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Spatial Mixing between Calling Males of Two Closely Related, Sympatric Crickets Suggests Beneficial Heterospecific Interactions in a NonAdaptive Radiation

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Abstract

Sympatry among closely related species occurs in both adaptive and nonadaptive radiations. Among closely related, sympatric species of a nonadaptive radiation, the lack of ecological differentiation brings species into continual contact where individuals are exposed to the risk of reproductive interference. Selection thus should cause divergence in multiple components mediating the reproductive boundary. Besides differentiation of reproductive signals *per se*, spatial segregation is a commonly proposed mechanism that can mitigate reproductive interference. Studying a pair of broadly sympatric, closely related cricket species from a nonadaptive radiation in Hawaii, we 1) quantified acoustic divergence of male songs and 2) tested alternative hypotheses of spatial distribution of calling males of the 2 species. Acoustic analyses of the recorded songs showed that, while the 2 species differed substantially in pulse rate, no spectral or fine temporal segregation of the pulse structure was evident, indicating the potential for acoustic masking. Moreover, we found that calling males of the 2 species are highly mixed both vertically and horizontally and showed the same preference for calling sites. More surprisingly, calling males were found to form mixed-species calling clusters where heterospecific males are closer to each other than conspecific males. Such an individual spacing pattern suggests low heterospecific aggression and/or high conspecific competition. Because females prefer higher sound intensity, heterospecific males may benefit, rather than interfere, with each other in attracting females. These findings offer a potential mechanism enabling species coexistence in sympatry.

Keywords: neighbor spacing, nonadaptive radiation, reproductive barrier, signaling site choice, spatial segregation, sympatric species.

Classic adaptive radiations are characterized by rapid ecological divergence and the consequential evolution of closely related, ecologically distinct species (Schluter 2000). In some cases, the build-up of species in sympatry accompanies the process, probably in its later stages (Streelman and Danley 2003). Familiar examples include lake-dwelling cichlids (Seehausen 2015), freshwater sticklebacks (McKinnon and Rundle 2002), *Anolis* lizards (Losos 2011), Hawaiian honeycreepers (Lerner et al. 2011), Hawaiian *Tetragnatha* spiders (Gillespie 2016), and the Hawaiian silversword alliance (Carlquist et al. 2003).

For many, ecological divergence is often the most captivating feature of adaptive radiations. However, as adaptive radiations also include speciation, the evolution of reproductive boundaries is equally important to the process. In cases where adaptive radiations lead to the build up of sympatric species that otherwise have the potential to hybridize, the evolution of reproductive barriers is especially crucial because in their absence, gene flow is expected to erode species boundaries.

In a nonadaptive radiation, where speciation is not fueled by the advantages of enhanced resource exploitation (Rundell and Price 2009; Czekanski-Moir and Rundell 2019), forces affecting divergence in reproductive phenotypes should be particularly important for preventing gene flow and maintaining species boundaries. For example, reproductive interactions between species can lead to reduced fitness due to reproductive interference or decreased reproductive success due to maladaptive hybridization (Pfennig and Pfennig 2012). Character divergence in allopatry (enabling species overlap) or in sympatry due to species interactions (character displacement) may take the form of evolution in sexual signals and preferences, differences in behavioral reproductive timing or differential use of space during reproductive interactions. In adaptive radiations, ecological differentiation may reduce encounter rates of heterospecific males and females as a consequence of evolved differences in habitat preference, host shifts or temporal segregation. In comparison, in nonadaptive radiations, reproductive interactions among sympatric species may lead to elevated selective pressures causing divergence across reproductive phenotypes due to a lack of ecological differentiation that might otherwise reduce heterospecific encounters. Differentiation along various reproductive phenotypic axes may set the stage for coexistence.

The endemic Hawaiian cricket genus *Laupala* conforms to the above description of a nonadaptive species radiation. Current diversity in *Laupala* results from a rapid radiation of 38 morphologically and ecologically similar species that display sexual barriers to gene exchange (Mendelson and Shaw 2005). Species in the genus are single island endemics and fall into 3 major lineages, i.e., the *kauai* (restricted to the island of Kauai), the *cerasina* (found on Oahu, Maui, and the Big Island of Hawaii), and the *pacifica* groups (found on Oahu, Molokai, Lanai, Maui, and the Big Island of Hawaii). Phylogenetic evidence suggests that speciation has occurred largely in allopatry and that sympatric communities have arisen as a consequence of secondary contact on numerous occasions (Otte 1994; Shaw 2002). Sympatry is commonly found between species from the *cerasina* and the *pacifica* groups, and sometimes between more closely related species within the same lineage (Otte 1994; Mendelson and Shaw 2005). Two species endemic to the Big Island of Hawaii, *L. pruna* (*pacifica* lineage) and *L. cerasina* (*cerasina* lineage) occur in sympatry across the eastern half of the Big Island of Hawaii (Otte 1994), exemplifying the common occurrence of secondary overlap among species of different *Laupala* lineages. These species last shared a most recent common ancestor (MRCA) roughly 3.7 million years ago (likely on Oahu). *L. pruna* is also sympatric with 2 other Big Island endemics of the *pacifica* lineage, *L. nigra* or *L. paranigra*; all 3 of these species likely shared a MRCA fewer than 0.43 million years ago (Otte 1994; Mendelson and Shaw 2005). Thus, while some definitions of nonadaptive radiation exclude the possibility of sympatry on the premise of competitive exclusion (e.g. Rundell and Price 2009), *Laupala* demands a more permissive definition because species of both close and more distant phylogenetic relationship often occur together despite ecological similarity.

Although *L. cerasina* and *L. pruna* are not sister species, they are very similar in morphological, behavioral and ecological attributes (Otte 1994; Shaw 2002), such that sympatric distributions might present conditions for reproductive interference. Moreover, shared variation in mitochondrial DNA sequences at both sympatric and allopatric locations, exemplified by *L. cerasina* and *L. pruna*, suggests persistent interspecific hybridization throughout the history of *Laupala* (Shaw 2002). Thus, although nuclear data support the phylogenetic placement of *L. cerasina* and *L. pruna* into different species groups (Shaw 2002; Mendelson and Shaw 2005), highly similar phenotypic attributes, along with highly similar or identical mtDNA and nuclear haplotypes, signify a close genetic relationship between these 2 cryptic species.

Like most crickets, reproduction in *Laupala* involves long distance acoustic communication where males sing and females respond to songs to locate potential mates. Four lines of evidence suggest the potential for reproductive interference between *L. cerasina* and *L. pruna*. First, despite differences in the pulse rate, the songs of *Laupala* species largely overlap in carrier frequency (i.e., the “pitch”), making it unlikely that female ears are tuned to species-specific frequencies (Otte 1994). This circumstance creates the risk of masking interference (Schmidt and Balakrishnan 2015), a phenomenon where acoustic signals in overlapping frequency bands mask each other during transmission, which has been shown to have a negative effect on female mate recognition, localization, and mate choice across a wide range of acoustic communities (Gerhardt and Klump 1988; Wollerman and Wiley 2002; Marshall et al. 2006; Kuczynski et al. 2010). Second, the acoustically active periods of both focal species are between mid-morning and mid-afternoon and overlap to a large extent (Shaw’s personal observation). Third, *Laupala* shows no plant host associations (Mendelson and Shaw 2005), removing a significant source of spatial partitioning that could otherwise reduce heterospecific encounters. Last, likely as a result of all of the above, the songs of *L. pruna* and *L. cerasina* exemplify hyper-dispersion in the pulse rate, which may reflect reproductive character displacement (Pfennig and Pfennig 2009), a pattern found when comparing the songs of numerous sympatric *Laupala* species (Otte 1994). These characteristics collectively suggest selective responses that would alleviate the negative effects of species interactions on reproductive activities.

Spatial segregation (i.e., physical separation in space) between signaling individuals of the sympatric species is a frequently proposed mechanism that mitigates masking interference between acoustic signals (Bee and Micheyl 2008; Schmidt and Römer 2011) and improves the efficiency of sexual communication (Gröning and Hochkirch 2008; Schmidt and Balakrishnan 2015). Spatial segregation can occur on multiple spatial scales. On a broad geographic scale, where the species within the local community is treated as the unit, we hypothesized that sympatric *L. pruna* and *L. cerasina* would be spatially segregated (i.e., species distributions in the local community show no overlap). The rationale is that such a spatial distribution may reduce reproductive interference. We quantified the components of acoustic niche partitioning and describe spatial distributions of both species in the horizontal and vertical dimensions as well as calling site types. Using the spatial distribution data, we first tested the alternative hypotheses that the 2 sympatric species are (1) spatially segregated and (2) spatially mixed at a broad geographic scale within the local community. Because we found that the 2 species were mixed on the community level, we further examined individual spacing patterns on the individual scale where individual calling males were treated as the unit (i.e., pattern of distances between neighboring individuals). We expected heterospecific males to be spatially more dispersed (i.e., farther apart in physical space) than conspecific males based on the similar rationale that such an individual spacing pattern would reduce reproductive interference. An alternative expectation, however, was that conspecific males would be more dispersed than heterospecific males if the cost of conspecific competition outweighs that of reproductive interference. We tested these alternative individual spacing patterns by comparing nearest neighbor distance between conspecific and heterospecific neighbors. We discussed the implications of the observed calling male distribution pattern on reproductive boundaries and the evolution of species coexistence in nonadaptive radiations.

Methods

Study Species and Study Site

Laupala cerasina and *L. pruna* frequently occur in sympatry across the windward slopes of the Big Island, Hawaii to which they are endemic. This study was conducted in the Kahauale'a Natural Area Reserve (19.41°N, 155.13°W) where both male and female *L. cerasina* and *L. pruna* of all life stages co-occur. Males of both species emit calling songs to attract females. The calling songs of these 2 species are characterized by trains of pulses produced by wing stridulation. Songs of these 2 species have distinctive pulse rates but similar carrier frequencies and pulse durations (Otte 1994; Shaw and Parsons 2002; Grace and Shaw 2011). Both species are widely distributed and can be heard in large numbers during the daytime in the forest at our study site. The main vegetation type at Kahauale'a is the hapu'u tree fern *Cibotium glaucum* and the 'Ōhi'a lehua (*Metrosideros polymorpha*) mixed forest with isolated patches of the invasive kāhili ginger *Hedychium gardnerianum*. We logged the temperature at the survey sites with UA001-08 HOBO pendant temperature data loggers (Onset Computer Corporation, Mourne, USA) every 15 min consecutively in August 9–11, 2016. Temperature loggers were placed in tree ferns near calling males.

Calling Male Localization

Surveys of calling males were conducted in 3 randomly chosen quadrats at the study site. The 3 quadrats covered 3 major habitat types: closed canopy forest, open canopy forest with small forest gaps, and tree fern-ginger mixed patches (Table 1). All quadrats were surveyed in the morning between 10:15 and 12:30, August 9–13, 2016, during which time the male singing behavior of both species overlap. Surveys were conducted in partial sun to overcast sky conditions. Light rain occasionally occurred; no survey was conducted in heavy rain. To locate calling males within each quadrat, a single observer systematically walked through the quadrat, locating singing males with the assistance of a unidirectional Enersound lav-100 microphone (Enersound, West Park, USA) mounted to a wooden dowel and connected to an Olympus VN 722PC digital audio recorder (Olympus America Inc., Center Valley, USA). The precise location of a calling male was determined by pinpointing the location at which the received song signal had the highest amplitude. Calling males were occasionally disturbed but resumed singing within a minute from the same location. The positions of calling males were marked with flags. After all males were marked on the first walk through, the observer re-walked the quadrat in order to identify any crickets missed on the first pass. For each calling male, species identity (unambiguously evident from song pulse rate), calling site type, height of calling position, and horizontal distance from nearest neighbor were noted. We recorded at least 30 s of calling song of 21 *L. cerasina* males and 19 *L. pruna* males with the above recording equipment. Recordings were not possible for 19 males due to rain that started before recordings could be obtained.

Acoustic Measurement and Analysis

To quantify the level of signal divergence between species, we measured the pulse rate (i.e., number of pulses / s), pulse duration, and carrier frequency from 5 independent samples in the recording of each male using Raven Pro 1.4 (http://ravensoundsoftware.com). Pulse rate and pulse duration measurements were made at a resolution of 0.001s, resulting in mean coefficients of variation at 0.8% and 3.7%, respectively. Because spectral similarity of the songs depends not only on the mean and range of the carrier frequency, but also on the relative power emitted at each frequency, we also measured the power spectra (i.e., distribution of relative acoustic power over the frequency range of the song) from both species. A higher proportion of overlap between the power spectra indicates a high level of spectral similarity between the 2 species. Power spectra were measured from 21 *L. cerasina* and 18 *L. pruna* males from field recordings as well as 2 *L. cerasina* and 2 *L. pruna* males recorded in a temperature-controlled, ETS-Lindgren predictable field acoustic enclosure (Acoustic Systems, Austin, USA) at Cornell University, Ithaca, NY. Specifically, we obtained the power spectrum from 1 representative pulse from each male at a frequency resolution of 1.3 Hz in Raven Pro. To standardize power spectra between values of 0 and 1, we normalized power measurements at each frequency by

$$x' = \frac{x - x_{min}}{x_{max} - x_{min}}$$

where x is the power measured at a specific frequency, x_{min} and x_{max} are the minimum and maximum values of power over the entire range of frequencies measured, respectively, and x' is the normalized power. To exclude nonsong noises, we subsequently filtered the normalized power spectra with a band pass filter between 3700 and 5700 Hz (the frequency range corresponding to the lower and upper boundaries of the carrier frequencies for both species as measured from data herein).

Table 1. Habitat type, areas of the quadrats surveyed, and the number of calling males of *Laupala cerasina* and *L. pruna* in each quadrat. R_{lc}, R_{lp}, and R_{pooled} are the clumping parameter for the horizontal distribution of *L. cerasina*, *L. pruna*, and pooled species, respectively. $R < 1$: clumped distribution, $R = 1$: random distribution, and $R > 1$: uniform distribution. The p values for the corresponding R are from Z-tests comparing R values with 1

Quadrat	Habitat type	Area (m^2)	No. of *L. cerasina* males	No. of *L. pruna* males	R_{lc}	p_{lc}	R_{lp}	p_{lp}	R_{pooled}	p_{pooled}
1	closed canopy forest	100	11	17	0.16	<0.001	0.11	<0.001	0.29	<0.001
2	open canopy forest	100	11	3	0.2	<0.001	0.24	<0.001	0.22	<0.001
3	fern-ginger mixed	75	7	6	0.71	0.2	0.23	<0.001	0.45	<0.001

Statistical Analysis

To investigate acoustic differentiation between the 2 species, we compared the pulse rates, pulse duration, and carrier frequencies using linear mixed models (LMMs) with males nested within quadrats. We also calculated the proportion of the overlap between the mean power spectra of the 2 species. In the above analyses, we excluded carrier frequency measurements of 2 males and pulse duration measurements of 2 males (one of which was also a carrier frequency outlier, marked in Figure 1b, c) that represent outliers on the left tail of the distribution from the respective models.

To examine whether calling males of the 2 species have overlapping or segregated spatial distributions, we first describe the horizontal distribution of calling males using a nearest neighbor framework. Specifically, we calculated the expected mean and standard error for the horizontal distance to the nearest neighbor under a random distribution (r_e and σ_{re}) and the observed median distances to nearest neighbors (r_o) for each quadrat according to the method described by (Clark and Evans 1954) with a correction for border effects (Donnelly 1978). Because the observed distances to nearest neighbors were positively skewed, medians, instead of mean distances, were used in the calculation. The ratio between the observed and expected distances to the nearest neighbor ($R = r_o / r_e$) was used to indicate a deviation from a random distribution and statistical significance was tested with a *Z*-test ($Z = (r_o - r_e) / \sigma_{re}$). An *R* value significantly less than 1 indicates a clumped distribution, an *R* value significantly greater than 1 indicates a uniform distribution, whereas an *R* value nonsignificantly different from 1 indicates a random distribution.

We then determined whether calling males of the 2 species spatially overlap or segregate on 1) the horizontal axis, 2) the vertical axis, or 3) by calling site type. On the horizontal axis, if males segregate according to species identity, we expect that a calling male's nearest neighbor is more likely than expected to be conspecific based on species abundance in the quadrat. If the 2 species overlap spatially, we expect the probability of species identity of a calling male's neighbor to be not significantly different from that expected from the species abundances in the quadrat. In addition, we compared the calling height of the 2 species using a generalized linear mixed model (GLMM) with Poisson error distribution and with males nested within quadrats and calling site type. Calling site type consisted of tree ferns, trees, ginger plants, and leaf litter on forest floor.

If the species demonstrate overlapping distributions, we further compared conspecific and heterospecifics neighbor distances using a linear mixed model (LMM) with neighbor pairs nested in quadrats and using log-transformed distance in 3D space. We also compared the variance in distance between conspecific and heterospecific neighbors using a Brown–Forsythe test that is robust to violation of the normal distribution.

Throughout, variable values are shown as mean and standard error for normal error distributions and as median and interquantile range (IQR) for nonnormal distributions. All statistical tests were conducted in R 3.3.0 (R Core Team 2016).

Results

At our study site, we estimated the relative frequencies of *L. cerasina* and *L. pruna* calling males to be 46.6% (*n* = 34) and 53.4% (*n* = 39), respectively. The daily mean temperature at the study site was 18.6 °C, ranging from 15.6 °C to 26.2 °C. The mean temperature during surveying hours was 21.0 °C, ranging from 18.2 °C to 22.7 °C.

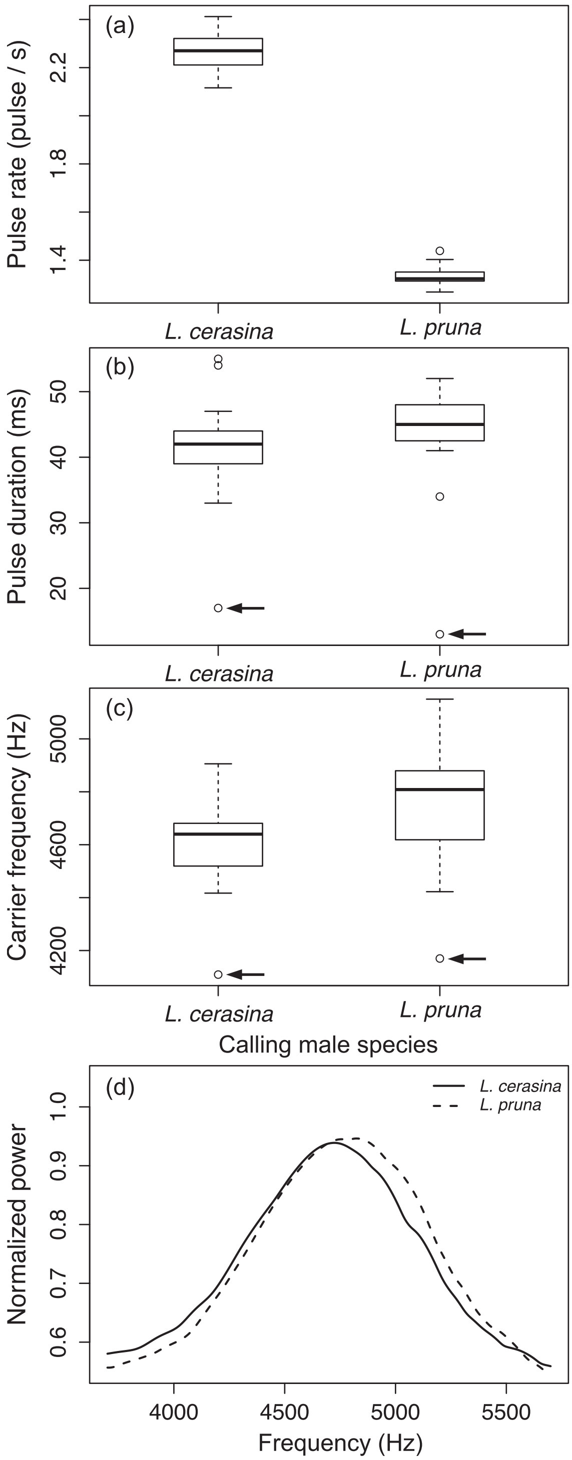

Figure 1. Pulse rate (a), pulse duration (b), carrier frequency (c), and normalized power spectra (d) of male *L. cerasina* (*n* = 21) and *L. pruna* (*n* = 19) from field recordings at a sympatric site in Kahauale'a, Hawaii. The arrows in (b) and (c) indicate outliers that were excluded from statistical analysis.

Compared to *L. pruna*, field recordings show that *L. cerasina* exhibits a significantly faster pulse rate (*L. cerasina*: 2.27 ± 0.02 pulse / s, *n* = 21; *L. pruna*: 1.33 ± 0.01 pulse / s, *n* = 19, $\chi^2 = 3074$, df = 1, $p < 0.0001$, Figure 1a), significantly shorter pulse duration (*L. cerasina*: 42.3 ± 1.2 ms, *n* = 20; *L. pruna*: 45.4 ± 1.0 ms, *n* = 18, $\chi^2 = 6.23$, df = 1, $p = 0.01$, Figure 1b), and significantly lower carrier

Table 2. The expected and observed proportions of nearest neighbors that were conspecifics for *L. cerasina* and *L. pruna* in sympatric quadrats and *p* values from binomial tests for deviation from a random distribution

Quadrat	*L. cerasina*				*L. pruna*			
	O(%con)	*E*(%con)	*n*	*p*	O(%con)	E(%con)	*n*	*p*
1	63.6	37.0	11	0.11	76.5	59.3	17	0.22
2	81.8	76.9	11	1.00	0.0	15.4	3	1.00
3	57.1	50.0	7	1.00	50.0	42.7	6	0.70

frequency (*L. cerasina*: 4638.9 ± 29.0 Hz; *L. pruna*: 4785.3 ± 43.2 Hz, $\chi^2 = 4.83$, df = 1, $p = 0.02$, Figure 1c). However, while pulse rates between the 2 species were substantially nonoverlapping, the phenotypic distributions of pulse duration and carrier frequency overlapped largely between the 2 species (Figure 1b and c). The mean power spectra did not differ between species in field recordings (overlapped for ~ 98.8% regardless of species); lab recordings of field caught males were likewise broadly overlapping (98%–99.6% regardless of species).

The horizontal distributions of calling males were significantly more clumped than expected by chance for both species, except for *L. cerasina* in the ginger-fern mixed quadrat (Table 1). When species were pooled, calling male distribution was also clumped in all 3 quadrats. Conspecific males were not more likely to be nearest neighbors than expected by chance, based on relative species abundance (Table 2). On average, *L. cerasina* called from significantly higher positions in the forest (median = 41.0 cm, IQR = 58.0 cm) than *L. pruna* (median = 19.5 cm, IQR = 49.3 cm, LMM: $\chi^2 = 4.63$, df = 1, $p = 0.03$, Figure 2). However, the height ranges occupied by the 2 species overlap broadly (Figure 2). The 2 species also did not differ in their calling site use, with the greatest proportions of both species found on tree ferns, followed by leaf litter and ginger for both species and none found on woody trees (Figure 3, $\chi^2 = 0.75$, df = 6, $p = 0.99$). Pooling data from both species, male distributions were significantly heterogeneous across calling site types ($\chi^2 = 39.03$, df = 3, $p < 0.001$). Out of all tree ferns ($n = 47$) and woody trees ($n = 40$) in the quadrats, 46.8% of tree ferns had at least 1 cricket calling from it, compared to 0% of trees.

Calling clusters consisted of both species. We found variance of conspecific neighbor distance to be significantly greater than variance in heterospecific neighbor distance ($F_{1,33.2} = 10.83$, $p = 0.002$). After log transformation, the nearest neighbor distance differed between conspecific neighbors and heterospecific neighbors (conspecific: median = 0.66 m, IQR = 0.77 m; heterospecific: median = 0.32 m, IQR = 0.09 m, $\chi^2 = 4.24$, df = 1, $p = 0.04$, Figure 4). Surprisingly, the nearest neighbor distance was smaller for heterospecific neighbors than for conspecific neighbors (Tukey's HSD test for LMM, z = 2.06, $p = 0.04$).

Discussion

The build-up of closely related species in sympatry amplifies the process of both adaptive and nonadaptive radiation by accumulating relatively more species in a given geographic area. Reproductive differentiation in sympatry is an integral component of both adaptive and nonadaptive radiations (Coyne and Orr 2004; Weber and Strauss 2016). However, in a nonadaptive radiation, the lack of ecological adaptation and ecologically segregated use of space among sympatric species may result in higher rates of heterospecific encounters relative to adaptive radiations, enhancing the opportunity for selection to strengthen reproductive barriers. We should therefore expect divergence in reproductive phenotypes in a nonadaptive radiation to be pronounced due to its diminished effect on reproductive interference between heterospecifics.

Sexual communication using the same signaling channels by closely related sympatric species can lead to signal interference and/or hybridization. In addition to divergence in the specific signal and preference phenotypes, the choice of signaling site can impact the level of conspecific and heterospecific noise a signaler must contend with (Wiley 2015), which in turn, may influence the potential for hybridization (Gerhardt and Klump 1988; Marshall et al. 2006; Kuczynski et al. 2010; Richardson and Lengagne 2010; Reichert and Ronacher 2015; Wiley 2015). Decisions on where to signal thus can have a strong impact on the reproductive boundary between sympatric species (Gröning and Hochkirch 2008; Burdfield-Steel and Shuker 2011). Using *L. pruna* and *L. cerasina*, a pair of congeneric species of Hawaiian crickets that exemplify secondary overlap of a nonadaptive radiation, we tested the hypothesis that calling males of sympatric species are spatially segregated within a local acoustic community. Below, we discuss the implications of the observed spatial distribution pattern on the efficacy of sexual communication, reproductive segregation, and species coexistence.

Acoustic analysis demonstrated the opportunity for interference between heterospecific songs. Although pulse rates differed substantially between *L. cerasina* and *L. pruna*, the acoustic signals of the 2 species are highly similar in other respects. Carrier frequencies among males of the 2 species are broadly overlapping, and the magnitude of the difference between the 2 species, while significant, is minor (~0.15 kHz). Moreover, the power spectra (distribution of emitted energy across the frequency range) of the 2 species overlap nearly 100% (Figure 1d), strongly suggesting that the signals of the 2 species compete in the same frequency channel. Due to the lack of frequency separation, it is highly likely that females can perceive the songs of both species. Frequency separation of signals and tuning of the receiver's ears are commonly observed among closely related species in acoustic communities (Chek et al. 2003; Schmidt et al. 2012) and have been proposed as an evolutionary response to mitigate signal masking and interference (Bee and Micheyl 2008; Schmidt and Balakrishnan 2015). Our data suggest the lack of such displacement in the spectral domain. The pulse durations of the 2 species also differed significantly but likewise to a minor degree (3.1 ms, ~ 7% of pulse duration, Figure 1b). Moreover, female *L. cerasina* have open-ended preference functions for pulse duration, always preferring longer durations even beyond their species range (Shaw and Herlihy 2000; Oh and Shaw 2013). Like carrier frequency, the pulse duration and preference for pulse duration are unlikely to alleviate interference between heterospecific signals. As the calling time of the 2 species overlap extensively, these acoustic features thus favor the hypothesis of segregation of heterospecific signalers in space.

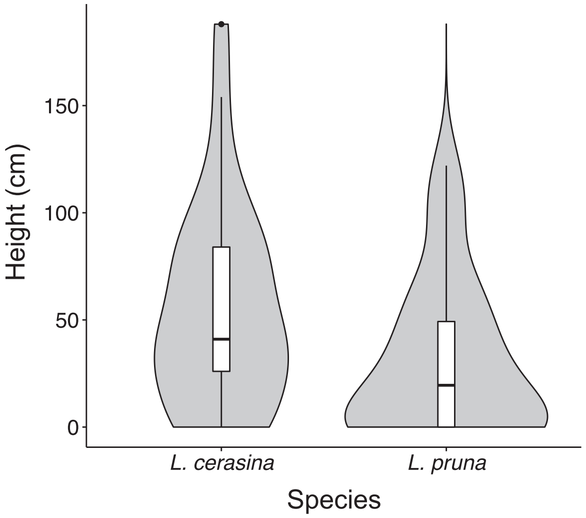

Figure 2. Height distributions of *L. cerasina* and *L. pruna* calling males relative to the ground in the 3 quadrats. The horizontal lines represent the median, the white boxes represent the interquantile range, the whiskers represent 25% and 75% quantiles and dots represent outliers. The widths of the violin plots (the grey shaded area) are proportional to the sample size at a certain height.

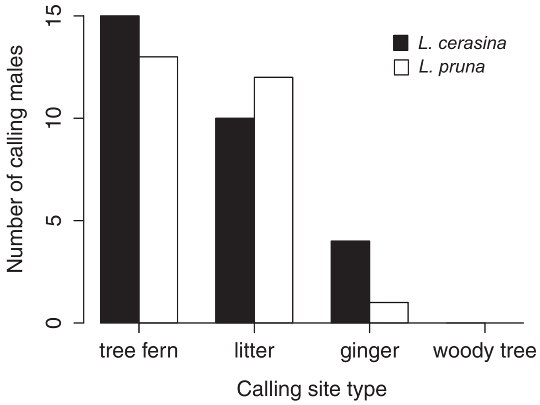

Figure 3. Number of calling males of *L. cerasina* and *L. pruna* found on 4 types of calling sites. The 4 calling site types are hapu'u tree fern *Cibotium glaucum* (treefern), the invasive kāhili ginger *Hedychium gardnerianum* (ginger), the ʻŌhiʻa lehua *Metrosideros polymorpha* (woody tree), and dead leaves on the ground (litter).

Surprisingly, however, the spatial distribution of calling males of the 2 species overlapped to a large extent. While males of the 2 species called from significantly different heights, the calling height distributions of the 2 species broadly overlapped (Figure 2), indicating lack of vertical stratification (i.e., nonoverlapping vertical strata occupied by different species) often observed in acoustic communities of frogs and insects (Ptacek 1992; Sueur and Aubin 2003; Diwakar and Balakrishnan 2007). Horizontally, calling males of both species showed significantly clustered distributions. However, identified calling clusters consisted of males of both species, indicating that the signals of the 2 species are mixed, rather than being sorted by clusters. Conspecific males are not more likely to be nearest neighbors than expected based on species abundance, indicating that calling males of the 2 species overlap in the horizontal dimension as well. Lastly, the 2 species did not differ in the type of calling sites they used. Males of both species preferred tree ferns, followed by leaf litter at the base of the ferns, and then by ginger plants; no individual called from woody trees (Figure 3). Therefore, we conclude that calling males of the 2 species spatially overlap to a large degree in all 3 dimensions (vertical, horizontal, and substrate type). This finding is in contrast to the segregated distribution patterns observed in many cases of adaptive radiation where sympatric species are sorted into different microhabitats (Bentzen et al. 1984; Seehausen 2006; Cocroft et al. 2009; Merrill et al. 2013).

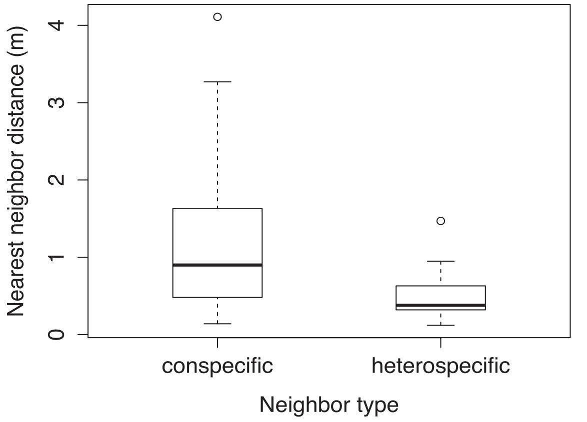

Figure 4. Distance between calling males and their nearest neighbors that were either conspecific or heterospecific.

The highly overlapping, clustered distribution of calling males from the 2 species may be due to similar signaling site preference, with the aggregating effect of shared signaling sites outweighing the segregating effect of acoustic resource competition. This is evident from the fact that both species prefer to call from tree ferns (Figure 3). Note that not all tree ferns had calling males. Therefore, it is unlikely that males of the 2 species were forced to occupy the same signaling site due to signaling site saturation. Tree ferns may be the most favored calling site for both species for 3 reasons. First, they offer vertical structures that likely facilitate sound propagation (Marten and Marler 1977). Second, although both woody trees and tree ferns can offer vertical structures, tree ferns provide greater protection against predators from the attached dense fibers, tubular-shaped stipes, and dead fronds. These structures can also provide protection from desiccation. Lastly, because females lay eggs in the fibers and young frond heads of tree ferns (Shaw personal observation), tree ferns may represent traffic hotspots where males are most likely to encounter sexually mature females. As these advantages are shared between both species, males of both species may be motivated to call from the same sites. In addition, a clustered distribution on the tree ferns may also benefit males by decreasing per capita predation risk, a consequence observed in both single-species leks and mixed-species aggregations (Turchin and Kareiva 1989; Wrona and Dixon 1991; Gibson et al. 2002; Kleindorfer et al. 2009; Brunel-Pons et al. 2011).

Interestingly, on the individual scale, heterospecific neighbors were significantly closer to each other than conspecific neighbors (Figure 4). On average, heterospecific neighbors were only 32 cm apart and in 2 cases, they were as close as 2 cm apart, suggesting weak or absent heterospecific aggression. This pattern again is at odds with the expectation of spatial segregation between heterospecifics to reduce reproductive interference. Such a neighbor spacing pattern suggests that the benefit of calling near heterospecific males and/or the cost of conspecific competition outweigh the potential risk of reproductive interference. It has been shown that males signaling in conspecific aggregations attract a larger numbers of females per capita within a certain density range (Walker and Forrest 1989; Klappert and Reinhold 2003; Ritschard et al. 2010; Pacheco and Bertram 2014). In crickets, females prefer louder songs (Huber et al. 1989) and 2 features emerging from the present study suggest that heterospecific songs may also attract females. First, the 2 species' songs are similar in pulse duration and overlap to a great extent in frequency. Second, from a distance, 2 closely located heterospecific neighbors would be separated by a very small angle relative to the position of a searching female. For example, the angle of approach toward 2 heterospecific neighbors 30 cm apart only differs by 6.4° to a female 3 m away. With such a small separation angle, the songs of the 2 neighboring males would benefit each other in boosting the attraction to their general vicinity. In such a case, compared to a single-species cluster of the same size, males in a mixed-species cluster face lower conspecific competition such that the per capita net benefit may be higher. Given these findings, it is possible that heterospecific neighbors effectively offer a net benefit, rather than a disadvantage. This finding is contrary to numerous reports of aggression towards heterospecific signalers in mixed-species signaling communities that alleviates reproductive interference through spatial segregation or character displacement (Given 1990; Grether et al. 2009; Malavasi and Farina 2013). Together, these results highlight the diversity of heterospecific social interactions in sympatric signaling communities (Sridhar and Guttal 2018).

In summary, our study revealed a highly mixed distribution of 2 closely related cricket species where males position themselves closer to heterospecifics than to conspecific males in an acoustic community. Such a distribution pattern suggests conspecific competition and an advantage to males in the proximity of heterospecifics in the acoustic attraction of females. Although genetic data from this location is unavailable, at 3 other locations, *L. cerasina* and *L. pruna* are genetically distinct based on nuclear sequences (Shaw 2002), suggesting that the reproductive barrier is stable in sympatry despite a mixed distribution. The effectiveness of the reproductive boundary appears to rely heavily on divergence in the signal form *per se* and less on how males are spatially distributed. Intriguingly, divergence of song pulse rate is also observed in the Hawaiian cave planthoppers, including 2 cryptic species that occupy the same cave (Hoch and Howarth 1993; Wessel et al. 2013). How calling individuals distribute in this sympatric community is unknown, yet it provides an evolutionarily independent replicate for future investigation.

While sympatry is common in *Laupala* (Otte 1994) and has persisted for more than 3 decades of observation (Otte 1989), it remains to be tested whether this represents true coexistence or co-occurrence by any number of causes (Siepielski and McPeek 2010). Low levels of heterospecific aggression and high levels of conspecific competition in a highly mixed community may generate negative frequency dependent selection on males. When males in such a mixed community are in the minority, they may receive a 2 part demographic benefit: lowered intraspecific competition and increased acoustic attraction of conspecific females due to the presence of heterospecific song. If true, the signaling site choice behavior of *Laupala* may reduce the risk of ecological drift and facilitate species coexistence when the ecology of the 2 species is similar (Svensson et al. 2018). More community ecological, behavioral, and population genetic data can help test this idea.

Supplementary Material

Supplementary data are available from the *Journal of Heredity* online.

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Author Contributions

MX designed the experiment, collected, and analyzed the data. MX and KS wrote the manuscript.

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Data Accessibility

Acoustic and spatial data and the script for statistical analyses in R are available at https://github.com/MingziXu/Spatial_distribution.

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