Multivariate sexual selection in a rapidly evolving speciation phenotype

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Estimating the fitness surface of rapidly evolving secondary sexual traits can elucidate the origins of sexual isolation and thus speciation. Evidence suggests that sexual selection is highly complex in nature, often acting on multivariate sexual characters that sometimes include non-heritable components of variation, thus presenting a challenge for predicting patterns of sexual trait evolution. Laupala crickets have undergone an explosive species radiation marked by divergence in male courtship song and associated female preferences, yet patterns of sexual selection that might explain this diversification remain unknown. We used female phonotaxis trials to estimate the fitness surface for acoustic characters within one population of Laupala cerasina, a species with marked geographical variation in male song and female preferences. Results suggested significant directional sexual selection on three major song traits, while canonical rotation of the matrix of nonlinear selection coefficients (g) revealed the presence of significant convex (stabilizing) sexual selection along combinations of characters. Analysis of song variation within and among males indicated significantly higher repeatability along the canonical axis of greatest stabilizing selection than along the axis of greatest linear selection. These results are largely consistent with patterns of song divergence that characterize speciation and suggest that different song characters have the potential to indicate distinct information to females during courtship.

1. Introduction

Secondary sexual characters are remarkable for both extreme degrees of elaboration and pronounced diversification among closely related taxa, suggesting that sexual selection is a potent evolutionary mechanism with a central role in speciation [1,2]. Evidence supporting this hypothesis has been inferred from both comparative [3] and empirical studies [4], yet there are few examples that link the evolution of reproductively isolating sexual traits with the form of sexual selection within populations in the early stages of lineage divergence.

For male phenotypes involved in attracting mates, the rate and direction of evolution will be influenced in part by the shape of female preference functions (i.e. the relationship between mating response and male sexual trait variation [5]), which can be broadly characterized as ranging from unimodal (intermediate male trait values elicit the strongest mating response) to open-ended preferences for extreme trait values [6]. The former is commonly interpreted as indicating the presence of stabilizing sexual selection, while the latter is equated with directional sexual selection [7]. However, the evolutionary response of sexually selected traits is difficult to predict from preference functions alone, as the precise form of selection depends also on the distributions within populations of both male trait and female preference variation. Indeed, the individual fitness surface for sexual traits can be highly complex, with linear and nonlinear components of selection acting simultaneously on traits. Additionally, it is increasingly apparent that morphologies and behaviours involved in sexual displays are often composed of multiple components [8,9] and/or signalling modalities [10], and thus univariate preference functions may fail to capture the complex and interacting effects of multivariate signals on mating decisions [11]. Furthermore, both theory and empirical studies suggest that some sexually selected traits show high degrees of phenotypic plasticity [12,13], exhibiting both state- and condition-dependent expression that may convey information about the individual to potential
mates [14]. As such phenotypic variation is non-heritable, the evolutionary response to sexual selection is likely to be difficult to predict [15,16]. Taken together, these observations suggest that linking female mating preferences with patterns of sexual trait evolution will require both explicit measurement of multivariate sexual selection gradients [11,17] and heritability of sexual trait expression [18]. Such an approach may be particularly insightful in resolving contradictions between observed and expected patterns of sexual trait diversification among recently diverged lineages.

Sword-tail crickets of the genus Laupala have undergone an explosive species radiation across the Hawaiian archipelago [19], exhibiting one of the most rapid rates of speciation in animals [20]. This radiation has been accompanied by extensive diversification of male courtship song, a conspicuous sexual signal produced by rhythmic stridulation of the forewings [19]. Most notably, pulse rate of the song exhibits pronounced variation among species [20], and recent studies in one species, Laupala cerasina, found significant geographical covariation with female preference, suggesting a role for differentiation in acoustic signalling in establishing barriers to gene exchange during speciation [21,22]. Such patterns are the hallmarks of directional sexual selection, yet behavioural studies show that female L. cerasina clearly express unimodal preference functions for pulse rate, with peak mating responses corresponding to average pulse rates of local males [6]. Nonetheless, pulse rate shows extensive diversification among populations of L. cerasina and species of Laupala. Preference functions for carrier frequency are also unimodal, though this song character shows little evidence of diversification within or among species. Likewise, pulse duration of male song exhibits little evolutionary lability [21], despite largely open-ended female preference functions for this character [6]. Thus, each of the three primary song characters is characterized by distinct preference functions and patterns of phenotypic evolution. Understanding how preference functions for these different features interact to generate sexual selection on male songs is thus critical for predicting the efficacy of sexual selection in facilitating speciation and acoustic elaboration among species. In particular, a multivariate study of acoustic preference and trait heritability requires the use of quantitative genetic methods for predicting the efficacy of sexual selection in facilitating speciation and acoustic elaboration among species.

In this study, we observe female acoustic preference in L. cerasina from Hawai‘i Island to test the form and strength of multivariate sexual selection on male song. We first estimate linear and nonlinear components of sexual selection using polynomial regression [17], followed by canonical analysis to evaluate the overall importance of nonlinear selection in this population [23,24]. We then examine heritability of sexually selected song characters by calculating repeatability (r), which indicates the proportion of variance attributed to differences among as opposed to within individuals [25] and, as such, reflects the degree to which individuals consistently differ. It is often used to approximate the upper limit of heritability in behavioural studies [26,27]. The resulting analyses suggest that songs experience a complex combination of multivariate stabilizing and directional selection owing to female preferences, and that song traits differ in repeatability. In combination, these results resolve observed patterns of evolutionary change in song elements with the form of preference functions both within and among species.

2. Material and methods

(a) Animal collection and rearing

Wild-caught, naturally mated L. cerasina females were collected in June 2009 from Pololu Valley of the Kohala Mountains, Hawai‘i Island. In the laboratory, all individuals were housed separately in plastic specimen cups at 20°C with a 12:12 light cycle and fed cricket chow (Fluker Farms, Port Allen, LA) ad libitum. Females were provided with a moistened tissue to maintain humidity and as an oviposition substrate. Eggs were collected weekly and stored in separate cups, from which newly hatched nymphs were collected. To maintain virgin status, male and female progeny were separated during the penultimate instar.

(b) Female acoustic preference

Beginning at age one month post-final moult, first-generation laboratory-reared females were subjected to two-choice phonotaxis trials using synthesized songs. Single virgin adults (n = 73, sampled from n = 10 maternal lines; average number of females per family = 7.3, s.d. = 4.9) were placed beneath a plastic specimen cup in the centre of a circular arena (47 cm diameter) in a temperature-controlled (20°C) anechoic chamber. Using a personal computer and custom software, two digitally synthesized songs (one a test song and the other a control) were played simultaneously through identical speakers (3.5 inch diameter; Radio Shack model no. 40-1218) placed at opposite ends of the arena. A pulsed, sinusoidal tone was generated via a 16 bit digital/analog converter (Tucker–Davis Technologies, Gainesville, FL) and synthesized song was filtered at 10 kHz to prevent aliasing (Krohn–Hite filter model 3322). For all trials, pulses had an amplitude envelope with rise and fall times of 10 ms each. Prior to trials, sound pressure levels from both speakers were measured using a Bruel and Kjaer SPL Meter (Type 4155) and equilibrated on a 4.0 pulses s⁻¹ pulsed tone using Tucker–Davis digital attenuators. After a 5 min acclimatization period during which stimulus playback was audible, the specimen cup was remotely retracted and female phonotactic behaviour was observed over a 5 min period. Phonotactic response (movement to within a 10 cm zone in front of each speaker) was scored as one (female responded to the test song) or zero (female responded to the control song), at which point the trial was immediately ended as females rarely responded to both speakers [28]. Females that failed to respond to either speaker within the 5 min trial were removed, and retested on the following day. While the majority of females responded after retesting, those that remained unresponsive were discarded from the analysis.

(c) Phonotaxis experiment design

Each female was subjected to a total of five phonotaxis trials between 10.00 and 14.00 h across 3–4 days, with a minimum of 1 h between trials and not more than two trials per day. In all trials, parameters for the control song were set to the observed average song values (pulse rate = 2.58 pulses s⁻¹, carrier frequency = 4879.3 Hz, pulse duration = 39.8 ms), based on a sample of published values for wild-caught males (n = 14) in this population [21]. Test songs were generated using a method following Brooks et al. [29], where values for pulse rate, carrier frequency and pulse duration were selected independently (i.e. assuring no correlation among traits) but in a manner that resulted in the same univariate distribution observed for each trait in the source population. Briefly, for each synthetic test song, a random number was drawn for each song character,
converted to a z-score using the NORMSINV function in Excel (Microsoft Corp., Redmond, WA), then multiplied by the observed population standard deviation and added to the trait mean to obtain the test song value. In total, 73 unique test songs were generated such that each song was presented to five different females and each female was tested with five distinct stimuli. Test song parameters and response data are included in the electronic supplementary material.

(d) Selection analysis

Linear and nonlinear sexual selection differentials were estimated using multiple regression analysis [17]. All three song characters were first standardized to a mean of zero and unit variance, while attractiveness (i.e. mean female phonotactic response) was standardized to a mean of one. The vector of standardized linear selection gradients (β) was constructed from the partial regression coefficients of a multiple linear regression model with linear terms only, while the matrix containing quadratic and correlational selection differentials (γ) was calculated from a full second-order polynomial regression. Quadratic selection gradients, which indicate the strength of stabilizing (negative sign) or disruptive (positive sign) selection, were estimated as double quadratic regression coefficients. To test for potential biases owing to the inclusion of multiple related females, we also performed bootstrap analysis in which each selection gradient was recalculated from a subsample consisting of a single, randomly drawn female per family. This was repeated for 1000 iterations to create a distribution of selection gradients. All of the coefficients reported in our study fell within the 95% CIs of these bootstrapped distributions, suggesting no significant bias owing to our sampling scheme.

To evaluate the overall importance of nonlinear sexual selection on song characters, we performed canonical rotation of γ [23,24] using the RSREG procedure in SAS v. 9.2 (SAS Institute, Cary, NC) to reveal the major axes of quadratic selection by eliminating cross-product terms (i.e. correlational selection) of the matrix. This analysis results in the matrix M, which contains the eigenvectors (mi) that explain the major axes of nonlinear selection. The elements of M describe the loadings of the original song characters along the transformed axes, thus facilitating the biological interpretation of these axes in a manner similar to principal components analysis [23]. Associated eigenvalues (λi) are equivalent to the strength of nonlinear selection along each canonical axis, while the strength of linear selection (hi) was evaluated by converting original trait values to canonical scores, and placing values into a new multiple regression.

For all regression analyses, statistical significance of selection gradients was evaluated using randomization tests in which female phonotactic response values were iteratively shuffled 1000 times among test songs and the regression coefficients recalculated to create a distribution of expected values under random choice. p-values were then obtained by evaluating the number of simulated regression coefficients out of 1000 with absolute values that were greater than or equal to the absolute value of the experimentally derived estimate. This process was carried out separately for the models containing only linear selection terms as well as the full polynomial regression model in both the untransformed song characters and the canonical traits.

Visualization of the fitness surface defined by the canonical analysis was performed by fitting a thin-plate smoothing spline (PROC TPSPLINE in SAS v. 9.2), a non-parametric regression approach that uses a penalized least-square method to generate a smooth multivariate surface. The smoothing parameter was determined by the generalized cross-validation function.

(e) Repeatability of song traits

The songs of male progeny (n = 10, six to nine weeks postfinal moult) from a mass-mated population of wild-caught adults were recorded using a Sony Walkman Professional (WM-DDC) from open plastic chambers fitted with screen covers. To estimate repeatability, 10 songs per male were sampled. Each recording represented a distinct singing bout, and individuals were not recorded more than three times each day. Unfiltered songs were digitized using SOUNDESCOPT/16 software (GWI Instruments, Cambridge, MA) at 44.1 kHz to generate oscillogram plots that displayed trains of pulses comprising the male song bout. Measurements were carried out across 10 sequential pulses following Shaw & Herlihy [6] and are included in the electronic supplementary material. Briefly, pulse rate was calculated as the inverse of pulse period (the beginning of one pulse to the beginning of the following pulse), carrier frequency was measured as the average peak frequency in the power spectrum plot for each pulse, and pulse duration was measured from the start to end of each pulse. To assess repeatability along major axes of sexual selection, resulting values were standardized as above and converted to canonical trait scores (γi) using the corresponding trait loadings for each eigenvector. Repeatabilities (r) were calculated as intraclass correlation coefficients [25] with 95% CIs estimated by bootstrapping, wherein song trait values were resampled with replacement (1000 iterations) within males to generate a distribution of r values.

3. Results

Results from the multiple regression analysis indicated significant linear selection on all three song characters (table 1), with females exhibiting increasing positive phonotaxis towards test songs with slower pulse rates (β = −0.160, randomization test: p = 0.001), higher carrier frequencies (β = 0.134, randomization test: p = 0.016), and longer pulse durations (β = 0.246, p < 0.001). In addition, there was significant negative (convex) quadratic selection on carrier frequency (γ = −0.111, p = 0.011), but not other traits (pulse rate: γ = −0.059, p = 0.062; pulse duration: γ = 0.004, p = 0.946). We observed no significant correlational selection for any of the trait pairs.

Canonical rotation of the γ matrix revealed one positive and two negative eigenvectors describing the major axes of nonlinear selection (table 2). Of these, we observed a single axis (m1) with significant convex quadratic sexual selection (λ = −0.147, p = 0.005). Inspection of the eigenvector coefficients indicated that this axis was most heavily influenced by carrier frequency, followed by pulse rate and pulse duration. There was also significant directional selection along the other two eigenvectors of M, with strong directional selection for larger values of m1 (θ = 0.301, p < 0.001) and directional selection for smaller values of m2 (θ = −0.111, p = 0.033). These axes were mostly weighted by pulse duration and carrier frequency (m1), and pulse rate and carrier frequency (m2), thus corroborating the directional selection patterns inferred from the initial multiple regression analysis (table 1).

Major features of the fitness surface were visualized by plotting the canonical axis of greatest nonlinear selection (m2) against the axis of greatest linear selection (m1). The resulting thin-plate spline surface (figure 1) reflects the overall convex curvature across m1 with a ridge of relatively high fitness at intermediate values, thereby suggesting the presence of stabilizing sexual selection [23,30]. In conjunction, there is also a conspicuous increase in fitness consistent with positive directional selection along m1 such that at extreme positive values, the effect of quadratic selection is apparently outweighed by positive linear selection.
Repeatability of male song characters along each of the major axes of sexual selection is shown in figure 2. Inspection of the bootstrapped 95% CIs indicated that repeatability along the axis of greatest nonlinear selection (\(m_3\)) was significantly greater than repeatability along the axis of greatest linear selection (\(m_1\)), which did not differ from zero. Repeatability of song traits along \(m_2\) was also significantly different from zero. The magnitude of repeatability along \(m_2\) was not statistically distinct from the magnitude of repeatability of \(m_3\) (i.e. axes shared overlapping CIs). Repeatabilities for untransformed song characters were also estimated and, given the loadings of each trait on the respective canonical axes, were largely concordant with these results: significant repeatability for pulse rate and carrier frequency (which contribute strongly to \(m_3\) and \(m_1\)), but not pulse duration (\(m_2\)).

4. Discussion
Elucidating the individual fitness surface for sexual signals within populations should provide insight into the evolutionary mechanisms operating during the initial stages of

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Table 2. Matrix (\(M\)) of eigenvectors (\(m_i\)) from canonical rotation of \(\gamma\). Elements of the matrix describe the loadings of original song variables on the transformed axes. Linear selection gradients (\(\theta_i\)) and quadratic selection gradients (equal to the eigenvalue, \(\lambda_i\), of each corresponding eigenvector) along each latent axis shown in last two columns. \(p\)-values for selection gradients estimated from randomization tests (1000 iterations).

Figure 1. Fitness surface for male courtship song characters, defined by two canonical axes with greatest nonlinear (\(m_3\)) and linear (\(m_1\)) sexual selection. The surface was estimated by fitting thin-plate spline to relative attractiveness (standardized mean female responsiveness) of digitally synthesized songs. Points represent songs of wild males from the same source population projected onto the experimentally derived fitness surface.

Repeatability of male song characters along each of the major axes of sexual selection is shown in figure 2. Inspection of the bootstrapped 95% CIs indicated that repeatability along the axis of greatest nonlinear selection (\(m_3\)) was significantly greater than repeatability along the axis of greatest linear selection (\(m_1\)), which did not differ from zero. Repeatability of song traits along \(m_2\) was also significantly different from zero. The magnitude of repeatability along \(m_2\) was not statistically distinct from the magnitude of repeatability of \(m_3\) (i.e. axes shared overlapping CIs). Repeatabilities for untransformed song characters were also estimated and, given the loadings of each trait on the respective canonical axes, were largely concordant with these results: significant repeatability for pulse rate and carrier frequency (which contribute strongly to \(m_3\) and \(m_1\)), but not pulse duration (\(m_2\)).

4. Discussion
Elucidating the individual fitness surface for sexual signals within populations should provide insight into the evolutionary mechanisms operating during the initial stages of
speciation in clades characterized by conspicuous divergence of sexual traits. While the rate and direction of evolutionary change in secondary sexual characters ultimately depends also on patterns of heritable variation and the influence of neutral processes (such as genetic drift), the rapid divergence of phenotypes known to be targets of mate choice suggests the action of strong contemporary sexual selection. However, sexual selection regimes can be highly complex, with linear and nonlinear selection acting on multivariate combinations of both heritable and non-heritable trait components. Moreover, empirical estimates of multivariate selection may be biased when based on observed male phenotypes, which can be truncated owing to historical selection [31] or constrained by strong correlations among characters [17]. Indeed, Lande & Arnold [17] emphasized the critical importance of using a multivariate approach, which can account for indirect selection on correlated characters, when analyzing nonlinear selection. As a consequence, the precise form of sexual selection is difficult to infer from univariate female preference functions, calling for studies that explicitly estimate both the multivariate fitness surface and heritability of male sexual traits.

In this study, analysis of female preference trials demonstrated significant linear selection on all three components of male song along with significant convex selection on carrier frequency (table 1). Furthermore, canonical rotation of the γ matrix (table 2) revealed significant convex selection along an axis (m3) comprised all three song traits, and inspection of the individual fitness surface (figure 1) indicated a fitness peak at intermediate values, thereby suggesting the presence of multivariate stabilizing selection. More specifically, these results predict directional trait evolution for slower pulse rates, higher carrier frequencies and longer pulse durations in response to linear selection, as well as reduced variance in carrier frequency, pulse rate and duration owing to stabilizing selection oriented along m3. At the same time, our results showed positive directional sexual selection along two canonical axes: m1, which was mostly influenced by pulse duration and carrier frequency; and m2, which corresponded with pulse rate and, again, carrier frequency. Interestingly, repeated measures of live singing males (figure 2) suggested no significant repeatability along m1, while there was significant repeatability along m2 as well as the axis of greatest stabilizing selection, m3.

Taken together, the results of our study indicate a combination of directional and stabilizing sexual selection on different components of a relatively simple male acoustic signal. Such patterns of selection on different song components are expected to lead to distinct patterns and rates of diversification for each character. Indeed, evidence consistent with such ‘atomized evolution’ of signal characters has recently been documented in a macroevolutionary study of cricket song in eneopterine crickets [32]. One general prediction is that characters subject to strong directional selection should exhibit rapid evolutionary change, whereas evolutionary stasis is predicted for components under stabilizing selection. In our study, directional sexual selection was strongest for pulse duration, while directional selection for carrier frequency and pulse rate was comparatively weaker, suggesting that we might expect corresponding diversification primarily with respect to pulse duration. However, divergence among populations of L. cerasina is dominated by pulse rate, whereas there is considerable overlap in phenotypic values for both pulse duration and carrier frequency [21]. Likewise, studies of evolutionary diversification in male song across the genus Laupala suggest that pulse rate is the most evolutionarily labile character [19,20].

This apparent discordance between patterns of selection with patterns of diversification might be explained, in part, by the genetic architecture underlying variation in each song character, as the evolutionary response of phenotypes to selection will depend not only on the form of the fitness surface, but also on its relationship to axes of heritable variation for traits under selection [33,34]. Likewise, if the dominant axis of selection involves combinations of traits, the predicted rate and the direction of evolutionary change will be contingent on heritability along these canonical axes. As a corollary, when genetic variation is oriented away from the direction of selection, or when there are strong genetic correlations among traits, the evolutionary response can be heavily biased or diminished [35]. While estimating heritability of behavioural traits remains challenging, repeatability can provide insight into the upper limit of heritability [33] (but see [36]), and thus analysis of repeated measures often presents a first step towards understanding inheritance of behavioural variation [27]. In our study, repeatability values for canonical traits m1 and m2 were significantly different from zero, and generally fell within the observed range for courtship behaviours (for a review see [26]), thereby suggesting a heritable basis for traits contributing to these axes, and thus an evolutionary response to sexual selection described by the fitness surface. Consequently, although directional selection on pulse rate was comparatively weaker (table 1), its higher repeatability is generally consistent with greater diversification in this character within L. cerasina and among congeners. In contrast, the absence of conspicuous evolutionary responses in pulse duration to strong directional sexual selection might be due in part to the low heritability along this axis (m3). Alternatively, particular song characters might be more or less constrained by genetic correlations with other traits, though at least among song characters, phenotypic correlations in this population are evidently weak [21]. It is also possible that divergence has been influenced by neutral genetic drift, which is expected to occur primarily in the direction concordant with additive genetic variation [37,38].

**Linear selection**

With respect to the mechanisms of sexual selection, our finding of strong directional selection on pulse duration is largely consistent with a previous study of female preference functions in L. cerasina [6], which reported open-ended preferences for the same character. Open-ended preference functions are indicative of attraction to extreme signal trait values, and are thus commonly associated with exaggerated male sexual traits [7]. Such preferences are thought to evolve when trait exaggeration serves as a signal of male quality or physiological condition [39–41]. In acoustic signalling systems, temporal properties of songs may often reflect the total energetic costs of production; thus by preferring costly songs, females might more often mate with vigorous or high-condition males [41,42]. This suggests that pulse duration in L. cerasina functions as a condition-dependent sexual signal, which is corroborated by the absence of significant repeatability along the canonical axis m1. Moreover, previous analysis of within-male variation found high levels of variability (measured as coefficient of variation) in pulse duration [6], and metabolic studies of male
calling in other gryllid species [43] have shown energetic costs to increase with longer pulse duration. However, the degree to which pulse duration is linked to male nutritional or physiological condition remains unexplored.

In contrast to pulse duration, the observed directional sexual selection on pulse rate and carrier frequency were perhaps more surprising, given previous work suggesting unimodal female preference functions for these characters [6]. The presence of directional selection can thus be interpreted as resulting from a disparity between peak female preferences and mean male trait values in this population. With respect to pulse rate, one hypothesis that might explain this disparity in our study population is interspecific acoustic interference with Laupala kohalensis, a sympatric congener that sings at a significantly faster pulse rate (approx. 3.72 pulses s⁻¹). Specifically, if interspecies interactions are costly, L. cerasina females that prefer slightly slower pulse rates will have higher fitness owing to fewer heterospecific encounters, leading to directional sexual selection on L. cerasina male songs. Indeed, previous work suggests strong assortative mating preferences associated with distinct genetic boundaries among these two species [44].

(b) Nonlinear selection

While both theory and empirical work on sexual trait evolution have traditionally focused on directional selection [45,46], the results of our study revealed stabilizing selection on carrier frequency and, to a lesser degree, pulse rate and duration, as evidenced from canonical rotation of the γ matrix. Thus, the major axis of nonlinear selection is oriented along linear combinations of song characters, as has been shown in other studies of acoustic sexual signalling [47]. Overall, these findings add to recent growing evidence of multivariate stabilizing sexual selection in both laboratory [29,47–50] and natural populations [51,52], and are suggestive of a general pattern of female preference for male songs with intermediate values as opposed to extreme male phenotypes. As some authors have observed [53], the tendency of empiricists to grossly underestimate quadratic selection gradients has probably contributed to a general underappreciation of stabilizing selection in evolutionary studies. It is important to note, however, that the observation of directional selection (as discussed earlier) does not preclude the presence of stabilizing selection as well. Indeed, such a pattern of selection is evident in our results with respect to carrier frequency (table 1).

Evidence of stabilizing selection in this study is broadly consistent with previous work in L. cerasina that reported unimodal female preference functions for pulse rate and carrier frequency [6]. Unimodal preferences for acoustic signals have been hypothesized to reflect auditory tuning of the female sensory organs for a particular range of male trait values [54]. In particular, considerable behavioural and neuroethological evidence suggests female hearing organs in diverse taxa are often highly specialized to spectral properties of acoustic songs in general, and carrier frequency in particular [55,56]. Likewise, our finding of stabilizing selection on pulse rate (to the degree it is involved in canonical axis m₂) is consistent with results from similar studies in other cricket species [29]. Unimodal preference functions for pulse rate may be common in species of Laupala owing to their frequent occurrence with sympatric congeners. While the presence of congeners may sometimes result in directional selection on pulse rate (see above), species may also occur in acoustic communities with both faster and slower singing congeners, as has been observed across the geographical range of L. cerasina [57]. Consequently, the presence of acoustic interference at both extremes might represent conditions for the evolution of unimodal preferences.

In conclusion, while sexual selection is commonly portrayed as a unidirectional and unrelenting evolutionary force favouring extreme elaboration, our results demonstrate that sexual selection can be highly complex and nuanced, even in rapidly diversifying clades characterized by evolutionarily labile sexual signals. The combination of distinct patterns of selection on different traits that probably vary in both signal content [14] and heritability suggests that this relatively simple sexual display might respond to persistent selection in a mosaic fashion [58], with each trait evolving along distinct trajectories. However, the degree to which mosaic evolution in this system may be constrained or facilitated by genetic covariances among traits remains to be examined. Overall, while the patterns of song diversity in Laupala may be perplexing with respect to univariate preference functions alone, they can be better understood in the context of the multiple factors considered in our study, thereby providing a link between microevolutionary process and macroevolutionary pattern.

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References


