duration of the interval between pulses in the chirp (*c*), the intercall duration (*d*), the dominant frequency of the call (not shown), and the number of times a call was repeated in a 5-min continuous loop. All other aspects of the call were kept constant. See table 1 for the mean values used.

whether, variation in calling effort affects selection on structural components of the call.

Here we use an experimental field study to estimate linear and nonlinear selection on the same five characters (hereafter collectively referred to as the "structural call characters"), plus a measure of calling effort (number of calls per 5 min). We wished to test whether the five structural characters manipulated in our laboratory study were under a similar pattern of multivariate stabilizing selection in the wild. Close-range (<1.5 m) two-choice phonotaxis experiments in the laboratory might provide estimates of selection of limited relevance to the field for several reasons, including the degradation of calls with certain structural properties over greater distances and in less controlled environmental conditions (Greenfield 2002) and the presence of potential predators (Bailey and Haythornthwaite 1998) and parasitoids (Cade 1975; Zuk and Kolluru 1998; Gray and Cade 1999). Further, we wished to test whether calling effort is under strong directional selection, as would be expected for a costly signal of genetic quality. Last, we wished to test whether selection operates largely independently on call structure and call effort or there is any correlational selection.

Methods

Call Construction

To estimate linear and nonlinear selection on male call properties, we conducted a large-scale field experiment in which we measured the number of wild female *Teleogryllus commodus* attracted to artificially constructed male calls. Many aspects of our current study are based on our previous laboratory-based phonotaxis study (Brooks et al. 2005), and we direct the reader to that study for a more detailed description.

Briefly, we constructed artificial calls that varied independently in the following five structural characters: chirp pulse number, trill number, intercall duration, chirp interpulse duration (see fig. 1), and dominant frequency. We calculated the natural distribution of these five structural characters from a random sample of field-caught males (see table 1; Brooks et al. 2005, p. 873). We then constructed 300 unique calls with values for each parameter selected independently from one another, each drawn at random from the univariate natural distribution of that parameter (these are the 300 calls constructed by Brooks et al. [2005]; see their article for further details on call distributions, sampling, and construction). Each unique call was then assigned to a 5-min playback loop with a randomly assigned number of call repeats (RN). To achieve this, we assigned each unique call a random number from a normal distribution of values between 1 and 50 and

Table 1: Call property values of the six manipulated call properties

Properties manipulated	Abbreviation	SD	Mean
Chirp pulse number	CPN	.82	6.42
Trill number	TN	1.12	2.78
Intercall duration (s)	ICD	435.37	501.52
Chirp interpulse duration (s)	CIPD	3.48	28.16
Dominant frequency (kHz)	DF	.14	3.83
Repeat number	RN	13.73	26.50

constructed a 5-min loop using this number of repeats of the call. Consequently, loops with calls that were longer in duration and that contained a greater number of calls had a longer duration of sound (i.e., bout length) and a shorter interval of silence between the end of the last call in the loop and the first call in the next playback of the loop (i.e., shorter interbout intervals). There were, therefore, no correlations among manipulated characters in our artificial call loops, and our selection analysis explores the full range of phenotypic combinations of call parameters, including those that may be biophysically impossible to generate, by removing any natural correlations that may exist between these traits (see also Brooks et al. 2005).

Playback Experiment

We conducted our field playback experiment at Smith's Lake Field Station, situated near Forster, New South Wales, Australia (32°22′S, 152°30′E), between March 1 and March 30, 2004. This corresponds with the peak breeding season of *T. commodus* (Otte and Alexander 1983).

We constructed a circular arena (figs. A1–A3) 20.4 m in diameter on a level patch of maintained pasture. We placed 13 playback units at regular 4.88-m intervals around the circumference. Each playback unit comprised a pair of 9-V speakers (placed back to back, one facing directly toward the center of the circle and one facing directly away from it), a CD player (Magnavox MPCD-12), and a tent cover over the top for weather protection (see appendix). The speakers were placed on a 60 × 60-cm sticky trap that consisted of a piece of Corflute board covered in Tangletrap insect trap coating, secured to the ground at each corner using tent pegs. The CD players and speakers were connected to the main power supply via cords that ran around the outside of the arena. Call playback intensity was calibrated daily to 80 dB (root mean square) at 0.8 m from each speaker using a Radio Shack (Fort Worth, TX) SPL meter.

Each night we ran a trial of the experiment from 9:00 p.m. to 5:30 a.m. One pair of speakers was a silent control in which the acoustic equipment was set up and powered

yet no call was played. A second pair of speakers played a control call, representing the mean call properties for this population (see table 1). Each of the remaining 11 pairs of speakers continuously played one of the 300 unique call loops. The 11 unique call loops, the control loop, and the silent control were assigned at random to playback stations in the arena each night before the trial commenced. The 11 unique call loops used each night were also drawn at random from the 300 constructed calls (of which only 275 were used).

Each night, we collected 50 females from the nearby (5 km away) Bungwahl graveyard and released them under cardboard egg cartons at the center of the arena immediately before the start of each trial. To ensure that females attracted to calls did not escape the sticky trap and/or were not consumed by predators while on the trap, we marked and monitored any captured females hourly between 10:00 p.m. and 1:00 a.m. and then at 5:00 and 5:30 a.m., after which the units were turned off. We used the total number of females caught on a sticky trap as our measure of the attractiveness of the call loop played from the corresponding speakers. The purpose of the two controls was to measure the background rate at which females were trapped in a silent control. The selection estimates do not differ substantially with the two controls included in the analyses, and we therefore omit them to avoid possible pseudoreplication.

Estimating Linear and Nonlinear Selection

We used standard multiple-regression-based linear and nonlinear selection analysis (Lande and Arnold 1983) to estimate gradients of linear and quadratic selection on the six traits and correlational selection on pairs of traits. We followed the usual practice of standardizing each trait to have zero mean and unit variance and dividing our response variable (number of females per call loop) by the mean across all samples to provide a measure of relative fitness (Lande and Arnold 1983). We then built separate multiple-regression models to estimate the vector of linear selection gradients, β, and the matrix of nonlinear selection gradients, γ, as described by Lande and Arnold (1983).

It can be difficult to obtain a full appreciation for the strength and significance of nonlinear selection by interpreting the size and sign of γ-coefficients individually (Phillips and Arnold 1989; Blows and Brooks 2003). We therefore performed a canonical rotation of the γ matrix to find the major axes of the response surface (Phillips and Arnold 1989; see method in app. 1 of Blows and Brooks 2003). This results in a matrix, M, comprising i eigenvectors, m_i, each describing a major axis of the response surface (where i is the original number of traits). The strength of nonlinear selection along each eigenvector

is given by its eigenvalue, λ_i. The strength of linear selection (θ_i) along each eigenvector and the significance of both linear and nonlinear selection along each eigenvector were obtained by including linear and quadratic forms of all eigenvectors in a new multiple-regression model (i.e., the double–linear regression [DLR] method described by Bisgaard and Ankenman [1996]). As our response variable was not normally distributed, we used randomization testing (as suggested by Mitchell-Olds and Shaw [1987]) to assess the significance of estimated selection gradients in both the original linear and nonlinear regressions and the second regression (DLR). The statistical methodology is outlined and applied in greater detail in Brooks et al. (2005).

Visualizing the Fitness Surface

We used nonparametric approaches to visualizing the fitness surface because they provide a less constrained view than the best quadratic approximation (Schluter 1988; Schluter and Nychka 1994). To view the fitness surface along a single axis, we used Schluter's GLMS 4.0 (available at http://www.zoology.ubc.ca/~schluter/splines.html) to fit a cubic spline with bootstrapped standard errors (Schluter 1988). To view the fitness surface along pairs of axes, we fitted thin-plate splines (Green and Silverman 1994), using the Tsp function in the *fields* package in R (available at http://www.r-project.org).

Results

Over 25 nights, we caught six females in the silent control trap (0.24 females trap-night^{-1}), 18 females in the trap at the standard control call (0.72 females trap-night^{-1}), and 192 females in the traps at the unique call loops (0.77 females trap-night^{-1}). There was significant linear selection favoring greater numbers of call repeats in the 5-min loop (table 2). Nonlinear selection analysis revealed significant negative quadratic selection on trill number, positive quadratic selection on chirp interpulse duration, and correlational selection between intercall duration and both dominant frequency and number of call repeats (table 2).

Canonical rotation of the γ matrix of nonlinear selection gradients resulted in three eigenvectors with positive eigenvalues (m_1–m_3) and three with negative eigenvalues (m_4–m_6; table 3). There was significant stabilizing selection along three of the four major axes of nonlinear selection, m_4–m_6, as illustrated in figure 2. The axis of strongest stabilizing selection, m_6, was heavily affected by intercall duration and dominant frequency. The remaining two axes of significant nonlinear selection are heavily weighted by a single structural character, chirp pulse number in the

Table 2: The vector of standardized linear selection gradients (β) and the matrix of standardized quadratic and correlational selection gradients (γ)

| Property | β | γ | | | | | |
		CPN	TN	ICD	CIPD	DF	RN
CPN	$-.037 \pm .094$	$-.122 \pm .068$					
TN	$.020 \pm .093$	$-.064 \pm .107$	$-.121 \pm .062^*$				
ICD	$.143 \pm .093$	$-.070 \pm .124$	$.008 \pm .101$	$-.081 \pm .046$			
CIPD	$.016 \pm .092$	$.110 \pm .086$	$-.021 \pm .090$	$-.161 \pm .109$	$.117 \pm .055^*$		
DF	$-.171 \pm .093$	$.154 \pm .090$	$-.058 \pm .094$	$-.349 \pm .086^{***}$	$.161 \pm .092$	$-.011 \pm .068$	
RN	$.385 \pm .093^{**}$	$.089 \pm .087$	$-.102 \pm .094$	$-.239 \pm .118^*$	$-.092 \pm .091$	$-.145 \pm .090$	$.071 \pm .100$

Note: CPN = chirp pulse number; TN = trill number; ICD = intercall duration; CIPD = chirp interpulse duration; DF = dominant frequency; RN = repeat number. Gradients are presented $\pm$ standard error.

* Randomization test $P < .05$.

** Randomization test $P < .01$.

*** Randomization test $P < .001$.

case of m_5 and trill number in the case of m_4, and by minor contributions from the other call attributes.

The axis of strongest linear and nonlinear selection was m_1 (table 3; fig. 3), which was heavily loaded by calling effort (repeat number) and three structural characters of the male call: intercall duration, chirp interpulse duration, and dominant frequency. Selection strongly favored low values of m_1, which would involve lower dominant frequencies, greater calling effort, longer intercall durations, and shorter interpulse durations in the chirp. The fitness function appears to flatten out at the lower boundary of values tested, and there is some indication that very high values are favored over average values (fig. 3). In figure 4, we present the fitness surface described by the two major axes of nonlinear selection, m_1 and m_6, which illustrates both stabilizing selection favoring intermediate values of m_6 and the extreme directional and concave-up selection favoring low values of m_1.

There was also significant directional selection toward high values of m_2, which are associated with greater calling effort, longer chirp interpulse duration, and more pulses per chirp (table 3). This provides further evidence for directional selection on both the duration of the chirp and the number of repeat calls in a looped bout. Intriguingly, however, fitness is not maximized at low values of m_1 and high values of m_2. Rather, there appears to be strong disruptive selection (fig. 5), with a peak at low values of m_1 and intermediate values of m_2 and another peak at high values of both m_1 and m_2. The presence and size of this second peak must be interpreted with caution, however, because it is based on only three data points (fig. 5b). This second peak arises when there is greater calling effort and longer chirps because of more pulses per chirp and longer gaps between pulses.

Discussion

Our analyses indicate that both linear and nonlinear selection are significant on the manipulated male call characters in the field. As predicted by pairwise choice trials in the laboratory (Brooks et al. 2005), there was strong stabilizing selection on structural call characters. Furthermore, our prediction that calling effort (the number of repeat calls in a looped bout) would be under strong di-

Table 3: The matrix **M** of eigenvectors from the canonical rotation of γ

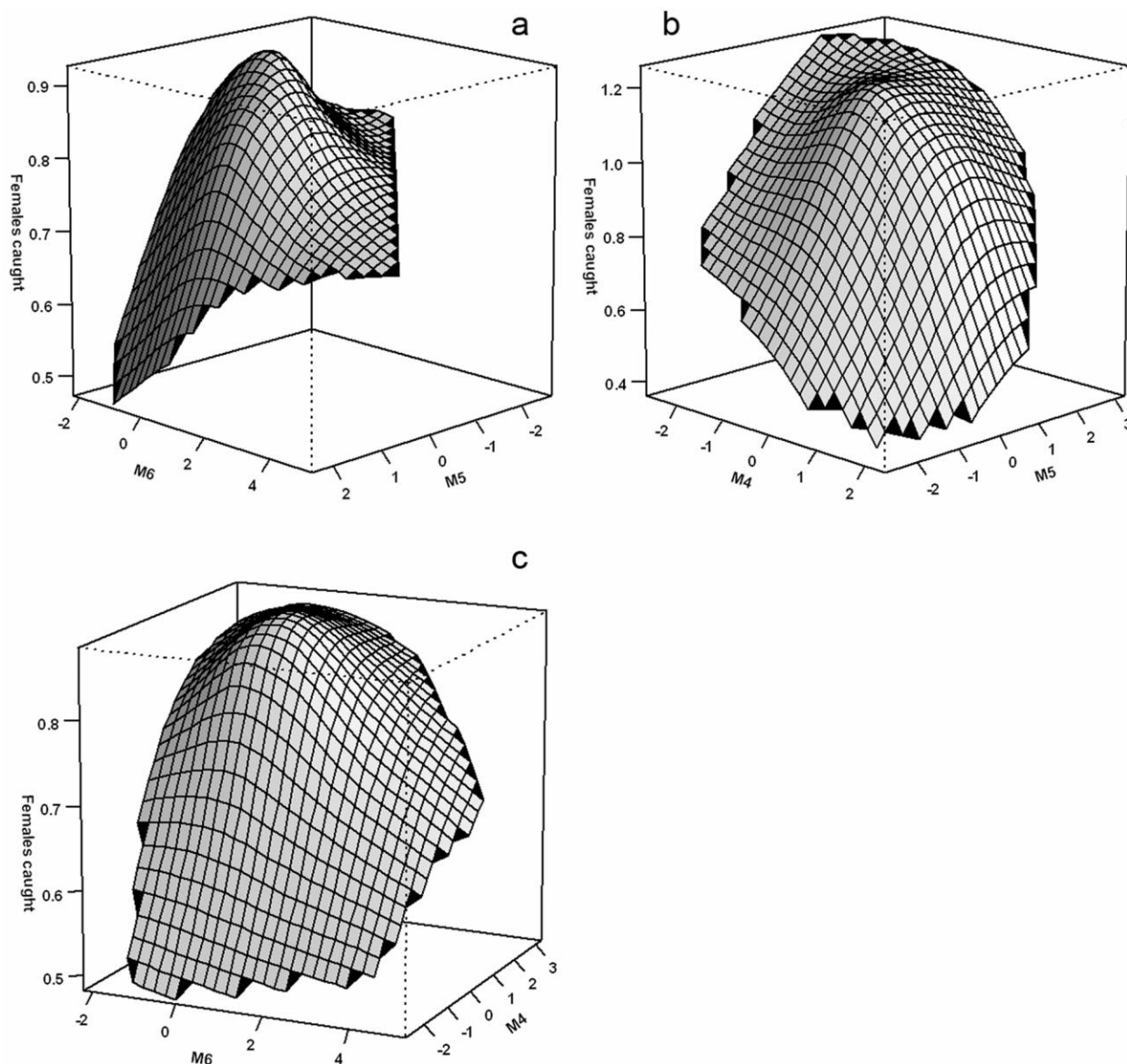| | **M** | | | | | | Selection | |
Eigenvector	CPN	TN	ICD	CIPD	DF	RN	θ	λ
m_1	.15	$-.01$	$-.48$	.53	.51	$-.45$	$-.456^{***}$	$.320^{***}$
m_2	.34	$-.26$	.11	.54	.06	.71	$.221^*$	.088
m_3	.28	$-.26$	$-.28$	$-.64$	.54	.25	$-.061$	.004
m_4	.18	.93	$-.08$	$-.02$	.15	.27	.160	$-.147^{**}$
m_5	.86	.00	.27	$-.08$	$-.24$	$-.36$	$-.116$	$-.178^{**}$
m_6	$-.13$	.03	.78	.05	.60	$-.13$	.118	$-.234^{***}$

Note: The linear (θ_i) and quadratic (λ_i, the eigenvalue) gradients of selection along each eigenvector (m_i) are given in the last two columns. CPN = chirp pulse number; TN = trill number; ICD = intercall duration; CIPD = chirp interpulse duration; DF = dominant frequency; RN = repeat number.

* Randomization test $P < .05$.

** Randomization test $P < .01$.

*** Randomization test $P < .001$.

Figure 2: Thin-plate spline visualizations of the fitness surfaces, which demonstrate significant convex stabilizing selection between $\mathbf{m}_5$ and $\mathbf{m}_6$ (*a*), $\mathbf{m}_4$ and $\mathbf{m}_5$ (*b*), and $\mathbf{m}_4$ and $\mathbf{m}_6$ (*c*).

rectional sexual selection was upheld. Interestingly, however, there were also strong interactions between structural characters and calling effort. This results in a complex pattern of multivariate sexual selection on male call properties, a finding that is becoming increasingly apparent for sexual signals of a variety of other animal taxa (*Ceratitis capitata*, Rodriguero et al. 2002; *Poecilia reticulata*, Blows et al. 2003; *Rhamphomyia tarsata*, LeBas et al. 2003; *Drosophila serrata*, Blows et al. 2004; *Gasterosteaus aculeatus*, Blais et al. 2004; *Teleogryllus commodus*, Brooks et al. 2005; *Junco hyemalis*, McGlothlin et al. 2005). More generally,

our findings highlight the need to view sexual selection in multiple dimensions and to examine both linear and nonlinear forms of multivariate selection (Lande and Arnold 1983; Blows and Brooks 2003). While this approach has long been advocated (Lande and Arnold 1983; Arnold and Wade 1984), it is still considerably underutilized in studies of sexual selection.

Structural Characters and Stabilizing Selection

Our results indicate that in the wild, female *T. commodus* exert significant multivariate stabilizing selection on three
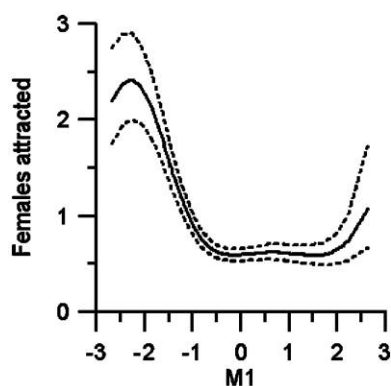
Figure 3: Cubic spline visualization of selection on m_1, the axis of strongest nonlinear and linear selection. Solid line shows the fitted spline; dotted lines represent bootstrapped upper and lower standard errors.

of the four major axes of nonlinear selection (m_4–m_6). These axes are heavily loaded toward four of the five structural call characters that we manipulated, trill number (m_4), chirp pulse number (m_5), intercall duration, and dominant frequency (m_6). This finding is consistent with our laboratory phonotaxis study (Brooks et al. 2005), in which the five structural characters that we manipulated were under multivariate stabilizing sexual selection. Further, the lack of evidence for stabilizing selection on chirp interpulse duration is consistent with the weak effect of this character on the major axes of the stabilizing selection surface identified in our laboratory study (table 3 of Brooks et al. 2005).

## Call Effort and Directional Selection

As predicted, there was strong directional selection on male calling effort, with females showing a strong preference for male calls with greater numbers of repeats. This finding is largely consistent with previous studies on field crickets (French and Cade 1989; Crnokrak and Roff 1995; Holzer et al. 2003) and frogs (Arak 1983; Ryan 1983; Klump and Gerhardt 1987; Passmore et al. 1992) that have demonstrated that call rate is a major determinant of male mating success. However, while call repeat number was the only trait for which we detected significant linear selection, it was not the only trait implicated in directional sexual selection. The major feature of the rotated fitness surface is the prominent and statistically well-supported peak at low values of m_1, which corresponds with high repeat numbers, long intercall durations, and low dominant frequencies. Long intercall durations and low dominant frequencies were also under strong but not significant linear selection, suggesting not only that m_1 is the major axis of nonlinear

selection but that it corresponds closely with the vector of linear selection gradients.

The secondary fitness peak at high values of both m_1 and m_2 also suggests strong selection favoring chirps containing large number of pulses and long interpulse intervals. The evidence that the number of pulses in the chirp and the duty cycle (proportion of the call in which sound is actually transmitted; Greenfield 2002) of the chirp may have directional effects on male attractiveness independent of bout length (i.e., repeat number) is consistent with evidence for the importance of the chirp component in the congeneric species *Teleogryllus oceanicus* (Hill et al. 1972; Simmons et al. 2001).

In our earlier univariate (Hunt et al. 2005) and multivariate (Brooks et al. 2005) laboratory experiments, female *T. commodus* preferred continuously looped calls with shorter intervals between calls (i.e., shorter intercall durations). We interpreted this as a preference for greater calling effort in much the same way as we have here for high repeat numbers. However, the results we present here demonstrate selection for longer intercall durations. The key difference between our earlier laboratory experiments and the current field-based study is that, in the laboratory study, the calls were on a continuous loop without any division into bouts of calling versus periods of silence. In contrast, in our current field study, calls were manipulated so that every 5 min there was one new bout of calling followed by silence. Consequently, both greater repeat numbers and longer intercall durations within each call extended the overall bout length and concomitantly shortened the interbout duration. It therefore appears that selection for shorter interbout durations overrides selection for shorter intercall durations within bouts. Such a finding would be expected if females prefer male calls with greater energy content or if it is easier for females to localize a male whose calls are spread over a longer time interval.

A point of difference between this study and our earlier univariate laboratory study (Hunt et al. 2005) is that Hunt et al. (2005) found that females preferred males with slightly higher than average call frequencies, whereas here we show that lower-frequency calls attract more females over larger spatial scales in the field. There are a number of differences between the two studies that may account for this discrepancy, one of which is the relationship between frequency and sound attenuation. Higher-frequency sounds typically attenuate more rapidly than lower-frequency sounds (Greenfield 2002). Therefore, even though call intensity was equal at 0.8 m from each speaker in our current study, beyond this range low-frequency calls are likely to have had a greater broadcast area and consequently reached and attracted more females. If this is indeed the case, it highlights the need for caution when interpreting female preferences measured in short-range
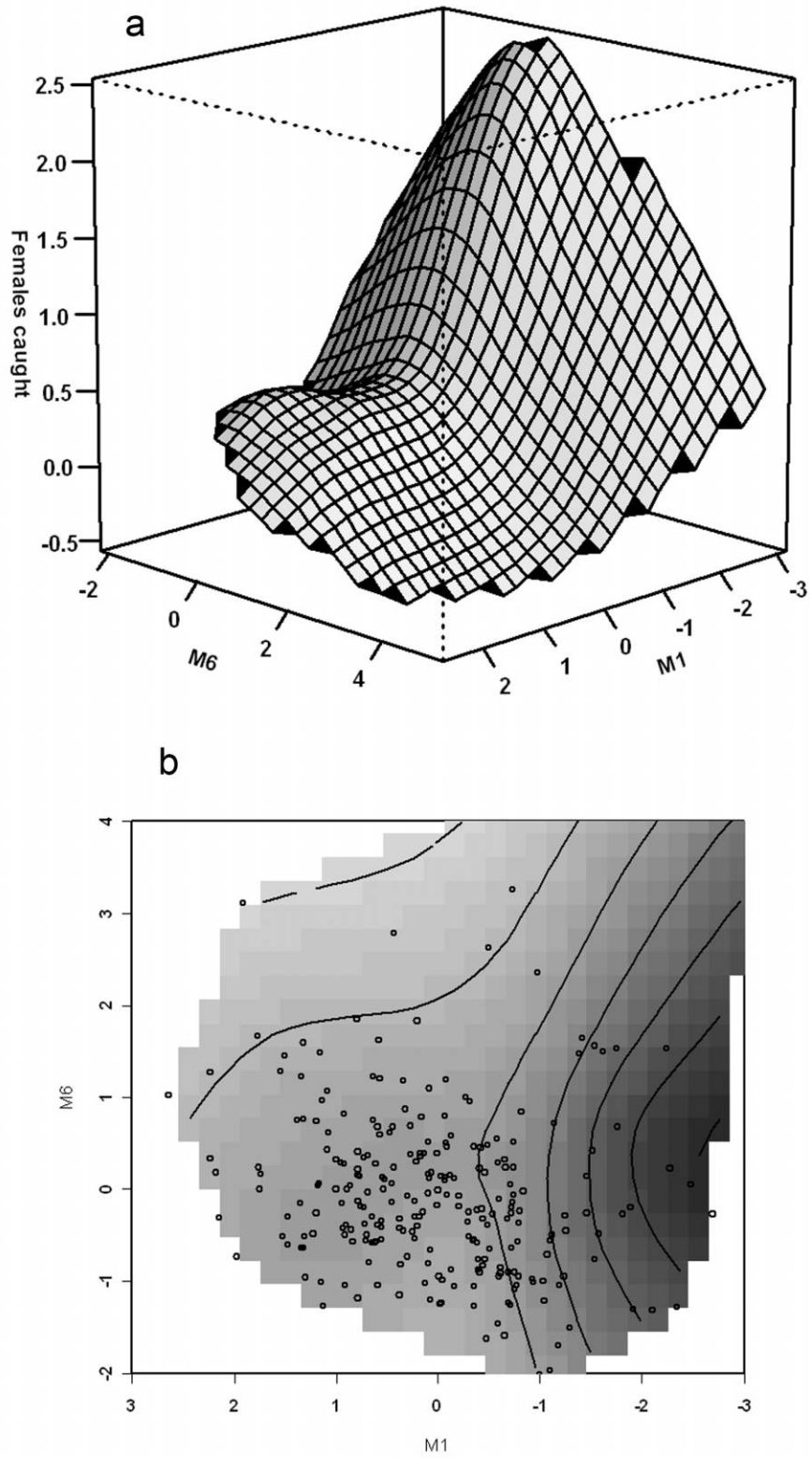
Figure 4: Thin-plate spline visualization of the fitness surface demonstrating the two major axes of selection, $\mathbf{m}_1$ and $\mathbf{m}_6$. *a*, Perspective view; *b*, shaded contour plot with supporting data points overlaid. Darker regions indicate higher fitness.

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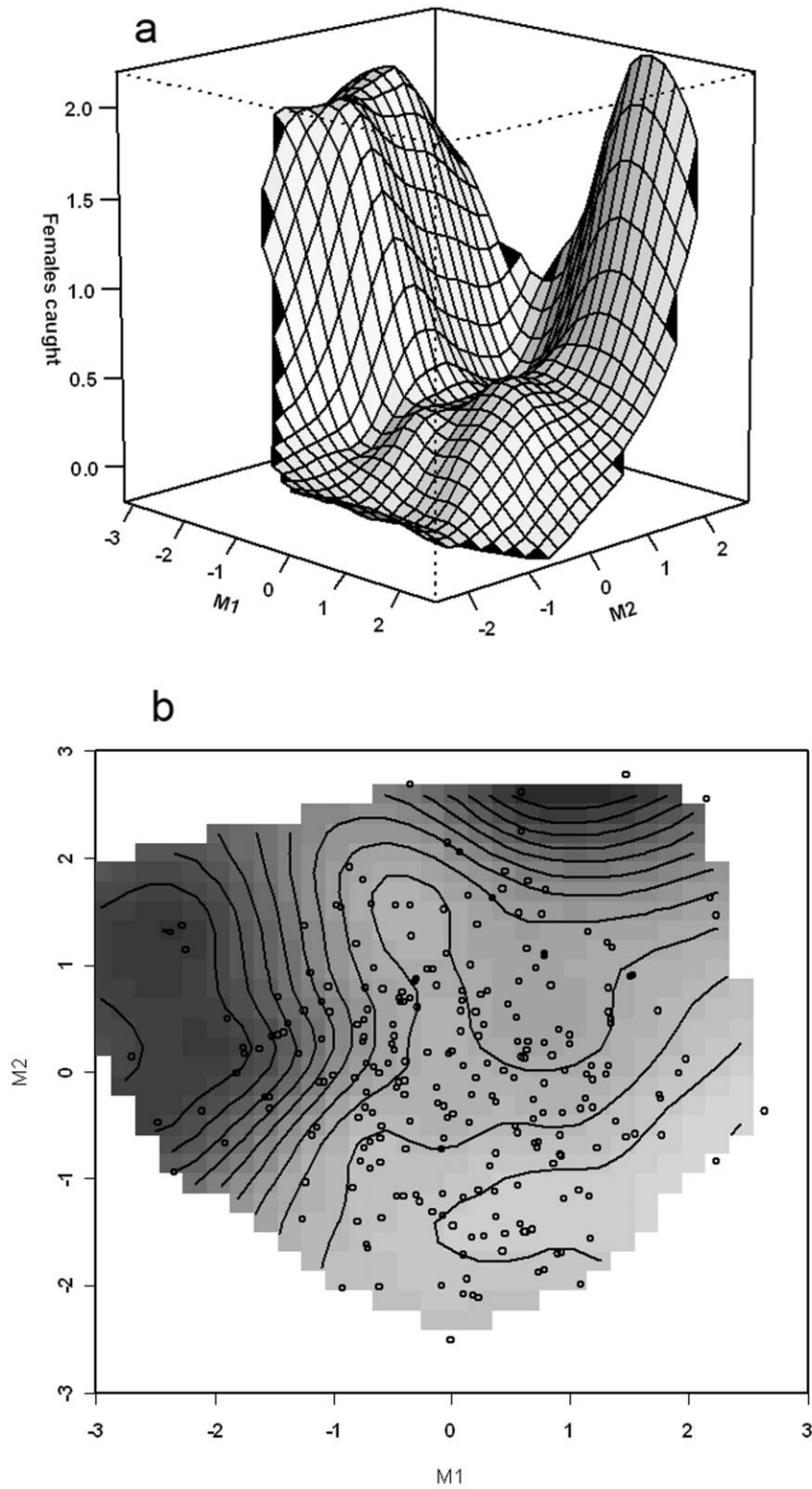
Figure 5: Thin-plate spline visualization of the fitness surface representing selection on $\mathbf{m}_1$ and $\mathbf{m}_2$. *a*, Perspective view; *b*, shaded contour plot with supporting data points overlaid. Darker regions indicate higher fitness.

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phonotaxis trials, particularly those that involve dominant frequency.

Interaction between Call Structure and Effort

Although we found evidence of stabilizing selection on call structure and directional selection on call effort, the most interesting feature of our results is the interaction between call structure and calling effort. This interaction is apparent from several strong correlational selection gradients in the γ matrix, involving all traits except trill number. Nowhere is this interaction more clear, however, than in the strong concave-up selection on the major axis of the fitness surface ($\mathbf{m}_1$). The substantial contributions of intercall duration, chirp interpulse duration, and dominant frequency to this axis indicate that in the wild, sexual selection is far more complex than the multivariate stabilizing pattern observed in the laboratory. Our findings suggest that laboratory-derived selection estimates based exclusively on call structure should be interpreted with caution when trying to understand how sexual selection operates on male signaling behavior.

In our recent laboratory study, we showed that the mean phenotype of Smith's Lake males closely matched the peak of the acoustic fitness surface for the five structural traits studied here (Brooks et al. 2005). This suggests that interactions between calling effort and call structure have not displaced the population mean phenotype from the most effective call structure. Whether the interaction between call structure and calling effort that we have documented here tends to displace the population away from the most acoustically effective signal or, conversely, whether it constrains the evolution of calling effort remains to be tested.

Honest Signaling of Genetic Quality

Call rate strongly influences the amount of time that a male spends calling in a given night. In a proximate sense, this provides females with a temporally larger "target" for passive attraction (sensu Parker 1983). From a more adaptive standpoint, females are expected to benefit from seeking out and mating with males that call for longer periods because such calling effort is expected to be condition dependent and thus more likely to signal genetic quality (Andersson 1982; Rowe and Houle 1996; Tomkins et al. 2004). Calling effort is consistently found to be subject to directional preference in univariate studies of acoustically signaling species (Klump and Gerhardt 1987; Ryan and Keddy-Hector 1992; Snedden and Sakaluk 1992; Crnokrak and Roff 1995; Welch et al. 1998; Castellano et al. 2000; Holzer et al. 2003; Klappert and Reinhold 2003; Hunt et al. 2005) and has been shown to be condition dependent

in wax moths (Jia et al. 2000; Greenfield and Rodriguez 2004), gray tree frogs (Welch 2003), and field crickets (Holzer et al. 2003). We have recently shown that the amount of time spent calling per night and the timing of this call effort during a male's adult lifetime are strongly condition dependent in *T. commodus* (Hunt et al. 2004*a*).

Signals that attract mates at a greater-than-linear rate per unit investment in signaling may be particularly likely to be honest signals of quality. Two recent studies, one on male mate choice for large males in the empidid dance fly *R. tarsata* (LeBas et al. 2003) and another on plumage in the dark-eyed junco *J. hyemalis* (McGlothlin et al. 2005), have demonstrated accelerating benefits of increased signaling and implicated correlational selection. Both studies argue that the measured signal traits are honest signals of male quality (LeBas et al. 2003; McGlothlin et al. 2005). Furthermore, analysis of lifetime lekking performance in black grouse *Tetrao tetrix* (Kokko et al. 1999) indicates that male mating success is disproportionately high among males with high lek attendance and high activity when on the lek. Although it is not presented in the form of standardized selection gradients, this result strongly resembles positive correlational selection (see fig. 2 of Kokko et al. 1999). Once again, the interaction between lek attendance and activity is thought to be an important feature underpinning signal honesty (Kokko et al. 1999).

In our study, selection on male calling effort and the interaction between calling effort and four out of five manipulated call structure characters generate a strong pattern of accelerating benefits of signaling (toward lower values of $\mathbf{m}_1$). Our results therefore uphold Getty's (1998*a*) prediction and, taken together with recent findings in species with very different mating systems (Kokko et al. 1999; LeBas et al. 2003; McGlothlin et al. 2005), suggest that accelerating benefits of signaling and correlational selection may be common features of honest signals.

More generally, correlational selection is expected to influence the evolution of the genetic covariance between traits by favoring linkage disequilibria and/or pleiotropy at the loci governing the traits (Arnold and Wade 1984; Brodie 1992; Blows et al. 2004; Blows and Hoffmann 2005). Strong and persistent correlational selection may also facilitate trait integration (Lande 1980; Cheverud et al. 1983; Cheverud 1984) and the common inheritance of functionally related traits as modular units (Schlosser and Wagner 2004). Quantitative genetic analyses, currently underway in our research group, of the six call traits that we have measured here will allow us to explore how a regime of strong correlational selection has shaped the genetic architecture of these traits. For multiple traits subject to complex selection, simply demonstrating that there is sufficient additive genetic variation in condition, or in the sexually selected trait itself, is insufficient evidence that

attractive signalers are of high genetic quality (Hunt et al. 2004b; Blows and Hoffmann 2005). Quantitative genetic analysis will allow us to determine a male's breeding value for the six call traits examined and, more important, whether there is sufficient multivariate genetic variation along the major axes of sexual selection for these call characters to evolve (Schluter 1996; Blows and Hoffmann 2005).

Conclusion

Here, we demonstrate that both linear and nonlinear selection play an important role in the evolution of male acoustic signals in *Teleogryllus commodus* under field conditions. Male calling effort is under strong linear selection, with a linear selection gradient ($\beta = 0.385$) that is well in excess of mean or median values reported for other traits in the published literature (median $|\beta| = 0.16$ [Kingsolver et al. 2001]; median $|\beta| = 0.15$ [Hoekstra et al. 2001]; mean $|\beta| = 0.28$ [Hereford et al. 2004]). Structural characters of male calls are associated strongly with the features of the fitness surface that are under stabilizing selection, lending support to findings that under laboratory conditions (Brooks et al. 2005), these traits are under multivariate stabilizing selection. However, there is also strong correlational selection due to complex interactions between call structure characters and calling effort. This adds complexity to the pattern of multivariate sexual selection acting on male acoustic signals. Our findings caution against using laboratory-derived selection estimates based exclusively on call structure characters that have been manipulated in a single dimension. Such studies dominate the literature in acoustic signaling and may underestimate the strength and complexity of selection operating on male acoustic signals.

Acknowledgments
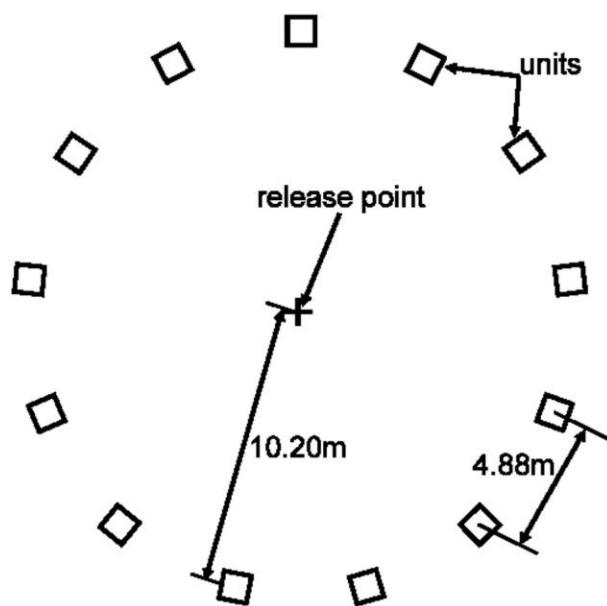
APPENDIX

Supplementary Figures

Figure A1: Schematic diagram of the design of the arena used for experimental phonotaxis trials at Smith's Lake Field Station.

Figure A2: Photograph of experimental arena at Smith's Lake Field Station, with all 13 playback units visible.

Figure A3: Photograph of a single playback unit from inside the circle.

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Literature Cited

Andersson, M. B. 1982. Sexual selection, natural selection and quality advertisement. Biological Journal of the Linnean Society 17:375–393.

Arak, A. 1983. Vocal interactions, call matching and territoriality in a Sri Lankan treefrog, *Philautus leucoirhinus*. Animal Behaviour 31:292–302.

Arnold, S. J., and M. J. Wade. 1984. On the measurement of natural and sexual selection: theory. Evolution 38:709–719.

Bailey, W. J., and S. Haythornthwaite. 1998. Risk of calling by the field cricket *Teleogryllus oceanicus*; potential predation by Australian long-eared bats. Journal of Zoology (London) 244:505–513.

Bennet-Clarke, H. C. 1970. The mechanism and efficiency of sound production in mole crickets. Journal of Experimental Biology 51:519–552.

Bentley, D. R., and R. Hoy. 1972. Genetic control of the neural network generating cricket (*Teleogryllus commodus*) song pattern. Animal Behaviour 20:478–492.

Bisgaard, S., and B. Ankenman. 1996. Standard errors for the eigenvalues in second-order response surface models. Technometrics 38:238–246.

Blais, J., C. Rico, and L. Bernatchez. 2004. Nonlinear effects of female mate choice in wild threespine sticklebacks. Evolution 58:2498–2510.

Blows, M. W., and R. Brooks. 2003. Measuring nonlinear selection. American Naturalist 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., R. Brooks, and P. G. Kraft. 2003. Exploring complex fitness surfaces: multiple ornamentation and polymorphism in male guppies. Evolution 57:1622–1630.

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. American Naturalist 163:329–340.

Brandt, L. S. E., and M. D. Greenfield. 2004. Condition-dependent traits and the capture of genetic variance in male advertisement song. Journal of Evolutionary Biology 17:821–828.

Brodie, E. D., III. 1992. Correlational selection for color pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. Evolution 46:1284–1298.

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.

Butlin, R. K., G. M. Hewitt, and S. F. Webb. 1985. Sexual selection for intermediate optimum in *Chorthippus brunneus* (Orthoptera: Acrididae). Animal Behaviour 33:1281–1292.

Cade, W. H. 1975. Acoustically orienting parasitoids: fly phonotaxis to cricket song. Science 190:1312–1313.

Castellano, S., A. Ross, F. Laoretti, S. Doglio, and C. Giacoma. 2000. Call intensity and female preferences in the European green toad. Ethology 106:1129–1141.

Cheverud, J. M. 1984. Quantitative genetics and developmental constraints on evolution by selection. Journal of Theoretical Biology 110:155–171.

Cheverud, J. M., J. J. Rutledge, and W. R. Atchley. 1983. Quantitative genetics of development: genetic correlations among age-specific trait values and the evolution of ontogeny. Evolution 37:895–905.

Crnokrak, P., and D. A. Roff. 1995. Fitness differences associated with calling behaviour in the two wing morphs of male sand crickets, *Gryllus firmus*. Animal Behaviour 50:1475–1481.

Endler, J. A., and A. L. Basolo. 1998. Sensory ecology, receiver biases and sexual selection. Trends in Ecology & Evolution 13:415–420.

Evans, A. R. 1988. Mating systems and reproductive strategies in three Australian gryllid crickets: *Bobilla victoriae* Otte, *Balamara gidya* Otte and *Teleogryllus commodus* Walker (Orthoptera: Gryllidae: Nemobiinae: Trigonidiinae: Gryllinae). Ethology 78:21–52.

French, B. W., and W. H. Cade. 1989. Sexual selection at varying population-densities in male field crickets, *Gryllus veletis* and *Gryllus pennsylvanicus*. Journal of Insect Behavior 2:105–121.

Gerhardt, H. C. 1991. Female mate choice in treefrogs: static and dynamic acoustic criteria. Animal Behaviour 42:615–635.

Gerhardt, H. C., and F. Huber. 2002. Acoustic communication in insects and anurans. Princeton University Press, Princeton, NJ.

Getty, T. 1998a. Handicap signalling: when fecundity and viability do not add up. Animal Behaviour 56:127–130.

———. 1998b. Reliable signalling need not be a handicap. Animal Behaviour 56:253–255.

———. 2002. Signaling health versus parasites. American Naturalist 159:363–371.

Grafen, A. 1990. Biological signals as handicaps. Journal of Theoretical Biology 144:517–546.

Gray, D. A., and W. H. Cade. 1999. Sex, death and genetic variation: natural and sexual selection on cricket song. Proceedings of the Royal Society of London B 266:707–709.

Green, P. J., and B. W. Silverman. 1994. Nonparametric regression and generalised linear models. Chapman & Hall, London.

Greenfield, M. D. 2002. Signalers and receivers: mechanisms and evolution of arthropod communication. Oxford University Press, Oxford.

Greenfield, M. D., and R. L. Rodriguez. 2004. Genotype-environment interactions and the reliability of mating signals. Animal Behaviour 68:1461–1468.

Hennig, R. M., and T. Weber. 1997. Filtering of temporal parameters of the calling song by cricket females of two closely related species: a behavioural analysis. Journal of Comparative Physiology A 180:621–630.

Hereford, J., T. F. Hansen, and D. Houle. 2004. Comparing strengths of directional selection: how strong is strong? Evolution 58:2133–2143.

Hill, K. G., J. J. Loftus-Hills, and D. F. Gartside. 1972. Pre-mating isolation between the Australian field crickets *Teleogryllus commodus* and *T. oceanicus* (Orthoptera: Gyllidae). Australian Journal of Zoology 20:153–163.

Hoekstra, H. E., J. M. Hoekstra, D. Berrigan, S. N. Vignieri, A. Hoang, C. E. Hill, P. Beerli, and J. G. Kingsolver. 2001. Strength and tempo of directional selection in the wild. Proceedings of the National Academy of Sciences of the USA 98:9157–9160.

Holzer, B., A. Jacot, and M. W. G. Brinkhof. 2003. Condition-dependent signaling affects male sexual attractiveness in field crickets, *Gryllus campestris*. Behavioral Ecology 353:359.

Hunt, J., R. Brooks, M. D. Jennions, M. J. Smith, C. L. Bentsen, and L. F. Bussière. 2004a. High-quality male field crickets invest heavily in sexual display but die young. Nature 432:1024–1027.

Hunt, J., L. F. Bussière, M. D. Jennions, and R. Brooks. 2004b. What is genetic quality? Trends in Ecology & Evolution 19:329–333.

Hunt, J., R. Brooks, and M. D. Jennions. 2005. Female mate choice as a condition-dependent life-history trait. American Naturalist 166:79–92.

Jia, F.-Y., M. D. Greenfield, and R. D. Collins. 2000. Genetic variance of sexually selected traits in waxmoths: maintenance by genotype × environment interaction. Evolution 54:953–967.

Johnstone, R. A. 1995. Sexual selection, honest advertisement and the handicap principle: reviewing the evidence. Biological Reviews of the Cambridge Philosophical Society 70:1–65.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. American Naturalist 157:245–261.

Klappert, K., and K. Reinhold. 2003. Acoustic preference functions and sexual selection on the male calling song in the grasshopper *Chorthippus biguttulus*. Animal Behaviour 65:225–233.

Klump, G. M., and H. C. Gerhardt. 1987. Use of non-arbitrary acoustic criteria in mate choice by female gray tree frogs. Nature 326: 286–288.

Kokko, H. 2001. Fisherian and "good genes" benefits of mate choice: how (not) to distinguish between them. Ecology Letters 4:322–326.

Kokko, H., P. T. Rintamäki, R. V. Alatalo, J. Höglund, E. Karvonen, and A. Lundberg. 1999. Female choice selects for lifetime lekking performance in black grouse males. Proceedings of the Royal Society of London B 266:2109–2115.

Kokko, H., R. Brooks, J. M. McNamara, and A. I. Houston. 2002. The sexual selection continuum. Proceedings of the Royal Society of London B 269:1331–1340.

Lande, R. 1980. The genetic covariance between characters maintained by pleiotropic mutations. Genetics 94:203–215.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

LeBas, N. R., L. R. Hockham, and M. G. Ritchie. 2003. Nonlinear and correlational sexual selection on "honest" female ornamentation. Proceedings of the Royal Society of London B 270:2159–2165.

Loher, W., and B. Rence. 1978. The mating behaviour of *Teleogryllus commodus* (Walker) and its central and peripheral control. Zeitschrift für Tierpsychologie 46:225–259.

McGlothlin, J. W., P. G. Parker, V. Nolan Jr., and E. D. Ketterson. 2005. Correlational selection leads to genetic integration of body size and an attractive plumage trait in dark-eyed juncos. Evolution 59:658–671.

Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. Evolution 41:1149–1161.

Murphy, C. G., and H. C. Gerhardt. 2000. Mating preference functions of individual female barking treefrogs, *Hyla gratiosa*, for two properties of male advertisement calls. Evolution 54:660–669.

Otte, D., and R. D. Alexander. 1983. The Australian crickets (Orthoptera: Gryllidae). Academy of Natural Sciences of Philadelphia, Philadelphia.

Parker, G. A. 1983. Mate quality and mating decisions. Pages 141–165 *in* P. Bateson, ed. Mate choice. Cambridge University Press, Cambridge.

Passmore, N. I., P. J. Bishop, and N. Caithness. 1992. Calling behaviour influences mating success in male painted reed frogs, *Hyperolius marmoratus*. Ethology 92:227–241.

Paterson, H. E. H. 1982. Perspective on speciation by reinforcement. South African Journal of Science 78:53–57.

———. 1985. The recognition concept of species. Pages 21–29 *in* E. Vrba, ed. Species and speciation. Transvaal Museum, Pretoria.

Patten, M. A., J. T. Rotenberry, and M. Zuk. 2004. Habitat selection, acoustic adaptation, and the evolution of reproductive isolation. Evolution 58:2144–2155.

Phillips, P. C., and S. J. Arnold. 1989. Visualizing multivariate selection. Evolution 43:1209–1222.

Polakow, D. A., P. R. Y. Backwell, N. Caithness, and M. D. Jennions. 1995. Stabilizing or directional selection in signalling systems: investigations in a population of painted reed frogs, *Hyperolius marmoratus*. South African Journal of Science 91:270–273.

Ritchie, M. G. 1996. The shape of female mating preferences. Proceedings of the National Academy of Sciences of the USA 93: 14628–14361.

Rodriguero, M. S., M. T. Vera, E. Rial, J.-P. Cayol, and J. C. Vilardi. 2002. Sexual selection on multivariate phenotype in wild and mass-reared *Ceratitis capitata* (Diptera: Tephritidae). Heredity 89:480–487.

Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. Proceedings of the Royal Society of London B 263:1415–1421.

Ryan, M. J. 1983. Sexual selection and communication in a Neotropical frog, *Physalemus pustulosis*. Evolution 37:261–272.

Ryan, M. J., and A. Keddy-Hector. 1992. Directional patterns of female mate choice and the role of sensory biases. American Naturalist 139(suppl.):S4–S35.

Ryan, M. J., and A. S. Rand. 1993a. Sexual selection and signal evolution: the ghost of biases past. Philosophical Transactions of the Royal Society of London B 340:187–195.

———. 1993b. Species recognition and sexual selection as a unitary problem in animal communication. Evolution 47:647–657.

———. 2003. Sexual selection in female perceptual space: how female túngara frogs perceive and respond to complex population variation in acoustic mating signals. Evolution 57:2608–2618.

Ryan, M. J., and W. Wilczynski. 1988. Coevolution of sender and receiver: effect on local mate preferences in cricket frogs. Science 240:1786–1788.

Ryan, M. J., S. A. Perrill, and W. Wilczynski. 1992. Auditory tuning and call frequency predict population-based mating preferences in the cricket frog, *Acris crepitans*. American Naturalist 139:1370–1383.

Schlosser, G., and G. P. Wagner. 2004. Modularity in development and evolution. University of Chicago Press, Chicago.

Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. Evolution 42:849–861.

———. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774.

Schluter, D., and D. Nychka. 1994. Exploring fitness surfaces. American Naturalist 143:597–616.

Shaw, K. L., and D. P. Herlihy. 2000. Acoustic preference functions and song variability in the Hawaiian cricket *Laupala cerasina*. Proceedings of the Royal Society of London B 267:577–584.

Simmons, L. W., M. Zuk, and J. T. Rotenberry. 2001. Geographic variation in preference functions and male songs of the field cricket *Teleogryllus oceanicus*. Evolution 55:1386–1394.

Snedden, W. A., and S. K. Sakaluk. 1992. Acoustic signalling and its relation to male mating success in sagebrush crickets. Animal Behaviour 44:633–639.

Stumpner, A., and D. von Helversen. 2001. Evolution and function of auditory systems in insects. Naturwissenschaften 88:159–170.

Tomkins, J. L., J. Radwan, J. S. Kotiaho, and T. Tregenza. 2004. Genic capture and resolving the lek paradox. Trends in Ecology & Evolution 19:323–328.

Welch, A. M. 2003. Genetic benefits of a female mating preference in gray tree frogs are context-dependent. Evolution 57:883–893.

Welch, A. M., R. D. Semlitsch, and H. C. Gerhardt. 1998. Call duration as an indicator of genetic quality in male gray tree frogs. Science 280:1928–1930.

Zahavi, A. 1975. Mate selection: a selection for a handicap. Journal of Theoretical Biology 53:205–214.

Zuk, M., and G. R. Kolluru. 1998. Exploitation of sexual signals by predators and parasitoids. Quarterly Review of Biology 73:415–438.