

Rapid diversification of sexual signals in Hawaiian *Nesosydne* planthoppers (Hemiptera: Delphacidae): the relative role of neutral and selective forces

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Abstract

Changes in sexual signals have the potential to promote rapid divergence and reproductive isolation among populations of animals. Thus, identifying processes contributing to variation in signals is key to understanding the drivers of speciation. However, it is difficult to identify the processes initiating changes in signals in empirical systems because (1) the demographic history of populations under study is usually unclear, and (2) there is no unified hypothesis-testing framework for evaluating the simultaneous contribution of multiple processes. A unique system for study in the Hawaiian Islands, the planthopper species *Nesosydne chambersi*, offers a clear demographic context to disentangle these factors. By measuring variation in male vibratory sexual signals across different genetic populations on the island of Hawaii, we found that that multiple signal traits varied significantly between populations. We developed a mixed modelling framework to simultaneously test competing hypotheses about which processes contribute to changes in signal traits: genetic drift, sensory drive or reproductive character displacement. Our findings suggest that signal divergence proceeds along different axes for different signal traits under the influence of both neutral and selective processes. They are the first, to our knowledge, to document the relative importance of multiple processes on divergence in sexual signals.

Introduction

The interplay of genetic drift and selection is central to models of how speciation proceeds, but is not well understood in most taxa (Ritchie, 2007; Butlin *et al.*, 2011; Safran *et al.*, 2013). Changes in the production and/or processing of sexual signals have the potential to promote rapid divergence among populations of animals (West-Eberhard, 1983) and changes in sexual communication systems are known to play a central role in shaping patterns of diversity (Claridge & de Vrijer, 1985; Gray & Cade, 2000; Masta & Maddison, 2002). Thus, identifying the processes contributing to

variation in signals and receiver preferences is key to understanding the drivers of speciation.

Initial changes in signal traits may result from a variety of different categories of processes acting in combination or in sequence (Kirkpatrick & Ryan, 1991; Bradbury & Vehrencamp, 1998). Neutral processes are known to be ubiquitous in evolution (Wright, 1931), and drift is thought to be able to initiate changes in signals (Fisher, 1958; Lande, 1976). Selection on signals can also come from a variety of agents. For example, ecological selection may act directly on signals through shifts in ecology that alter the signalling channel, which is known as sensory drive (Endler, 1992; Boughman, 2002; Elias *et al.*, 2010). Sexual selection may also operate on signals in a variety of ways, including runaway processes (Fisher, 1958; Lande, 1981) or selection against mismatching leading to reproductive character displacement (Brown & Wilson, 1956; Howard, 1993; Pfennig &

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Pfennig, 2009). In addition, neutral and selective processes may act in combination or in sequence to promote changes in signalling. For example, drift may initiate changes in mean signal phenotypes between populations that are then driven farther apart via sexual selection through female preference (Uyeda *et al.*, 2009) or reproductive character displacement (Pfennig & Pfennig, 2009).

Determining how neutral and selective processes are acting and with what magnitude is difficult. In part, this is because the demographic history of the populations under study is often complex or unclear (Tregenza *et al.*, 2000), creating a challenge for pinpointing the processes that drove the initial changes in signals. Furthermore, we lack a unified hypothesis-testing framework to evaluate the relative contributions of multiple evolutionary processes that are acting simultaneously (Wilkins *et al.*, 2013). Here, we use the planthopper species *Nesosydne chambersi* that is clearly in the early stages of diversification on the island of Hawaii to explore the simultaneous contribution of different evolutionary processes on the diversification of sexual signals.

Study system

Planthoppers in the Delphacidae family use substrate-borne signals to locate and court mates (Ossiannilsson, 1949; Ichikawa, 1976; de Vrijer, 1984; Claridge, 1985a, b; Claridge & de Vrijer, 1985). Planthopper courtship signals consist of a 'whine' portion followed by a series of pulses (Fig. 1) that travel as substrate-borne vibrations through the host plant tissue. Signals are species-specific (Claridge, 1985a,b), and several temporal traits in the male call can vary among geographic populations (Claridge *et al.*, 1984, 1985; O'Connell, 1991; Butlin, 1993). Although the genetics underlying signal traits have not been studied extensively in this family, temporal traits (including interpulse interval and pulse repetition frequency) have been shown to have a significant heritable component and thus should respond to selection (Heady & Denno, 1991; De Winter, 1995; Butlin, 1996).

The genus *Nesosydne* is distributed throughout islands in the eastern Pacific, with an adaptive radiation of more than 80 species endemic to the Hawaiian Islands (Zimmerman, 1948; Fennah, 1958; Asche, 1997) that specialize on a wide variety of host plants from 20 different host plant families (Zimmerman, 1948; Fennah, 1958; Wilson *et al.*, 1994; Asche, 1997; Roderick, 1997; Roderick & Metz, 1997; Drew & Roderick, 2005; Hasty, 2005). *Nesosydne chambersi* is endemic to the island of Hawaii, where it is relatively widespread. Hawaii Island is the youngest of the Hawaiian archipelago and is composed of five shield volcanoes formed in an age progression from approximately 0.5 million years old to flowing in the present day. While habitats of the older

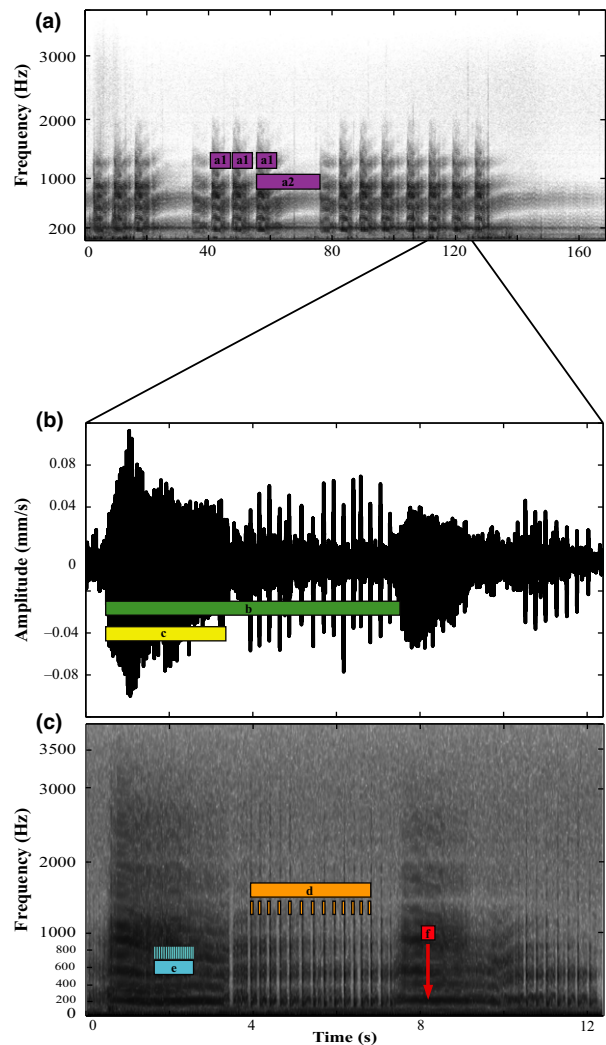


Fig. 1 Vibratory signals (signal traits) of male planthoppers measured in this study. (a) $a1/a2$ = ratio of number of whines per bout/interval between bouts (RSI); (b) b = intersignal interval, c = whine length, (c) d = pulse rate (no. of slow pulses/unit time), e = whine pulse rate (no. of pulses in whine/unit time), f = fundamental frequency.

volcanoes (Kohala, Hualali and Mauna Kea) have remained relatively stable over the last 1500 years, the surfaces of the younger volcanoes (Mauna Loa and Kilauea) are very dynamic and have been covered and recovered in a complicated matrix of different aged lava flows (Trusdell *et al.*, 1996). The genetic structure and phylogeography of *N. chambersi* suggest that it is a species in the process of diversification whose large amount of genetic structure is driven by natural fragmentation and colonization due to volcanic activity within the last few thousand years (Goodman *et al.*, 2012). Populations in this species form a stable zone of secondary contact with clear genetic differences, which

suggests that although individuals are within dispersal range of one another and hybrids can be observed, one or more barriers are limiting gene flow that prevent the populations from fusing (Goodman *et al.*, 2012). Across Mauna Kea and Mauna Loa, *Nesosydne chambersi* feeds on three closely related but architecturally distinct host plants in the silversword alliance (Asteraceae): *Dubautia ciliolata glutinosa*, *D. ciliolata ciliolata* and *D. scabra*. *D. ciliolata glutinosa* are restricted to Mauna Kea, whereas *D. ciliolata ciliolata* and *D. scabra* are each edaphically specialized to lava flows of different ages and are distributed in a patchwork across the Mauna Loa landscape (Robichaux, 1984).

To assess the relative contribution of multiple processes potentially acting to promote signal divergence in *Nesosydne chambersi*, we exploited individual-level differences in signal traits among populations (using a mixed modelling framework) to examine the evolution of vibratory signal traits. In this system, populations are closely related but genetically distinct, and divergence in sexual signals may result from either neutral or selective processes. We constructed a model using signal data from allopatric and sympatric populations of *N. chambersi* that vary in host plant use. We predicted that in signal traits whose divergence is promoted by (a) drift, changes should be associated with time since divergence between populations; (b) sensory drive, changes would be associated with differences in host plant signalling environments; and (c) reproductive character displacement (RCD), changes would be greater in sympatry than in allopatry. Our analysis indicates that these complex acoustic signals diverge rapidly under the influence of multiple processes, both neutral and selective.

Materials and Methods

Collections

Four genetically distinct populations of *N. chambersi* were selected for this study: one from Mauna Kea (collected from *D. ciliolata glutinosa*), one from Mauna Loa (collected from *D. ciliolata ciliolata*) and two from the Saddle region between Mauna Loa and Mauna Kea – Saddle Road High (collected from *D. scabra*) and Saddle Road Low (collected from both *D. ciliolata ciliolata* and *D. scabra*) (Table 1, Fig. 2). Mitochondrial data indicate that the Mauna Loa and two Saddle Road populations each represent an independent colonization to the newly formed habitat on Mauna Loa, which extends into the Saddle region between the two volcanoes (Goodman *et al.*, 2012; Fig. 2). The two populations from the Saddle region form a zone of secondary contact. However, despite occasional hybridization, relatively little contemporary or historic gene flow has occurred (Goodman *et al.*, 2012), suggesting that some mechanism has maintained the integrity of these popu-

lations. *N. chambersi* individuals were collected live from the four populations described above. Males and females were separated upon returning to the laboratory and maintained on cuttings of the host plant species (and subspecies) from which they were collected in the field.

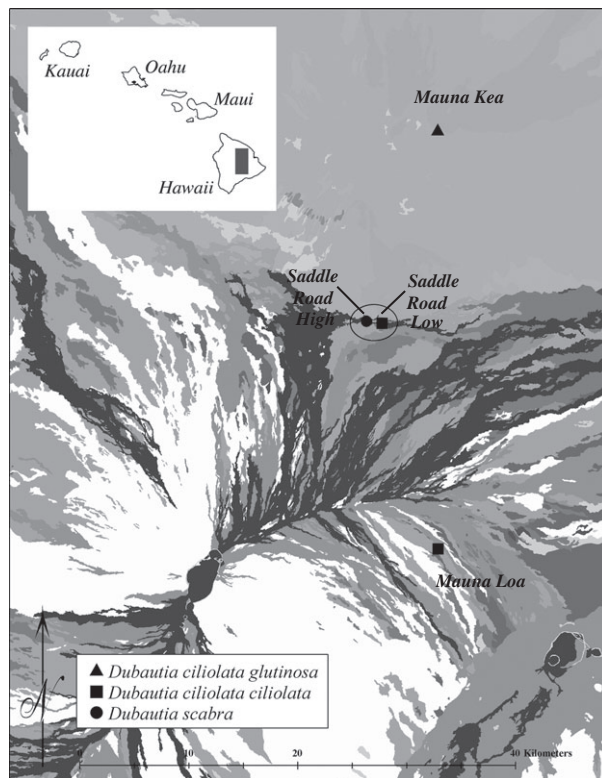
Male signal recording

Nesosydne chambersi's vibrational signals were recorded on site at the USGS Biological Resource Division laboratory facilities in Hawaii Volcanoes National Park in May and September of 2009. Experimental chambers were built using insect rearing cages. The cages were positioned on boxes, and plant cuttings were placed inside with the stems emerging from a hole in the bottom. The cuttings were stabilized in sand, and the space around the hole was sealed with cotton to prevent planthoppers from escaping. One or two field-collected males were released into each cage and allowed to settle onto the vegetation. After approximately 10–30 min, one female was released into the cage and activity was monitored until calling began. If calling did not commence, an additional one or two females were released into the cage. Signals were analysed from a single male per plant. If two males were introduced to a given plant, males were distinguished on the basis of call intensity. All experiments were conducted on the same host plant species or subspecies from which the individuals were collected in the field.

Two recording methods were used to obtain mating signals: an accelerometer and a laser vibrometer. For accelerometer recordings, a USB Powered-Dual Channel ICP Sensor Signal Conditioner (Model 485B36) connected to a pre-amplifier (iMic, Griffin Technology) was secured to a wire clip and then attached to the plant stem below the cage. Signals were recorded using AUDACITY software (version 1.2, 44.1 kHz sampling rate: Audacity, 2008) on a Mac PowerBook G4. For laser vibrometer recordings, we used a Portable Digital Vibrometer (PDV-100, Polytec) connected to a High Resolution Audio Recorder (Sound Devices 722, 48 kHz sampling rate). The laser beam was focused onto reflective tape at the centre of the plant, and laser recordings were resampled to a 44.1 kHz sampling rate prior to trait scoring. Before performing statistical analyses, we determined that there were no differences in fundamental frequency for the two recording methods (general linear model, $F_{1,13}=0.022$, $P=0.885$). Some traits were corrected for temperature, a variable known to influence some signal traits (Gerhardt & Huber, 2002). Specifically, we used linear regression to determine whether temperature was related to the variation in signal traits of interest; variables that had significant associations with temperature were corrected to 22.5 °C.

Table 1 Sampling Locations and Site Characteristics. Host plant indicates the host plants that individuals were collected from in the field and on which recordings were made in the laboratory.

Population name	Host plant	Elevation (m)	Age of substrate (years)	Latitude	Longitude
Mauna Kea (<i>Pu'u Kanakaleonui</i>)	<i>Dubautia ciliolata glutinosa</i>	2850	14 000–65 000	–155.391	19.847
Saddle Road High	<i>Dubautia scabra</i>	1950	<200–3000	–155.452	19.689
Saddle Road Low	<i>Dubautia scabra</i>	1950	<200–3000	–155.439	19.686
	<i>Dubautia ciliolata ciliolata</i>				
Mauna Loa Trail (<i>Hawaii Volcanoes National Park</i>)	<i>Dubautia ciliolata ciliolata</i>	2085	1500–3000	–155.385	19.499

**Fig. 2** Map of collecting locations on the island of Hawaii. Grey box on the inset map of the Hawaiian Islands indicates the extent of the sampling map on the island of Hawaii. The base layer shows lava flow and soil substrates of varying ages, indicated by the shades of grey (Trusdell *et al.*, 1996; Table 1). Black symbols indicate host plants (Table 1).

Sound analyses

Temporal traits

Temporal traits that have been demonstrated to be variable among treehoppers and planthoppers (Claridge *et al.*, 1985; Sattman & Coccoft, 2003; Rodriguez *et al.*, 2006) were selected for analysis in this study. Multiple signals per male were scored and averaged. Traits measured included (with units and sample sizes for n =number of individual males and x =mean number of samples

scored per individual): whine duration (seconds: $n = 78$, $x = 15.1$); whine pulse rate (count/second: $n = 67$, $x = 8.9$), slow pulse rate (count/second: $n = 55$, $x = 9.0$), interpulse interval (seconds: $n = 72$, $x = 12.3$) and ratio of the number of signals per bout to the interbout interval (RSI) ([count/second]: $n = 44$, $x = 6.7$) (Fig. 1). Temporal traits of signals were visualized and measured using AUDACITY software (Audacity, 2008). For each trait, 6–12 samples were measured for each individual male and then averaged to obtain a single score for each individual.

Frequency traits

N. chambersi signals are generated using a fundamental frequency with a significant amount of additional signal energy concentrated in harmonics (Fig. 1). We examined frequency characteristics by comparing fundamental and dominant frequency among populations. All frequency spectra were measured using normalized power spectra (window size: 4096) calculated in Matlab (The Mathworks).

We obtained measurements of the fundamental and dominant frequencies of whines for 59 individuals and calculated power spectra of each signal by averaging 1-s segments from the centre of five separate whines, avoiding the frequency modulations found at the beginning and end of the whines. Peak intensities were normalized to the maximum intensity within each measurement, and peak positions identified using custom written Matlab scripts. Whereas background noise was strong between 0 and 95 Hz in closely related and similarly sized insects (treehoppers, Hemiptera: Membracidae), fundamental frequency ranges between 100 and 450 Hz (Sattman & Coccoft, 2003; Rodriguez *et al.*, 2004; McNett & Coccoft, 2008), and thus it is likely the important frequency elements were captured in our model.

Statistical analysis

Associations between signal traits within individuals

To estimate the degree to which signal traits covary with each other, we evaluated all pairwise combinations using generalized additive mixed models (GAMMs). This modelling framework uses nonparametric

smoothing of continuous terms to assess associations between traits without imposing relationships on the data (e.g. linear, quadratic). For each model, we included a random effect of population to account for nonindependence and clustering in raw data caused by different numbers of individuals recorded within each population. We specified a Gamma error structure to account for strong positive skew of some variables. The residuals of all final models were normal. Significance tests for smoothed terms in GAMMs are approximate and should be treated conservatively (Wood, 2006). Therefore, each trait pair was categorized as to have a strong, weak or no association if $P < 0.01$, $0.10 \geq P \geq 0.01$ and $P > 0.1$, respectively. Pairwise associations between signal traits are reported in Appendix S2 and Fig. S2.

Differences between populations in signal traits

We examined variation in signals using pairwise comparisons among each population pair for each signal trait separately (sample sizes, means and standard errors for each signal trait by population are presented in Table S1). Using a generalized linear model with population identity as the sole independent fixed effect and appropriate error structure (e.g. Gamma), we examined the impact of population identity on signal traits. We tested pairwise differences between each unique population–plant combination and adjusted P -values accordingly to account for multiple comparisons (Tukey post hoc tests) using the ‘multcomp’ package in R (Hothorn & Bretz, 2008; R Core Development Team, 2010). We log-transformed ($\log[x + 1]$) and standardized all response variables after checking the normality of residuals.

Relative importance of genetic drift, sensory drive and RCD

We developed a model to simultaneously assess the relative influence of genetic drift, differences in host plant (sensory drive) and geographic arrangement (sympatry vs. allopatry, a variable we named RCD). We predicted that in traits whose changes are promoted by: (a) drift, changes would be associated with increased time since divergence between populations, if promoted by (b) sensory drive, changes would be associated with differences in host plant, and if promoted by (c) RCD, changes would be greater in sympatry than in allopatry.

Drift (Time)

We used the amount of sequence divergence at the mitochondrial locus cytochrome oxidase I (COI) as a proxy for time, a neutral process that could promote interpopulation differences in signal traits. Incorporating data from previous work (Goodman *et al.*, 2012), we calculated the amount of uncorrected pairwise

sequence divergence at COI among all population pairs in PAUP* (Swofford, 2002).

Sensory drive (Host Plant)

We evaluated the influence of signalling environment, the host plant, on signal trait divergence by testing how signal traits varied between recordings taken from individual planthoppers on different host plant species or subspecies (hereafter referred to as species). We conducted our analyses based on 10 population comparisons (henceforth, ‘population’), designated by each unique geographic population–plant identity combination (e.g. Saddle Road Low – *Dubautia ciliolata ciliolata* vs. Saddle Road High – *Dubautia scabra*). For each population comparison, we included a two-level factor that designated whether comparisons were from recordings taken from individual planthoppers on the same or different host plant species. Because the three species of host plant did not exist in all four locations, it was not possible to test the influence of same or different host plants on signal divergence between each population pair (whereas two species were present at Saddle Road Low (*Dubautia scabra* and *D. ciliolata ciliolata*), only one species was found at Mauna Kea (*Dubautia ciliolata glutinosa*) and Mauna Loa Trail (*D. ciliolata ciliolata*; Fig. S1).

Reproductive character displacement

A hypothesis of reproductive character displacement leads to the prediction that trait divergence would increase more in sympatric than allopatric populations. We tested whether absolute trait divergence between populations was greater in sympatric than allopatric populations or vice versa. A total of three (of 10) population comparisons were designated as sympatric for host species in the model: Saddle Road High (*D. scabra*) versus Saddle Road Low (*D. ciliolata ciliolata*), Saddle Road High (*D. scabra*) versus Saddle Road Low (*D. scabra*), and Saddle Road Low (*D. ciliolata ciliolata*) versus Saddle Road Low (*D. scabra*). The remaining population comparisons ($n = 7$) were designated as allopatric for species of host plant.

We used generalized linear mixed model (GLMMs) to quantify the variation in interpopulation signal trait divergence explained by each of these three factors. To quantify signal trait divergence between each population, we calculated absolute trait differences for each unique pair of individuals (one from each population). For example, for a pair of populations, A and B, the absolute difference in a trait value was calculated between individual i in population A and all individuals in population B. This process was repeated for all individuals in population A for a total of $n_A n_B$ data rows for each population comparison. As this data set contained multiple pairwise differences comprised of individual i , we used a mixed-effect approach with individual identity included as a random effect, nested within each

unique population comparison. The resultant data set included 1321 pairwise comparison from 58 individuals across the 10 populations.

We fit a candidate set of eight models, including one null model (no fixed terms) as well as seven models comprised of all unique combinations of: time, host plant (same or different) and RCD (allopatry versus sympatry). Due to sample size constraints, models with interactions were not tested. In GLMMs, the reference levels (model intercept) for host plant differences and RCD were 'different plant' and 'allopatry', respectively. To facilitate comparisons of the relative impact of these factors, we standardized all responses prior to analysis (mean=0, standard deviation =1). Mitochondrial sequence divergence, the only continuous predictor, was likewise standardized. We log-transformed absolute signal trait divergence for three signal traits (dominant frequency, interpulse interval and slow pulse rate) to achieve normal residuals; all other signal traits were untransformed prior to standardization. For two signal traits (whine duration and RSI), models with mitochondrial sequence divergence as a fixed effect exhibited high heterogeneity of residual variance. Therefore, to meet the assumptions of generalized linear models, we specified a fixed and inverse variance structure for mitochondrial sequence divergence in all models of whine duration and RSI, respectively.

We used Akaike's information criterion corrected for small sample sizes (AIC_c) to compare models (Burnham & Anderson, 2002). We calculated change in AIC_c (ΔAIC_c) relative to the best-supported model, or the model with the lowest AIC_c , as well as Akaike weights for each model. For all response variables, no singular model had an Akaike weight exceeding 0.90. Therefore, we calculated model-averaged parameter estimates using the entire candidate set (Tables S3a,b), weighted by each model's AIC_c (Burnham & Anderson, 2002). Significance of model-averaged parameter estimates is based on a z-test; thus, no degrees of freedom are reported. We calculated relative importance, ranging from 0 to 1, of each factor by summing the Akaike weights of those models in which the factor appeared. All models included individual identity as a random effect, nested within each unique population comparison, as described above. Inference is drawn from model-averaged β and standard errors and whether or not confidence intervals overlap zero; *P*-values are provided as an approximation of term significance.

All statistical analyses were conducted in R 2.12.1 (R Core Development Team, 2010). Generalized linear mixed models (GLMMs) were fit using the lmer function in package 'lme4' (Bates *et al.*, 2011). Generalized additive mixed models (GAMMs) were implemented using the gamm function in package 'mgcv' (Wood, 2006, 2011). Examination of residuals of global and final models confirmed that assumptions of residual normality and homoscedasticity were met.

Results

Differences in signal traits across populations

All signal traits except dominant frequency and whine pulse rate differed significantly in pairwise comparisons of populations across the island, indicating that signals have diversified among geographic populations (Table 2, Fig. 3).

Relative role of neutral vs. selective processes: genetic drift, sensory drive and RCD

Drift (Time)

Mitochondrial sequence divergence (our proxy of time) was related to divergence in several signalling traits, both in the direction we predicted and in the opposite direction. Increased mitochondrial divergence corresponded to increased signal trait divergence for slow pulse rate ($\beta = 0.12$, $SE = 0.05$, $z = 2.35$, $P = 0.019$; relative importance=0.87). Conversely, increased mitochondrial divergence corresponded to reduced trait divergence in interpulse interval ($\beta = -0.14$, $SE = 0.07$, $z = -2.13$, $P = 0.033$; relative importance=0.72), whine pulse rate ($\beta = -0.25$, $SE = 0.09$, $z = -2.87$, $P = 0.004$; relative importance=0.87) and weakly in fundamental frequency ($\beta = -0.14$, $SE = 0.08$, $z = -1.82$, $P = 0.080$; relative importance=0.68) (Table 3, Fig. 4).

Sensory Drive (Host Plant)

We found no significant effect of signalling environment, the host plant, on signal trait divergence (fundamental frequency: $\beta = -0.14$, $SE = 0.14$, $z = -1.02$, $P = 0.317$; dominant frequency: $\beta = -0.12$, $SE = 0.22$, $z = 0.56$, $P = 0.574$; interpulse interval: $\beta = -0.07$, $SE = 0.15$, $z = 0.46$, $P = 0.645$; whine duration: $\beta = -0.14$, $SE = 0.15$, $z = -0.92$, $P = 0.356$; whine pulse rate: $\beta = -0.18$, $SE = 0.18$, $z = -0.97$, $P = 0.333$; slow pulse rate: $\beta = 0.08$, $SE = 0.10$, $z = 0.75$, $P = 0.456$; RSI: $\beta = -0.02$, $SE = 0.36$, $z = -0.07$, $P = 0.948$; Table 3, Fig. 4).

Reproductive character displacement

Whine pulse rate was significantly influenced by the variable RCD in the opposite direction from our prediction; it showed increased divergence in allopatric population comparisons ($\beta = -0.64$, $SE = 0.20$, $z = -3.27$, $P = 0.001$; relative importance=0.93). Furthermore, three additional traits showed evidence of marginally significant influence of this variable and warrant discussion: fundamental frequency showed increased divergence in allopatric population comparisons ($\beta = -0.28$, $SE = 0.16$, $z = -1.72$, $P = 0.080$; relative importance=0.64), whereas whine duration ($\beta = 0.23$, $SE = 0.14$, $z = 1.62$, $P = 0.106$; relative importance=0.37) and RSI ($\beta = 0.41$, $SE = 0.33$, $z = 1.24$, $P = 0.062$; relative importance=0.43) showed increased divergence in the sympatric population comparisons. In these traits, small

Table 2 Summary of differences among population–plant combination in signal traits. Host plant identity: *Dubautia ciliolata glutinosa* (DCG), *Dubautia ciliolata ciliolata* (DCC), *Dubautia scabra* (DS). Population identity: Mauna Kea (MK), Mauna Loa (MLT), Saddle Road High (SRH), Saddle Road Low (SRL). Shown are β parameters (on the first line) and standard errors (on the second) obtained from generalized linear model comparing each population and host plant combination. Wald's Z-test statistics are shown on the third line and approximate P-values on the fourth. Pairwise differences were assessed from generalized linear models accounting for multiple comparisons (Tukey post hoc test). For each population comparison, the reference population in the comparison is indicated first. Samples sizes for each population are indicated below the population names (sample size of reference population, sample size of second population). For example, for fundamental frequency in the 'MK-DCG, MLT-DCC' comparison (row 1), fundamental frequency is lower in MLT-DCC ($\beta = -0.884$). Bold indicates that comparisons are significant at the <0.05 level.

Population–host plant comparison (n_{pop1}, n_{pop2})	RCD	Fundamental frequency	Dominant frequency	Interpulse interval	Whine duration	Whine pulse rate	Slow pulse rate	RSI
MK-DCG MLT-DCC (12,15)	Allopatry	-0.884 (0.368) $z = -2.401$ $P = 0.016$	-0.151 (0.428) $z = -0.354$ $P = 0.724$	1.288 (0.314) $z = 4.109$ $P < 0.001$	0.687 (0.312) $z = 2.200$ $P = 0.028$	-1.268 (0.313) $z = -4.050$ $P < 0.001$	-0.121 (0.408) $z = -0.297$ $P = 0.766$	-0.546 (0.351) $z = -1.553$ $P = 0.120$
MK-DCG SRH-DS (12,14)	Allopatry	-0.866 (0.374) $z = -2.317$ $P = 0.021$	0.427 (0.417) $z = 1.024$ $P = 0.306$	0.524 (0.318) $z = 1.648$ $P = 0.099$	-0.628 (0.316) $z = -1.985$ $P = 0.047$	-0.860 (0.323) $z = -2.659$ $P = 0.008$	0.469 (0.408) $z = 1.149$ $P = 0.251$	0.679 (0.365) $z = 1.862$ $P = 0.063$
MK-DCG SRL-DCC (12,6)	Allopatry	-1.319 (0.475) $z = -2.774$ $P = 0.006$	-0.509 (0.500) $z = -1.018$ $P = 0.309$	1.207 (0.351) $z = 3.436$ $P = 0.001$	0.399 (0.338) $z = 1.180$ $P = 0.238$	-1.641 (0.337) $z = -4.874$ $P < 0.001$	0.608 (0.485) $z = 1.253$ $P = 0.210$	-0.997 (0.431) $z = -2.312$ $P = 0.021$
MK-DCG SRL-DS (12,11)	Allopatry	-0.654 (0.397) $z = -1.647$ $P = 0.099$	0.182 (0.428) $z = 0.425$ $P = 0.671$	1.176 (0.335) $z = 3.507$ $P < 0.001$	-0.320 (0.326) $z = -0.981$ $P = 0.327$	-1.175 (0.345) $z = -3.409$ $P = 0.001$	0.626 (0.441) $z = 1.420$ $P = 0.156$	-0.823 (0.494) $z = -1.667$ $P = 0.095$
MLT-DCC SRH-DS (15,14)	Allopatry	0.018 (0.353) $z = 0.050$ $P = 0.960$	0.578 (0.437) $z = 1.324$ $P = 0.185$	-0.764 (0.308) $z = -2.479$ $P = 0.013$	-1.315 (0.302) $z = -4.354$ $P < 0.001$	0.408 (0.313) $z = 1.304$ $P = 0.192$	0.590 (0.372) $z = 1.584$ $P = 0.113$	1.225 (0.342) $z = 3.581$ $P < 0.001$
MLT-DCC SRL-DCC (15,6)	Allopatry	-0.435 (0.459) $z = -0.946$ $P = 0.344$	-0.358 (0.516) $z = -0.693$ $P = 0.488$	-0.081 (0.342) $z = -0.237$ $P = 0.812$	-0.288 (0.325) $z = -0.885$ $P = 0.376$	-0.372 (0.327) $z = -1.140$ $P = 0.254$	0.730 (0.456) $z = 1.600$ $P = 0.110$	-0.451 (0.412) $z = -1.095$ $P = 0.274$
MLT-DCC SRL-DS (15,11)	Allopatry	0.230 (0.377) $z = 0.610$ $P = 0.542$	0.333 (0.447) $z = 0.746$ $P = 0.456$	-0.112 (0.326) $z = -0.344$ $P = 0.731$	-1.007 (0.312) $z = -3.225$ $P = 0.001$	0.093 (0.335) $z = 0.277$ $P = 0.781$	0.747 (0.408) $z = 1.831$ $P = 0.067$	-0.278 (0.477) $z = -0.582$ $P = 0.560$
SRH-DS SRL-DCC (14,6)	Sympatry	-0.452 (0.459) $z = -0.984$ $P = 0.325$	-0.936 (0.516) $z = -1.813$ $P = 0.070$	0.683 (0.342) $z = 1.994$ $P = 0.046$	1.028 (0.325) $z = 3.161$ $P = 0.002$	-0.781 (0.327) $z = -2.389$ $P = 0.017$	0.140 (0.456) $z = 0.307$ $P = 0.759$	-1.676 (0.412) $z = -4.067$ $P < 0.001$
SRH-DS SRL-DS (14,11)	Sympatry	0.213 (0.377) $z = 0.563$ $P = 0.573$	-0.245 (0.447) $z = -0.548$ $P = 0.583$	0.652 (0.326) $z = 2.000$ $P = 0.046$	0.308 (0.312) $z = 0.987$ $P = 0.324$	-0.315 (0.335) $z = -0.941$ $P = 0.347$	0.157 (0.408) $z = 0.385$ $P = 0.700$	-1.503 (0.477) $z = -3.148$ $P = 0.002$
SRL-DCC SRL-DS (6,11)	Sympatry	0.665 (0.377) $z = 1.761$ $P = 0.078$	0.691 (0.447) $z = 1.546$ $P = 0.122$	-0.031 (0.326) $z = -0.094$ $P = 0.925$	-0.719 (0.312) $z = -2.304$ $P = 0.021$	0.465 (0.335) $z = 1.389$ $P = 0.165$	0.017 (0.408) $z = 0.042$ $P = 0.966$	0.173 (0.477) $z = 0.363$ $P = 0.717$

sample sizes resulted in a lack of power (Table 3, Fig. 4).

Discussion

Our results reveal a complex communication system that is rapidly diversifying in multiple dimensions

under the influence of both neutral and selective processes. Some of our results were consistent with our original predictions – we saw evidence of increased trait divergence with increasing genetic divergence (drift), and we also saw some evidence of greater trait displacement in sympatry than in allopatry (RCD). However, some signal traits were influenced in the

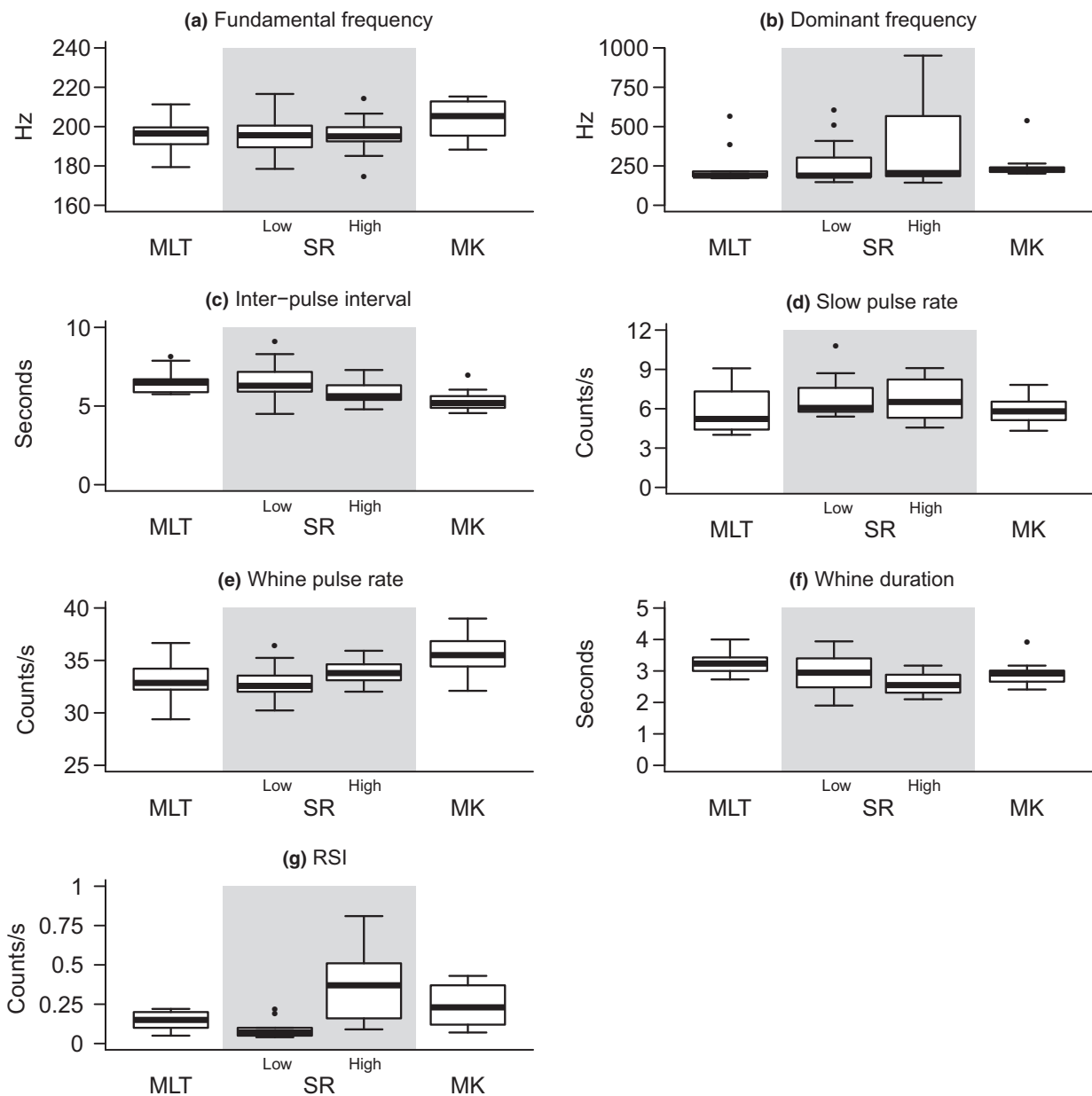


Fig. 3 Distribution of signal trait values by site. Populations in the zone of secondary contact are highlighted in gray. ML, Mauna Loa, SR, Saddle Road, SRL, Saddle Road Low, SRH, Saddle Road High, MK, Mauna Kea. Boxes are Tukey-style boxplots. Thick centre line of each box shows median value. Lower and upper extents of each box represent the first and third quartiles. Whiskers represent the lowest and highest datum within $1.5 \times$ the interquartile range from the first and third quartiles, and all other data appear as points.

opposite direction of our predictions: some traits became more similar with genetic divergence, and some traits were more distinct in allopatry than in sympatry. This variation is consistent with the hypothesis that in multidimensional signals, different components of the signals can serve different purposes (e.g. species recognition, mate quality assessment) and that signal diversification may occur in response to different evolutionary processes acting on different signal

traits (Candolin, 2003; Hebets & Papaj, 2005). These results have several implications for our understanding of how signals evolve.

First, genetic drift is important in signal divergence. Although drift is ubiquitous in evolution, it is typically thought to act slowly in large populations and therefore not be a major force in divergence in most systems (Coyne & Orr, 2004; Sobel *et al.*, 2010). Despite this, we found it to be the sole factor associated with

Table 3 Final models for factors influencing absolute divergence of seven signal traits. For host plant difference, model intercept (or reference level) was 'different plant species', whereas the RCD reference level was allopatry. Parameter estimates (β), standard errors (SE), P -values and 95% confidence intervals are obtained from model averaging of all candidate models. The last column indicates the relative importance, or the summed AIC_c weights across the entire candidate model set, for each predictor. Bold indicates factors for which the standard deviations do not overlap with zero.

Signal trait	Model term	β	SE	z	95% CI		P	Relative importance
					Lower	Upper		
Fundamental frequency	(Intercept)	0.07	0.08	0.98	-0.07	0.22	0.326	-
	time	-0.14	0.08	-1.82	-0.28	0.01	0.069	0.68
	host plant	-0.14	0.14	-1.02	-0.41	0.13	0.307	0.39
	RCD	-0.28	0.16	-1.72	-0.60	0.04	0.086	0.64
Dominant frequency	(Intercept)	-0.05	0.10	-0.53	-0.24	0.14	0.598	-
	Time	-0.02	0.10	-0.18	-0.21	0.18	0.854	0.27
	Host plant	-0.12	0.22	-0.56	-0.55	0.30	0.574	0.30
	RCD	0.14	0.20	0.67	-0.26	0.53	0.503	0.31
Interpulse interval	(Intercept)	0.03	0.07	0.40	-0.11	0.16	0.687	-
	Time	-0.14	0.07	-2.13	-0.27	-0.01	0.033	0.72
	Host plant	-0.07	0.15	-0.46	-0.37	0.23	0.645	0.29
	RCD	-0.01	0.18	-0.07	-0.36	0.33	0.948	0.30
Whine duration	(intercept)	-0.03	0.08	-0.43	-0.18	0.12	0.666	-
	Time	0.04	0.08	0.46	-0.12	0.20	0.644	0.32
	Host plant	-0.14	0.15	-0.92	-0.43	0.15	0.356	0.37
	RCD	0.23	0.14	1.62	-0.05	0.51	0.106	0.56
Whine pulse rate	(intercept)	0.19	0.09	2.01	0.00	0.37	0.044	-
	Time	-0.25	0.09	-2.87	-0.43	-0.08	0.004	0.87
	Host plant	-0.18	0.18	-0.97	-0.53	0.18	0.333	0.37
	RCD	-0.64	0.20	-3.27	-1.03	-0.26	0.001	0.93
Slow pulse rate	(Intercept)	-0.03	0.05	-0.56	-0.13	0.07	0.577	-
	Time	0.12	0.05	2.35	0.02	0.22	0.019	0.87
	Host plant	0.08	0.10	0.75	-0.13	0.28	0.456	0.33
	RCD	0.08	0.13	0.65	-0.17	0.34	0.516	0.35
RSI	(Intercept)	-0.14	0.17	-0.84	-0.48	0.19	0.403	-
	Time	0.05	0.18	0.30	-0.30	0.40	0.765	0.29
	Host plant	-0.02	0.36	-0.07	-0.73	0.68	0.948	0.27
	RCD	0.41	0.33	1.24	-0.24	1.07	0.214	0.43

divergence in one signal trait (slow pulse rate, as measured by increased divergence with time). In populations of limited size such as in islands, drift may be more likely to influence trait diversification (Mayr, 1954; Lande, 1976; Carson, 1978; Kaneshiro, 1980; Templeton, 2008). Theoretical work has shown that sexual isolation may evolve quite rapidly (in as few as 1000 generations) due in part to the neutral processes of drift with population sizes in the range of 1000–5000 (Uyeda *et al.*, 2009). Although the two Saddle Road populations are only estimated to have diverged approximately 2600 years ago, tropical planthoppers have multiple generations per year and thus have undergone several thousand generations since divergence. Furthermore, the smallest of the populations, Saddle Road Low, has an estimated effective population size of approximately 5600 (Goodman *et al.*, 2012). Together, the demographic parameters of the Saddle Road populations (sufficiently small populations and sufficiently long enough time) are consistent with those

that would be needed for drift to play a role in trait evolution leading to reproductive isolation (Uyeda *et al.*, 2009). Divergence with time is also compatible with a number of other scenarios, such as constant population-specific selection or mutation order effects (Mani & Clarke, 1990; Schluter, 2009). Although this is the case, we feel that drift is the most likely scenario considering the geographic and demographic parameters of this group.

Second, sensory drive is not important in signal divergence. Host plants provide channels through which signals propagate, constraining the variation possible in acoustic signals. It is possible that host plants did not play a role in signal divergence because the transmission properties between different host plants were similar. In this case, similar transmission properties in host plants may drive the convergence of courtship traits through time, possibly after post-zygotic mechanisms of isolation evolved (Wilkins *et al.*, 2013). These possibilities require future study.

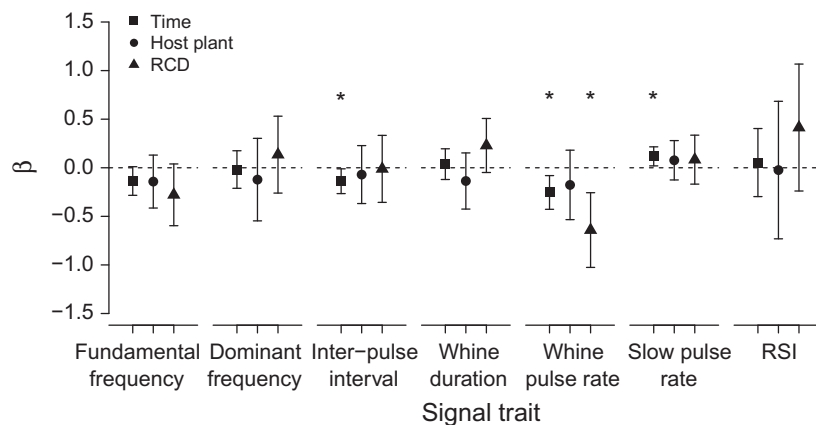


Fig. 4 Effect of modelled variables on signal traits. Units are scaled within and among signal trait models so that all results may be directly compared. For host plant difference, model intercept (or reference level) was 'different plant species', whereas the RCD reference level was allopatry. Asterisks indicate that the error bars do not overlap zero, indicating a significant result.

Third, some signalling traits become more similar over time. We found a negative association between time and divergence in a subset of traits (inter-pulse interval, whine pulse rate and fundamental frequency). This unusual pattern is one that we had not predicted, and suggests an unanticipated source of selection that drives convergence in signalling traits. Two potential sources of ecological selection that may have led to this pattern are eavesdropping predators (Endler, 1980, 1995; Basolo & Wagner, 2004; Zuk *et al.*, 2006; Trillo *et al.*, 2013) and wind noise (McNett *et al.*, 2010; Wu & Elias, 2014). Wind is an especially intriguing possibility in this system as all these sites are quite windy, resulting in significant levels of background noise. Noisy signalling environments have been shown to shape signalling characters in other systems (Brumm & Slabekoon, 2005; Ord *et al.*, 2011), and the spectral characteristics in noise tend to be similar across habitats.

Fourth, some signal traits are more divergent in allopatry than sympatry. This unexpected result demonstrates a pattern we would expect if selection on isolated populations resulted in divergent signalling trajectories (Fisher, 1930). We suggest that sexual selection may have led to this pattern. Recent work has pointed out that the null expectation for the way sexual selection drives changes in signal traits among populations is arbitrary (Prum, 2010), and thus by chance, it is possible that sexual selection acting on isolated populations could have driven more signal divergence in allopatry. Sexual selection, particularly selection acting directly on courtship signals, is an important force in driving male signal trait diversification in many groups (West-Eberhard, 1983; Rodriguez *et al.*, 2006; Boul *et al.*, 2007). A strong relationship has been demonstrated in *Enchenopa* treehoppers between female preferences and several male signal traits (frequency, whine length, pulse number, pulse rate, signal number; Rodriguez *et al.*, 2006), many of which we also studied here. Given the similarities in lifestyle and communication systems between treehoppers and planthoppers, it is likely that female

preference plays a strong role in shaping trait evolution in this system as well.

Fifth, some traits were more divergent in sympatry than allopatry. These effects are weak, but intriguing because they are suggestive that these traits are functioning in premating population discrimination in response to post-zygotic isolation (Brown & Wilson, 1956; Howard, 1993). Regions of secondary contact are of great interest to evolutionary biologists because they provide a way to examine the efficacy of reproductive isolating factors in their natural setting (Barton & Hewitt, 1985). Saddle Road Low and Saddle Road High are very similar ecologically, and planthoppers there form a stable zone of secondary contact with clear genetic differences and extremely low gene flow (estimated at <0.1 individual/generation). Furthermore, divergence among these populations has been conservatively estimated at only 2600 years ago (Goodman *et al.*, 2012). This indicates that a barrier has quickly developed to maintain isolation between the populations, despite geographic contact. Signal divergence in other arthropod taxa from this island is also fast. Signals have diverged to the point that they may be used for species level classification in the monophyletic species groups of seven cave planthoppers (Wessel *et al.*, 2013) and six *Laupala* crickets (Mendelson & Shaw, 2005), both of which have arisen within the recent time frame of Hawaii Island of approximately 0.5 million years. Data from *Nesosydne* provide a reference for the speed of within-species trait divergence.

Our study demonstrates that signal diversification in *Nesosydne chambersi* occurs along different axes for each signal trait, sometimes in unexpected ways. It is the first study, to our knowledge, to simultaneously look at the influence of multiple processes on divergence in sexual signals and to document their relative importance. The analytical framework we have developed is flexible and can be used on different taxa and incorporate a wide range of hypotheses. Determining the

mechanisms that drive species diversity is a long-standing goal in evolutionary biology. Rapid divergence and reproductive isolation among populations of animals is known to be driven by changes in sexual signals; however, understanding what drove those changes can be difficult. Here, we show that a study system with clear demography provides an opportunity for making significant progress on this problem. More empirical examples from a wide range of taxonomic groups are necessary to understand basic principles in signal diversification.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Summary of signal data.

Table S1. Summary of signal traits by population.

Appendix S2. Associations between signal traits.

Figure S2. Bivariate associations between signal traits.

Appendix S3. Results of Model selection.

Table S3. (a) Model selection for factors influencing absolute trait divergence of frequency-domain signal traits. (b) Model selection for factors influencing absolute trait divergence of time-domain signal traits.

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