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Title: Social selection parapatry in Afrotropical sunbirds

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Abstract

The extent of range overlap of incipient and recent species depends on the type and magnitude of phenotypic divergence that separates them, and the consequences of phenotypic divergence on their interactions. Signal divergence by social selection likely initiates many speciation events, but may yield niche-conserved lineages predisposed to limit each others' ranges via ecological competition. Here we examine this neglected aspect of social selection speciation theory in relation to the discovery of a non-ecotonal species border between sunbirds. We find

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that *Nectarinia moreaui* and *N. fuelleborni* meet in a ~6 km wide contact zone, as estimated by molecular cline analysis. These species exploit similar bioclimatic niches, but sing highly divergent learned songs, consistent with divergence by social selection. Cline analyses suggest that within-species stabilizing social selection on song-learning predispositions maintains species differences in song despite both hybridization and cultural transmission. We conclude that ecological competition between *moreaui* and *fuelleborni* contributes to the stabilization of the species border, but that ecological competition acts in conjunction with reproductive interference. The evolutionary maintenance of learned song differences in a hybrid zone recommend this study system for future studies on the mechanisms of learned song divergence and its role in speciation.

Introduction

Social selection, or selection on signals due to interactive intraspecific competition for resources (West-Eberhard 2014), can drive evolutionary divergence between populations in the presence or absence of ecological differences (West-Eberhard 1983). Our definition of social selection is inclusive of sexual selection. In the absence of ecological differences, the capacity of social selection to drive signal divergence stems from the potential for runaway selection via choice mechanisms (Fisher 1930), an absence of finite limits to social competition advantages from trait modification (Darwin 1871; West-Eberhard 1983; West-Eberhard 2014), and the efficacy of novel signals (West-Eberhard 1983). Social selection-driven signal divergence can directly lead to speciation when it causes sufficient pre-zygotic reproductive isolation (West-Eberhard 1983) or socially mediated post-zygotic reproductive isolation (Kawata and Yoshimura 2000; Seehausen and Schluter 2004; Stelkens et al. 2008; van der Sluijs et al. 2008).

Because social selection speciation could result in species with little to no ecological divergence, the products of speciation by social selection may be predisposed to intense ecological competition when in contact. Accordingly, West-Eberhard (1983) hypothesized that speciation with a strong social selection component should frequently predispose resultant lineages to mutual exclusion from ecological competition. By this "social selection parapatry" hypothesis, signal divergence can occur with minimal or no ecological divergence, yielding lineages that engage in strong resource and interference competition when they interact, stabilizing parapatry via ecological competition (see also (Case and Taper 2000; Case et al. 2005)). Further, lineages subject to social selection divergence may be prone to preferential aggregation with conspecifics (Stamps 1988; Hahn and Silverman 2006), which should reinforce

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stable parapatry, or decrease cline width (Payne and Krakauer 1997). While the roles of both social selection and niche conservatism in speciation have recently been central foci of research (Peterson et al. 1999; Wiens and Graham 2005; Pyron et al. 2015), the particulars of West-Eberhard's social selection parapatry hypothesis have received little attention.

Parapatric boundaries should be most frequently encountered in the tropics, where biological factors should set range limits more frequently than in temperate regions (Darwin 1859; MacArthur 1972). Such boundaries may form where ecological gradients are shallow or absent, as their existence need not depend on advantages accrued via local adaptation on either side of the boundary (Barton and Hewitt 1985; Case and Taper 2000; Case et al. 2005). As is true generally of parapatric boundaries, non-ecotonal parapatric boundaries (those with shallow or no environmental gradient) may occur more frequently in the tropics, where strong niche conservatism should be more common because of higher climatic constancy through time (Fjeldså et al. 2012). While several narrow, tropical parapatric boundaries without obvious environmental gradients have been described (see e.g. Hall and Moreau 1970; Krabbe and Schulenberg 1997; Sanchez-Gonzalez et al. 2015), detailed studies of the maintenance of these non-ecotonal parapatric distributions are uncommon (but see Brumfield et al. 2001).

Here we examine the divergence and shared range boundary between the sunbird sibling species *Nectarinia moreaui* and *N. fuelleborni* in southern Tanzania in the context of the social selection parapatry hypothesis. These taxa exhibit similar habitat requirements, being found exclusively in sky island montane forests in eastern Africa. Their parapatric boundary occurs within a mountain block of the Eastern Arc Mountains known as the Udzungwa Mountains. Though only subtle differences in plumage exist between the two species (Bowie et al. 2004), males of the two species sing strikingly different territorial songs (McEntee 2014). Their abutting distributions present the opportunity to examine the processes that stabilize the species border of sibling species with highly divergent signals and similar habitat requirements.

We propose that testing "social selection parapatry" would minimally require examining the following sub-hypotheses: 1) social selection is the primary mechanism for signal divergence; 2) each species' range is not limited by aspects of the environment outside of the presence of the sibling species, either by an abiotic gradient or a geographic barrier to dispersal (Case et al. 2005); 3) ecological competition is intense where there is local co-occurrence; and 4) reproductive interference, inclusive of selection against hybrids, is insufficient to explain the totality of fitness loss for either species at their shared range boundary. The last two of these

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criteria together distinguish social selection parapatry from the tension zone model of hybrid zones, wherein viability selection against hybrids alone is responsible for reinforcing parapatry (Barton and Hewitt 1985).

We address the first of these sub-hypotheses by examining several alternative hypotheses to explain the striking song divergence between *N. moreaui* and *N. fuelleborni*. The songs are used in male-male competition (McEntee 2014), and likely also attract or manipulate potential mates (Catchpole and Slater 2008), however we do not yet have evidence on how songs influence mate choice. As the songs are extremely complex, and as sunbirds are oscine songbirds, the songs are almost certainly developed through learning (Catchpole and Slater 2008). We therefore consider the possibility that these signals evolve by cultural mechanisms in addition to those mechanisms that would apply to unlearned signals. We consider the following hypotheses to explain song divergence (Podos and Warren 2007; Wilkins et al. 2013): 1) acoustic adaptation, 2) a byproduct mechanism where song divergence is a consequence of morphological change, 3) genetic and/or cultural drift, and 4) social selection independent of ecological differences. Evidence consistent with the last of these possibilities would constitute the best support for social selection as a driver of divergence.

With respect to the second sub-hypothesis, we 1) use bioclimatic niche modeling to test whether bioclimatic niches decline in suitability past the parapatric boundary, which would indicate that an abiotic gradient plays a role in limiting the species range, and 2) we use molecular data to test for local syntopy of the two morphologically cryptic species, where syntopy would discount a geographic barrier to dispersal as the sole cause of parapatry. We discuss the third sub-hypothesis based on a null-model based test of bioclimatic niche similarity between species (this study) and evidence from a previous study indicating that interspecific interference competition is strong between territorial males of the two species (McEntee 2014). With respect to the fourth criterion, we test for hybridization by examining support for admixture using DNA sequence data from 132 individuals. A lack of evidence for hybridization despite strong sampling in the range boundary vicinity would suggest that reproductive interference has a limited role in the stabilization of parapatry, whereas rampant hybridization would suggest that reproductive interference instead of ecological competition is primarily responsible for the stabilization of parapatry.

Beyond providing a possible case of social selection parapatry, the meeting of recent or incipient species with highly divergent learned song provides the opportunity to investigate

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learned song divergence's role in speciation. Learned song has received a great deal of attention as a signal that may be important to bird speciation (Baptista and Trail 1992; Grant and Grant 1996; Slabbekoorn and Smith 2002; Lachlan and Servedio 2004; Verzijden et al. 2012; Wilkins et al. 2013), both because song-learning birds are especially diverse and because learned songs appear especially labile. Essential for song to have a central role in speciation, though, is its robustness to convergence during secondary contact of divergent species (Cody 1969; Haavie et al. 2004; Seddon and Tobias 2010; Secondi et al. 2011; Verzijden et al. 2012). If learned song differences help prevent the merging of incipient species, accrued differences must be maintained when interspecific interactions make cultural transmission across species and/or gene flow of song-determining alleles possible. Here we examine the robustness of song divergence to contact using cline analyses of molecular and phenotypic data. If cultural transmission across species is frequent where *moreaui* and *fuelleborni* interact, song phenotype clines should be gradual compared to molecular clines, and the song cline may be displaced from molecular clines. If cultural transmission is limited or absent across species, then the two clines should be coincident and concordant. The latter possibility would suggest that learned song behaves much like other quantitative traits under stabilizing selection within species, and further that the genetic basis of learned song predispositions are distinct for each species.

Study taxa and geographic setting

Nectarinia (née *Cinnyris*) *moreaui* and *N. fuelleborni* (alternately classified as the subspecies *N. (Cinnyris) mediocris fuelleborni*) are sunbirds of Eastern Afromontane forest and forest edges. Bowie et al. (2004) found that *moreaui* and *fuelleborni* are characterized by 4-6% mtDNA sequence divergence, that mtDNA gene trees are reciprocally monophyletic, and provided evidence that both mtDNA lineages are found within forest habitats of the Udzungwa Mountains, Tanzania (see also (Stuart et al. 1987; Fjeldså et al. 2010; McEntee 2013; Werema et al. 2014)). The Udzungwa, much like the other Eastern Arc Mountain blocks (e.g. Ukaguru, Usambara, Uluguru), had largely been considered a single biogeographic sky island unit for forest-dependent birds (Fjeldså and Rabol 1995; Fjeldså and Bowie 2008; Fjeldså et al. 2010), such that the existence of two divergent taxa within the northeastern Udzungwa was unexpected.

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The forest areas inhabited by both species are constant environments that persisted in a similar state through the climatic fluctuations during the Pleistocene (Mumbi et al. 2008). The build-up of avian paleo- and neo-endemic diversity in the northeast Udzungwa provides further evidence that these areas have maintained stable environments through time (Fjeldså and Bowie 2008; Fjeldså et al. 2012). In contrast to contact zones where inter-specific interactions have been attributed to recent anthropogenic changes to local environments, the *moreaui* - *fuelleborni* contact zone occurs largely in tracts of mature forest with high environmental stability.

Methods

Molecular methods – sampling, laboratory procedures, and molecular analyses

Tissue and blood samples were acquired from multiple field expeditions and museum collections. We sampled 132 individuals encompassing 14 different populations (Table S5). DNA extraction was performed using Qiagen DNEasy blood and tissue kits. Sanger sequencing was performed for the mtDNA gene ND2, the Z-linked intron loci CHDZ, MUSK, and BRM, and the autosomal introns 11836 and 18142 (Goodwin 1997; Griffiths and Korn 1997; Sorenson et al. 1999; Backstroem et al. 2008; Kimball et al. 2009) (further details in Supplementary Materials). Sequences were checked for quality and aligned using the MAFFT alignment algorithm (Katoh et al. 2005) within Geneious Pro 5.1.6 (2010). Nuclear introns were phased probabilistically into haplotypes using the program PHASE (Stephens et al. 2001). Population structure and individual population membership were investigated using the Bayesian population assignment algorithm of the program *structure* (Hubisz et al. 2009). The number of populations *K* was set to 2 based on initial results where *K* was allowed to take values from 1 to 8 (Table S1). Results of Bayesian population assignment are depicted from a) a *structure* run where only nuclear intron haplotypes were included (Figure 2), compared against mtDNA haplotypes scored as either *fuelleborni* or *moreaui* in origin; and b) a *structure* run where nuclear intron and mtDNA haplotypes were both included (Figure S1). We used a cutoff value of .90 posterior probability of assignment to designate 'pure' individuals of either species from *structure* output. Individuals were dichotomously scored for mtDNA species origin by inspecting the alignment.

To provide an indication of the level of within-species population structure, multi-locus K_{st} indices (Hudson et al. 1992) were calculated in DNAsp (Librado and Rozas 2009). Because we

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sought to understand whether populations structure exists *within* species in these analyses, individuals with mixed ancestry, as evidenced by low probability of assignment (<90%) to both *moreaui* and *fuelleborni* in structure analyses, were excluded from the calculation of multi-locus K_{st}. Inclusion of mixed-ancestry individuals could prevent accurate inference of within-species population structure by inflating within species diversity. To prevent over-estimation of structure because of limited sampling, only populations with sampling of five or more individuals were included in this analysis. As we were interested in the magnitude of population structure in the vicinity of the contact zone, only those from within the cline-fitting area were included when estimating multi-locus Kst indices (Figure 1), which resulted in the exclusion of the Misuku Hills population of *N. fuelleborni*. Inclusion of this population may have resulted in higher estimates of population structure within *fuelleborni*, as this population is distant from our nearest sampled population (~60 km), and well isolated from it by unsuitable habitat.

Song recording and analysis

Sound recordings were made in the field in 2008-10 using Sennheiser ME-67 shotgun microphones and Marantz solid-state recorders (models PMD660, PMD670, and PMD671; 16 bit precision, 44.1 or 48 kHz sampling rate). Two additional *N. fuelleborni* recordings were obtained from the Macaulay Library, Cornell University Lab of Ornithology. Values for song variables were obtained for each of 14 measurements from sonograms visualized in Luscinia (Lachlan 2007), see Supplementary Materials for further recording details, measurement definitions and procedures). Mean individual values were then calculated from the set of songs measured for each individual, with the individual as our unit of analysis. Each vector of mean individual values was scaled by its standard deviation in R (R Core Team 2012) before MANOVA and principal components analysis in JMP 9 (SAS Institute Inc 2010). The first principal component was used to represent song variation in cline analyses. We present MANOVA results only for allopatric populations, to avoid inclusion of individuals of mixed-ancestry.

Analyzed song number per individual varied from 1 – 30 songs (mean=3.3, SD=3.2), which were exclusively sampled from songs sung in bout form (i.e. multiple songs were sung consecutively with a fairly regular inter-song interval). Some individuals were banded prior to sound recording, and were identified by band combination. For unbanded individuals, a minimum distance of 60m between recording localities was used to establish separation between individual samples. This minimum distance threshold was selected based on extensive

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observation of territorial behavior (McEntee 2013, McEntee 2014), including of banded males, and from observations of the typical densities of territorial males (JPM pers. obs.).

Bill measurements

Culmen length, defined as the distance between the distal tip and the notch where the culmen meets the skull, was measured for male museum specimens using digital calipers. A single observer (JPM) measured 141 male bills. We retained the 122 measurements derived from populations along the cline transect (Figure 1) for cline analyses.

Cline analyses

To analyze individual- and population-level geographic variation in genotypes and phenotypes, we first reduced the spatial dimensionality of the data by assigning each population a position along a circular arc transect. We fixed the northernmost and southernmost sampling populations as the endpoints of the circular arc, and then adjusted the arc's radius in ArcMap 10 (ESRI 2011) such that it evenly split the set of populations at the center of the contact zone (Figure 1). Population positions in one dimension were then assigned by finding the nearest point along the 504.5 km arc for each sampled population.

The goals of cline analyses were two-fold: to estimate the center and width of molecular and phenotypic clines as parameters of cline models, and to test for coincidence and concordance among molecular and phenotypic clines. Using the R package HZAR (Derryberry et al. 2014), we fit and compared clines for three traits: a molecular hybrid index (q-scores from STRUCTURE analyses of nuclear molecular variation, see e.g. Yannic et al. 2008; Devitt et al. 2011; Gowen et al. 2014; Johnson et al. 2015 for the use of a molecular hybrid index in cline analysis), song PC1 scores, and culmen length (see Supplementary Materials for additional cline fitting details). To ease visualization of the results, we normalized each of the three trait distributions prior to fitting so that their ranges were [0,1] using the following equation:

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$$z_i = \frac{x_i - \min(x)}{\max(x) - \min(x)},$$

where x is a trait vector, x_i is the *i*th observation of a vector, and z_i is the normalized value of the *i*th observation.

In HZAR, clines were fit with five different possible architectures: a sigmoid cline with or without exponential tails (Szymura and Barton 1986; Szymura and Barton 1991; Barton and Gale 1993; Brumfield et al. 2001; Derryberry et al. 2014). We then compared models representing each of the five architectures using model selection by AICc to determine a single preferred model architecture for each trait, and then extracted the maximum likelihood model parameters. For each architecture-trait combination, we made initial model fits (chain length = $1x10^6$ generations, burn-in = $5x10^5$ generations) to optimize the covariance matrix used in three separate subsequent MCMC chains (Derryberry et al. 2014). These three secondary MCMC chains were then concatenated ($9x10^6$ generations) for post-processing and analysis. We checked for adequate mixing and convergence in cline parameter estimation by visualizing sampling trajectories with the R package coda (Plummer et al. 2006).

Niche modeling and niche similarity test

Strong ecological competition between species in secondary contact requires that the niches of the two species are strongly conserved. To assess the hypothesis that our focal species' niches are strongly conserved, we test whether their bioclimatic niches are more similar than expected under a null hypothesis. In the test we employ, the "background similarity" test of Warren *et al.* (2008), rejecting the null hypothesis indicates that the niches of compared taxa are more similar than expected by chance, where we explicitly account for the environmental variation present in the area where the species distributions occur. To generate a null distribution of similarity scores, we compare the geographically projected bioclimatic niche model of species X, where the niche model is generated from known occurrences of species X, with a set of niche models generated from pseudo-randomly selected points from a buffer area (background) around the

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occurrence points of species Y. These null distributions are generated. Sets of background samples for each species were selected from buffer areas that were created by generating circles with 100-km radii around each of the true occurrence points. We use two niche similarity metrics: the ecological niche overlap metric Schoener's *D* (Schoener 1968), and a distance metric *I* (Warren et al. 2008; Warren et al. 2010; see Supplementary Materials).

We built niche models from 19 and 25 occurrence points for *N. moreaui* and *N. fuelleborni*, respectively. Background sample niche models were constructed from 19 background samples in the buffer area of *N. fuelleborni*, and 25 from the buffer area of *N. moreaui*. This process was repeated 100 times to generate null distributions. We used the maximum entropy modeling software Maxent to build niche models (Phillips et al. 2006). For each occurrence point, we extracted seven BIOCLIM variables (BIO1: annual mean temperature, BIO2: mean diurnal temperature range, BIO5: maximum temperature of warmest month, BIO6: minimum temperature of coldest month, BIO12: annual precipitation, BIO13: precipitation of wettest month, BIO14: precipitation of driest month) at a resolution of 2.5 arc-minutes (Hijmans et al. 2005). These seven BIOCLIM variables were chosen because they capture a large proportion of the climatic variation across space, and their levels of correlation are low over large areas of the globe (Jiménez-Valverde et al. 2009). We projected resulting niche models onto a geographic space including the range of both species.

Results

Molecular sequence data

Of the six molecular loci, *N. moreaui* exhibited a small but statistically significant K_{st} for MUSK (.073), with small and statistically insignificant K_{st} for the other loci (Table S2). *N. fuelleborni* exhibited small but statistically significant K_{st} for ND2 (.066) and MUSK (.110), with statistically insignificant Kst for the other loci (Table S2). Population structure for ND2 and MUSK in *fuelleborni* was driven by differences between the Rungwe and Udzungwa mountain blocks, which are separated by the Makambako Gap, a known biogeographic break (Burgess et al. 2007). The minimal structure was somewhat unexpected, given that many bird species with similar distributions exhibit strong structure among the same mountain blocks (Bowie and

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Fjeldså 2005; Fjeldså et al. 2010). The minimal structure in both species suggest that dispersal has maintained connectivity among geographically isolated populations, and further suggest that the parapatric boundary is unlikely to be explained by highly limited dispersal among forest patches.

In the combined mitochondrial and nuclear sequence data set, population assignment in *structure* revealed that seven of 132 sampled individuals have mixed ancestry. Five individuals had mixed nuclear genomes, and two individuals exhibited cyto-nuclear discordance. The seven individuals of mixed ancestry came from four populations: Kihulula, Nyumbanitu, Image, and Ndundulu (Figure 2). Sympatry of pure parentals species was revealed from molecular analyses at two localities: Nyumbanitu and Ikokoto (Figures 1 and 2).

Species-level phenotypic divergence

Songs Representative sonograms can be found in Figure 3 (a & b). MANOVA following scaling and centering of all 14 variables was significant for differences between allopatric populations of the two species (n_1 = 38 *fuelleborni*, n_2 = 41 *moreaui*, F = 268.87, df = 1, $p < 1 \times 10^{-15}$). The first principal component corresponded strongly with species differences. Loadings for PC1 for all nine variables with statistically significant differences in individual ANOVAs (Tables S3 and S4) had absolute value >.45, indicating correlation among these variables along the axis of species differences.

Bills Mean ± SD male bill lengths were 23.29 ± 1.02 for *N. fuelleborni* and 24.89 ± 1.01 for *N. moreaui* (n_1 = 51 *fuelleborni*, n_2 = 92 *moreaui*, linear mixed model with population nested within species and Satterthwaite approximations to df, t = 5.728, residual d.f. = 135, $p = 1.22 \times 10^{-5}$, Hedges' g for comparison of species means = 1.58). Individuals with mixed ancestry were excluded from this comparison. There is substantial overlap in bill length between species (Figure S2).

Ecological niche Species distribution models from Maxent had high prediction value for the training set for both species (*fuelleborni* AUC: .966; *moreaui* AUC: .988). In relativized

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contribution and permutation tests performed using Maxent, temperature variables had a higher impact than precipitation variables.

For both species, response curves for Maxent models developed independently for individual predictor variables generally indicate increasing suitability with decreasing mean annual temperature, maximum temperature of warmest month, and minimum temperature of coldest month. Suitability for both species is highest at intermediate diurnal temperature ranges relative to the background. For *moreaui*, suitability was highest at intermediate values of annual precipitation and precipitation of the wettest month, and for low values of precipitation of the driest month. For *fuelleborni*, suitability increased with annual precipitation and precipitation of the wettest month.

Predictions projected into the range of the sister species resulted in remarkably high concordance between the projected niche and the sister species' localities (Figure 4a), including of localities maximally distant (~900 km, see Figure 4) from the contact zone. Results of the background similarity test, performed in ENMTools (Warren et al. 2010), are shown in Figure 4b. Compared to the null distributions of niche similarity scores generated from comparing background point models of species X with the actual distribution model for species Y, niche models for *fuelleborni* and *moreaui* had higher similarity scores for both ecological indices calculated: Schoener's *D* and *I*. We reject the null hypothesis in all four comparisons, indicating bioclimatic niche conservatism.

Clines

Relative support for cline architectures by trait are shown in Table 1. Cline center and width estimates for the molecular index and song clines were coincident and concordant, with center ~163 km and width ~6 km. The center of the molecular and song clines corresponded closely to the positions of Nyumbanitu and Kihulula forests along the transect.

The culmen length cline was not coincident with the molecular and song clines (95% credible intervals did not overlap). The culmen length cline's center was shifted ~8 km north of the molecular index and song cline centers. The culmen length cline was also broader in width than the molecular index cline (95% credible intervals did not overlap), while there was a small amount of overlap between the 95% credible intervals for culmen length and song cline widths.

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Discussion

Evidence for social selection parapatry

West-Eberhard's (West-Eberhard 1983) "social selection parapatry" hypothesis is an appealingly plausible explanation for how stable range boundaries could arise between species with similar ecological niches. Models support the notion that (incipient) species with similar, even identical, ecological niches may establish stable mutual species borders or "mosaic sympatry", and may thus persist for long time periods within a finite domain (M'Gonigle et al. 2012). As indicated in the Introduction, we suggest that support for the social selection parapatry hypothesis would minimally require support for these four sub-hypotheses: 1) social selection is the primary mechanism for signal divergence; 2) each species' range is not limited by aspects of the environment outside of the presence of the sibling species, either by an abiotic gradient or a geographic barrier to dispersal (Case et al. 2005); 3) ecological competition is intense where there is local co-occurrence; and 4) reproductive interference, inclusive of selection against hybrids, is insufficient to explain the totality of fitness loss for either species at their shared range boundary. We review the evidence for each of these hypotheses from the *moreaui* – *fuelleborni* parapatric boundary.

With respect to the first sub-hypothesis above, we show that a signal, male territorial song, is highly divergent between *moreaui* and *fuelleborni* (see Table S3 and Figure S2), whereas an eco-morphological trait (culmen length, see Results and Figure S3), and bioclimatic niche (Figure 4) are more subtly divergent. Here we assess the evidence that each of the following mechanisms has contributed to the described song divergence: acoustic adaptation, byproduct mechanism from natural selection on other traits (e.g. morphology that constrains sound production, Podos 1996), genetic and/or cultural drift (Podos and Warren 2007; Wilkins et al. 2013), and social selection independent of environmental differences experienced by populations. We argue that, of these possibilities, social selection independent of ecological or morphological differences is the most plausible primary mechanism for song divergence. However, the action of social selection together with genetic drift may be important, as suggested by models incorporating both processes (Lande 1981; Uyeda et al. 2009).

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Under the "acoustic adaptation hypothesis", aspects of the environment serve to alter the efficacy of acoustic signals by impeding or promoting the transmission of certain sounds (Morton 1975, Endler 1993). Selection can then drive the evolution of acoustic signals by sorting sounds that vary in transmission. The acoustic adaptation hypothesis predicts divergence when the acoustic environments experienced by different populations are consistently different. With respect to sound transmission properties of the habitats or micro-habitats used by *N. moreaui* and *N. fuelleborni*, it is unlikely that such differences exist for two reasons: the species have similar niches, and each species sings across a large variety of microhabitats that must have different transmission properties. In this study, we provide evidence regarding the former, in the form of the background similarity tests indicating that the focal species use extremely similar bioclimatic niche space. With respect to the latter, natural history observations indicate the two species sing from a broad variety of forest and forest edge microhabitats, suggesting broad variation within each species and extensive overlap between species in the acoustic properties that each experiences (McEntee 2013). Overall, the constraints on the transmission of song are likely to be quite similar, and highly variable within species, for *moreaui* and *fuelleborni*. Mediation of divergence by shifted acoustic constraints is then unlikely to contribute substantively to their strong song divergence.

The "byproduct hypothesis" requires phenotypic change in a trait with pleiotropic effects on song. Multiple studies have shown that bill morphology divergence is associated with song divergence (Podos 2001; Seddon 2005). The mean of *N. moreaui*'s culmen lengths is greater than that of *N. fuelleborni*, suggesting that pleiotropic effects on trills are possible. However, one prediction of the byproduct hypothesis for song is that "ecologically" selected traits and song must co-vary among populations. Cline analyses show that *N. moreaui* populations at Ndundulu and Selebu sing species-typical songs despite possessing smaller (more *fuelleborni*-like) bills relative to other *N. moreaui* populations, indicative of a subtle decoupling of bill length and song phenotype variation. This decoupling runs counter to the prediction of the byproduct hypothesis that song and bill phenotypes should tightly co-vary among populations (Wilkins et al. 2013, Table 2). Additionally, the dramatic divergence in song duration (Table S3) and fine-scale spectro-temporal aspects of song structure (e.g. coefficient of variation in interval

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duration, Table S3) cannot easily be explained by pleiotropic effects of bill divergence. Our study thus discounts a strong role for bill morphology in song divergence.

Random genetic drift can explain song divergence if different alleles underlying song variation fix in different species from ancestral standing genetic variation. A prediction from this genetic drift model is isolation-by-distance in song phenotype aspects that are vertically inherited. While we do not specifically test this hypothesis here, we note that the song phenotype dimension presented (PC1) is strongly conserved across space within species (Figure 5) while being strongly divergent among species. This pattern suggests that different processes are at play within versus between species (Campbell et al. 2010), which is confirmed in a separate study specifically examining population-level song variation (McEntee et al. in prep). Strong conservatism in song phenotypes among spatially isolated populations, including some that have accrued substantial divergence at molecular markers (Table S2, McEntee et al. in prep), suggests that stabilizing selection prevents random genetic drift alone from driving song phenotype divergence among population isolates within species. Cultural drift, meanwhile, could explain the use of different song elements among populations within species, but is unlikely to account for the gross structural divergences of *moreaui* and *fuelleborni* song (Table S3), which are likely underlain by distinct, innate predispositions (Marler and Sherman 1985).

The failure of the acoustic adaptation, byproduct, and drift mechanisms to explain song divergence, together with evidence that niches are strongly conserved, leaves social selection independent of environmental differences as the most plausible primary mechanism of divergence, consistent with the social selection parapatry hypothesis. What is perhaps most interesting about song divergence in this case is strong within-species conservatism despite dramatic divergence between *N. moreaui* and *N. fuelleborni* (Figure 5), and despite geographic isolation within species providing the potential for further divergence (Bowie et al. 2004; Table S2). Intra-specific conservatism despite geographic isolation is suggestive of stabilizing selection (Charlesworth et al. 1982; Estes and Arnold 2007). However, if stabilizing selection on song prevents among-population divergence within species, what processes can explain strong divergence between species? We suggest range expansion-associated divergence as a possibility (Mila et al. 2007). We hypothesize that *N. fuelleborni* arose as a daughter species of *N. moreaui*

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during an ancient southward range expansion, with a concomitant relaxation of stabilizing selection on song phenotype that permitted rapid divergence (Kaneshiro 1976), followed by secondary contact. This hypothesis could account for both the striking divergence in song between *N. fuelleborni* and *N. moreaui*, and the comparatively limited geographic variation within each species.

With respect to the second sub-hypothesis of social selection parapatry, neither a geographic barrier nor an abiotic gradient can explain the range border of either species in the northeast Udzungwa. Evidence for local syntopy and hybridization precludes the possibility that a geographic barrier divides the two species ranges. An abiotic gradient, meanwhile, is extremely unlikely to be more important than the presence of heterospecifics in limiting each species' range. The results of the niche models and background similarity tests presented here (Figure 4) indicate that the environment is suitable for each species well beyond the range edge, extending to the opposite edge of the range of the sibling species. We acknowledge that we have not tested an additional possibility, that biotic factors outside of the sibling species interactions (e.g. plant food availability) play a major role in limiting the range of either species. However, the striking ecological similarity of the two species suggests that their populations are unlikely to be independently regulated. With respect to food plant species, both species are generalists in their exploitation of nectar (Dowsett-Lemaire 1989, JPM pers. obs.), and both frequently feed on the same widespread plants (*Tecoma capensis*, *Halleria lucida*, *Dombeya* sp., *Leonotis spp.*, JPM pers. obs.) in the vicinity of the parapatric boundary. Consequently, we conclude that the range of each species is limited primarily by the presence of the sibling species, and not by other aspects of the environment.

For the third sub-hypothesis, that the two species are in strong ecological competition where they locally co-occur, our evidence comes from a previous experimental territorial intrusion study (McEntee 2014) and from natural history observation. In the territorial intrusion study, populations of both species near the species border exhibited strong aggressive responses to heterospecific mounts following approaches initiated by heterospecific or conspecific song playback (see Figure 5 in McEntee 2014). This result suggests that interspecific interference competition for territories should be strong where there is local occurrence. At the only site

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where pure individuals of both species hold adjacent territories, we have observed extended aggressive chases among males of the two species. Such territorial disputes are further suggestive of resource competition, where suitable breeding territory is a shared resource. Collectively, the experimental and observational evidence is consistent with the social selection parapatry hypothesis, which requires strong ecological competition between species.

With respect to the fourth sub-hypothesis above, the combination of incomplete reproductive isolation and selection against hybrids can generate Allee effects for the locally rare species via reproductive interference (Hochkirch et al. 2007, Gröning and Hochkirch 2008) near mutual species borders (Case et al. 2005). It is theoretically possible then for reproductive interference to limit species ranges even where ecological competition is not strong. However, because reproductive interference may combine with ecological competition to set Allee effects in nature, teasing out their relative effects is likely to be difficult. The strongest support for West-Eberhard's social selection parapatry hypothesis, which specifies ecological competition and less so reproductive interference as the driver of mutual exclusion, would come from parapatric distributions where no reproductive interference occurred. In this study, individuals of mixed ancestry comprise 7 of the 75 individuals (9.3%) sampled from sites where genes from both species were sampled (Figure 2). While this proportion is likely to be an overestimate of contact zone hybrid formation because of intensive sampling effort where syntopy of parental forms was discovered, it is nonetheless clear that pre-zygotic reproductive isolation is incomplete. This result raises two issues: the role of signal divergence in speciation and whether parapatry could be stabilized entirely by reproductive interference in this case. While social selection against hybrids due to poor signaling traits may occur, the existence of hybridization raises the possibility that genetic incompatibilities may contribute to the fitness disadvantages of individuals with mixed ancestry, perhaps indicating a less important role for signal divergence in the speciation process. However, genetic incompatibilities appear to accumulate slowly in many birds (Price and Bouvier 2002), such that even if genetic incompatibilities occur at present, early signal divergence may have caused lowered rates of hybridization during contact earlier in the speciation process. Population-level studies examining fitness outcomes in the hybrid zone would be helpful to tease out some of these issues. With respect to the stabilization of parapatry, we must conclude that reproductive interference via selection against hybrids is likely a contributor to the range limits of *fuelleborni* and *moreaui* at their shared

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range boundary. However, because evidence indicates strong ecological similarity between species, reproductive interference and ecological competition can be seen as combining to limit the range of each species. Teasing out the relative strength of effects of reproductive interference and ecological competition on range limits would be a productive avenue of future research (Thum 2007; Kishi et al. 2009). Of the four examined sub-hypotheses for social selection parapatry, the insufficiency of reproductive interference to explain range limits remains the most challenging to support with evidence. It is, moreover, surprising that birds with such different songs do not exhibit more complete prezygotic reproductive isolation. Even dramatic divergence in signals is insufficient then to completely prevent hybridization attempts, such that reproductive interference could play a role in stabilizing avian parapatric boundaries even where (attempted) hybridization has not yet been documented.

Robustness of learned song divergence to secondary contact and hybridization

The role of learned song in bird speciation remains an open question that falls into the larger debate regarding plasticity's importance in divergence (West-Eberhard 2005; Pfennig et al. 2010; Verzijden et al. 2012). The combination of learned song's lability and its role in mate choice suggests it could promote speciation by reducing or impeding gene flow. However, the song learning process can result in heterospecific copying (Helb et al. 1985; Laiolo 2012). Divergent learned song may then be a weak barrier to hybridization (Secondi et al. 2003; Haavie et al. 2004; Qvarnström et al. 2006; Secondi et al. 2011; Vokurkova et al. 2013). There is more potential for learned song differences to prevent gene flow if song pre-dispositions and adult song phenotypes have co-diverged with preferences or response functions. Experiments have shown that certain aspects of song phenotypes tend to be constrained by innate song predispositions, namely duration, frequency range, organization (gross structure), and element (syllable) morphology (Thorpe 1958; Nottebohm 1968; Marler and Peters 1977; Marler and Sherman 1985; Soha and Marler 2000; Catchpole and Slater 2008). Thus (incipient) species bearing divergence in these aspects are less likely to converge on contact. *N. moreaui* and *fuelleborni* song differences include divergence in all of the listed aspects likely to be constrained by innate predispositions, rendering these species' contact zone an important case study for how song predisposition divergence impacts the robustness of learned song divergence to secondary contact with hybridization.

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The coincidence and concordance of molecular and song clines (Tables 1 and S4, Figure 5) suggest that song behaves much as a quantitative trait under within-species stabilizing selection across the *N. moreaui* – *N. fuelleborni* contact zone, and indeed suggests that heterospecific copying has not broadened the song cline, as would be predicted from inter-specific cultural transmission in parapatry. It is important to note, though, that not all intermediate values for learned song come from mixed-ancestry individuals. At least one of the intermediate values for song in the contact zone was exhibited by an individual that scored as pure *moreaui* at a molecular level in our *structure* analyses. Therefore, those individuals with intermediate song phenotypes may represent hybrids as well as pure individuals. Thus, in the geographic context analyzed here, song behaves much like a typical quantitative trait, but heterospecific copying is apparent within one contact zone population. If heterospecific copying is disadvantageous (Qvarnström et al. 2006), selection against locally rare heterospecifics exhibiting mis-matched genotypes and phenotypes might complement selection against hybrids in reinforcing the stability of the cline.

Conclusions

We have provided evidence that *N. moreaui* and *N. fuelleborni* may be viewed as a case of social selection parapatry. Our evidence includes demonstrating 1) that the two species have diverged strongly in a signal (learned song) while showing strong conservatism in bioclimatic niche, and 2) that interspecific interactions, not an ecological gradient or geographic barrier, play a primary role in limiting the range of each species. We add this evidence to experimental evidence from a previous paper (McEntee 2014) indicating that interference competition between species is intense where these species make contact. While interspecific competition is apparent and clearly plays some role in limiting the range of each species, we cannot yet rule out the possibility that reproductive interference in the form of selection against hybrids is involved in restricting the range of each species. The relative contribution of ecological competition and reproductive interference in limiting the ranges of parapatric species may be difficult to distinguish, but doing so should be a focus of future research. We advocate, however, that investigators of hybrid zones study competitive interactions, individual movement, and

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relative population densities across hybrid zones in addition to studying hybridization, to understand which factors stabilize parapatry.

The abrupt spatial turnover in highly divergent song phenotypes, concordant with genotypes, recommends the *moreaui-fuelleborni* hybrid zone for future study on song divergence and speciation. Strong divergence in multiple aspects of song phenotype in these two species, together with the possibility of examining the phenotypes of hybrids, may allow for identification of the molecular changes underpinning divergent song predispositions in recent species.

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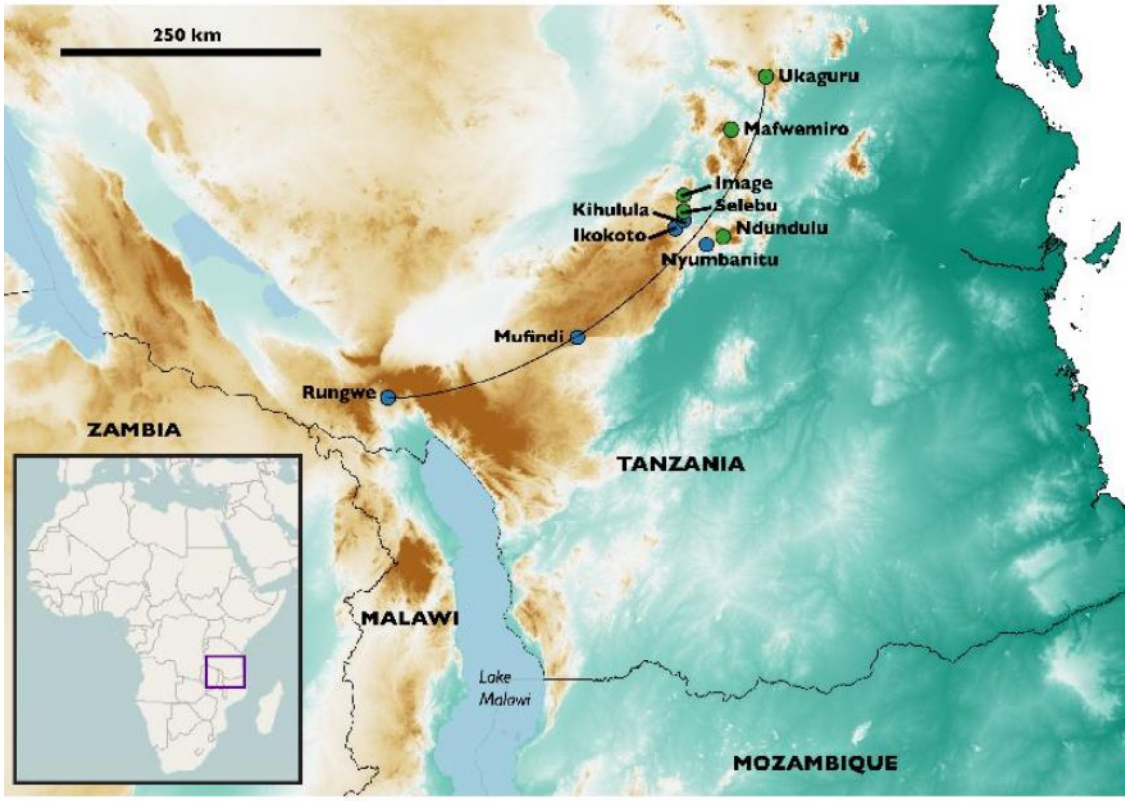
Explorers' Club Exploration Fund grants, and a Louise Kellogg Grant and Joseph Maillard Fellowship from the Museum of Vertebrate Zoology. Part of this research was conducted using funds made available to RCKB from the UC Berkeley.

Data archiving

Molecular data will be contributed to GenBank. Song and bill measurements will be made accessible via the Dryad Digital Repository datadryad.org. Song recordings will be made available through the ARCTOS data portal.

Figure legends

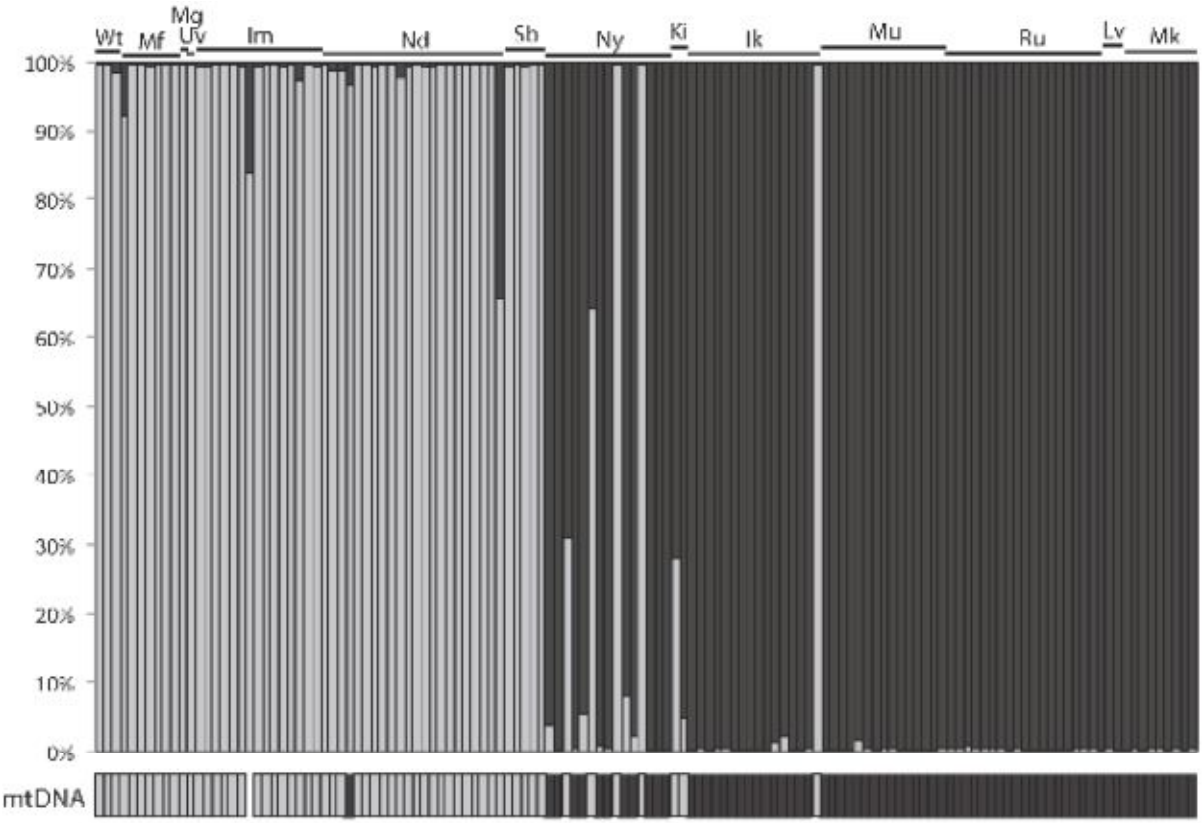
Figure 1: Map of the study area, with named localities mentioned in the text. The circular arc transect shown was used to reduce the spatial dimensionality of the data for cline analyses. Colouration indicates elevation: green shades = lower elevation and brown shades = higher elevation. Green dots are sampling localities dominated by *N. moreaui*. Blue dots are sampling localities dominated by *N. fuelleborni*.



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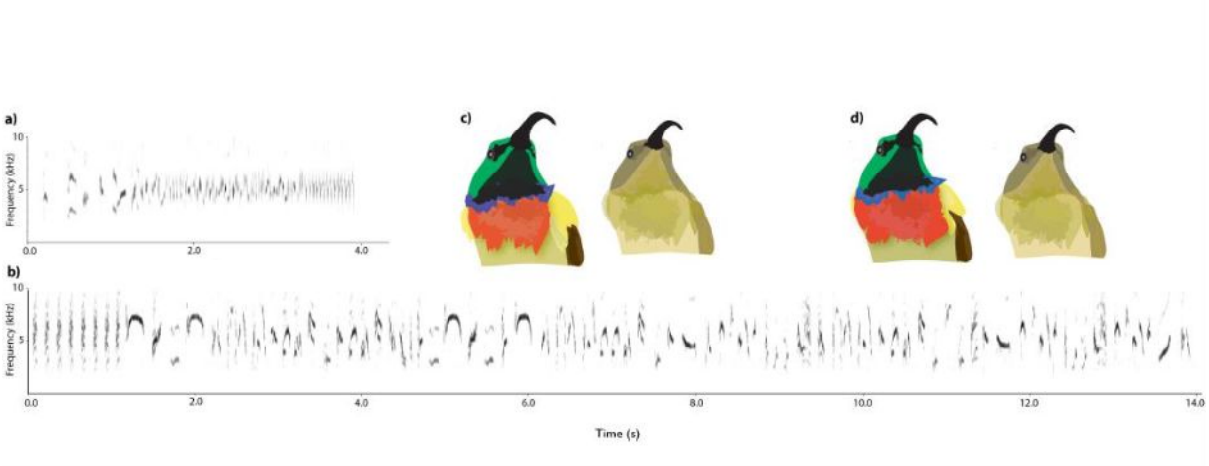
Figure 2: Probability of assignment to *N. moreaui* (light gray) and *N. fuelleborni* (dark gray) as determined from a *structure* analysis using nuclear intron haplotypes (top). The corresponding mtDNA lineage (*moreaui* or *fuelleborni*) for each individual is also shown (bottom). Note two instances of mito-nuclear discordance. Population abbreviations are as follows: Wt = Wota, Mf = Mafwemiro, Mg = Mang'alisa, Uv = Uvidunda, Im = Image, Nd = Ndundulu, Sb = Selebu, Ny = Nyumbanitu, Ki = Kihulula, Ik = Ikokoto, Mu = Mufindi, Ru = Rungwe, Lv = Livingstone Mountains, Mk = Misuku Hills.



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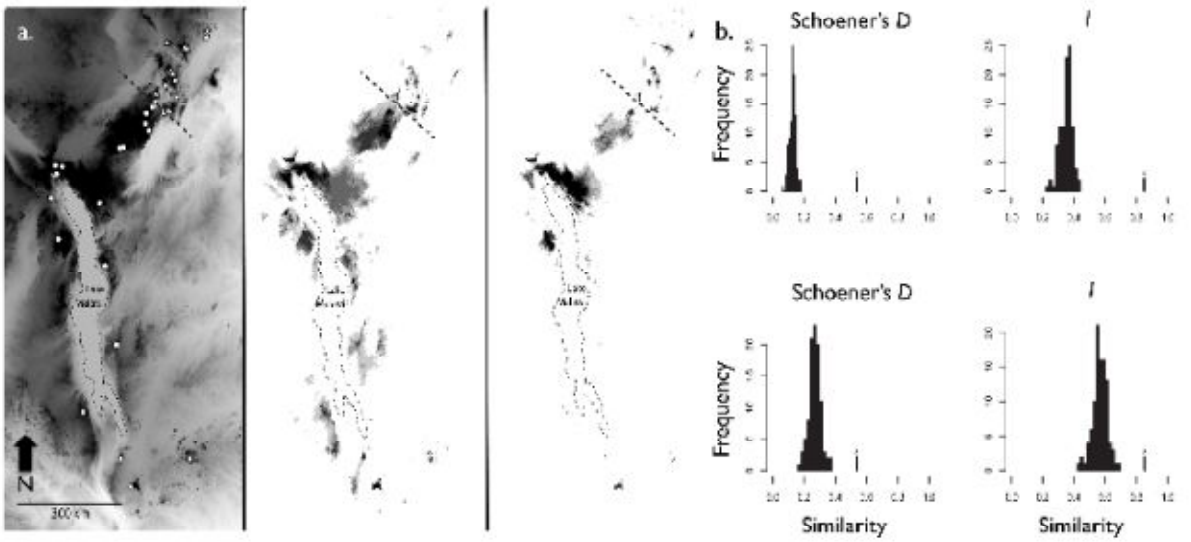
Figure 3: Representative sonograms and depictions of *Nectarinia moreaui* (a & c, male on left and female on right) and *N. fuelleborni* (b & d, same) respectively (reprinted from McEntee 2014 with permission from Oxford University Press). The large difference in song duration is representative of species differences.



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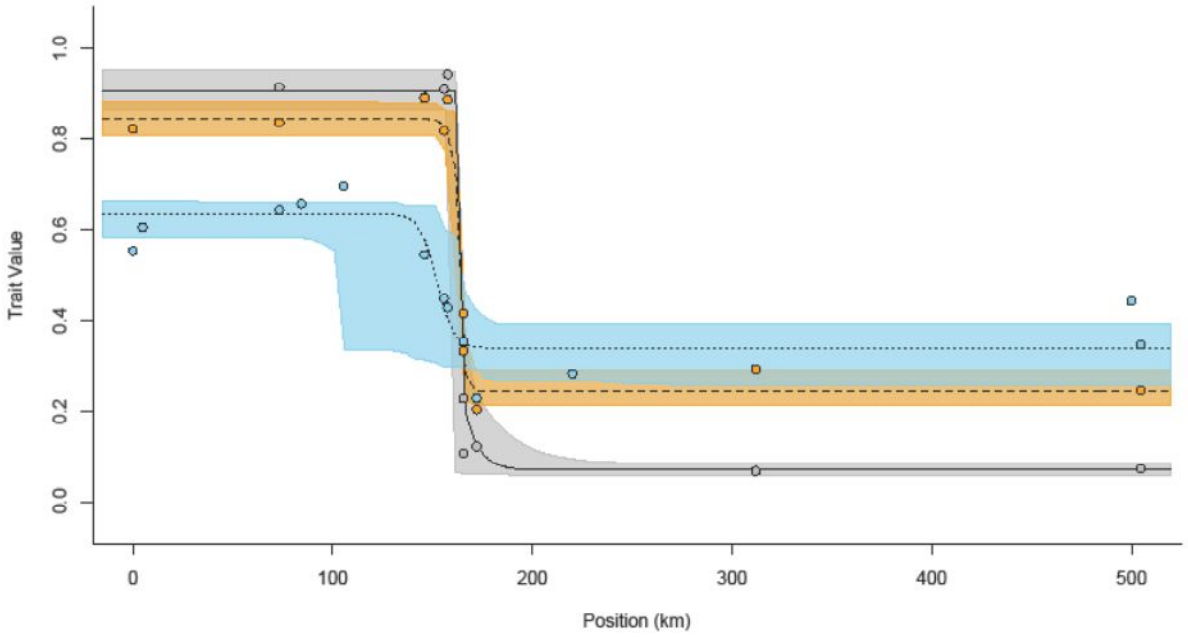
Figure 4: a) From left to right: occurrence points for *Nectarinia moreaui* (triangles) and *N. fuelleborni* (circles) with elevation shown across their distributions (darker colours at higher elevations); Maxent niche model output for *fuelleborni*, with darker colour indicating higher suitability; equivalent model for *moreaui*. b) Results of background similarity tests performed in ENMTools. Starred bars for the niche similarity indices Schoener's *D* and *I* are similarity scores from niche models predicting the distribution of the other species (top: *fuelleborni* model predicting *moreaui* suitability, bottom: *moreaui* model predicting *fuelleborni* suitability). Distributions shown are null distributions generated from niche models developed for sets of 'background' points within 100 km buffers around occurrence points. The greater similarity seen in niche models generated from the actual sister taxa distributions as compared to the null distributions indicates strong niche conservatism. See Methods for additional details.



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Figure 5: Cline analysis (HZAR) results for the molecular hybrid index (gray), song PC1 (orange), and culmen length (blue). Lines indicate the maximum likelihood cline for each trait, from the model with the minimum AICc value for that trait. Shaded areas represent 95% confidence intervals. Points are mean values for populations included in the cline analyses. When the clines are restricted to a common scale, the culmen length cline occupies the narrowest range of trait values because the maximum and minimum individual culmen length values lie far from the population means.



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LITERATURE CITED

Backstroem N., S. Fagerberg, and H. Ellegren. 2008. Genomics of natural bird populations: a gene-based set of reference markers evenly spread across the avian genome. Mol. Ecol. 17:964-980.

Baptista L. F., and P. W. Trail. 1992. The role of song in the evolution of passerine diversity. Syst. Biol. 41:242-247.

Barton N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Syst. 16:113-148.

Bowie R. C. K., and J. Fjeldså. 2005. Genetic and morphological evidence for two species in the Udzungwa forest partridge *Xenoperdix udzungwensis*. Journal of East African Natural History 94:191-201.

Bowie R. C. K., J. Fjeldså, S. J. Hackett, and T. M. Crowe. 2004. Systematics and biogeography of double-collared sunbirds from the Eastern Arc Mountains, Tanzania. Auk 121:660-681.

Brumfield R. T., R. W. Jernigan, D. B. McDonald, and M. J. Braun. 2001. Evolutionary implications of divergent clines in an avian (Manacus : Aves) hybrid zone. Evolution 55:2070-2087.

Burgess N. D., T. M. Butynski, N. J. Cordeiro, N. H. Doggart, J. Fjeldså, K. M. Howell, F. B. Kilahama, S. P. Loader, J. C. Lovett, B. Mbilinyi, M. Menegon, D. C. Moyer, E. Nashanda, A. Perkin, F. Rovero, W. T. Stanley, and S. N. Stuart. 2007. The biological importance of the Eastern Arc Mountains of Tanzania and Kenya. Biol. Conserv. 134:209-231.

Campbell P., B. Pasch, J. L. Pino, O. L. Crino, M. Phillips, and S. M. Phelps. 2010. Geographic variation in the songs of Neotropical singing mice: Testing the relative importance of drift and local adaptation. Evolution 64:1955-1972.

Case T. J., and M. L. Taper. 2000. Interspecific competition, environmental gradients, gene flow, and the coevolution of species' borders. Am. Nat. 155:583-605.

Case T. J., R. D. Holt, M. A. McPeek, and T. H. Keitt. 2005. The community context of species' borders: ecological and evolutionary perspectives. Oikos 108:28-46.

Catchpole C. K., and P. J. B. Slater. 2008. Bird Song. Cambridge University Press, Cambridge, United Kingdom.

Charlesworth B., R. Lande, and M. Slatkin. 1982. A Neo-Darwinian commentary on macroevolution. Evolution 36:474-498.

Cody M. L. 1969. Convergent characteristics in sympatric species - a possible relation to interspecific competition and aggression. Condor 71:223-&.

This article is protected by copyright. All rights reserved.

Accepted Article

Darwin C. 1871. The Descent of Man and Selection in Relation to Sex. John Murray, London.

--- 1859. On the Origin of Species. John Murray, London.

Derryberry E. P., G. E. Derryberry, J. M. Maley, and R. T. Brumfield. 2014. hzar: hybrid zone analysis using an R software package. Molecular Ecology Resources 14:652-663.

Devitt T. J., S. J. E. Baird, and C. Moritz. 2011. Asymmetric reproductive isolation between terminal forms of the salamander ring species *Ensatina eschscholtzii* revealed by fine-scale genetic analysis of a hybrid zone. BMC Evolutionary Biology 11.1: 245.

Dowsett-Lemaire F. 1989. Food plants and the annual cycle in a montane community of sunbirds (*Nectarinia* spp.) in northern Malawi. Tauraco 1:167-185.

Endler, J.A. 1993. Some general comments on the evolution and design of animal communication systems. Phil. Trans. Roy. Soc. B. 340:215-225.

ESRI. 2011. ArcGIS Desktop: Release 10. Environmental Systems Research Institute, Redlands, CA.

Estes S., and S. J. Arnold. 2007. Resolving the paradox of stasis: Models with stabilizing selection explain evolutionary divergence on all timescales. Am. Nat. 169:227-244.

Fisher R. A. 1930. The Genetical Theory of Natural Selection. The Clarendon Press, Oxford.

Fjeldså J., and J. Rabol. 1995. Variation in avian communities between isolated units of the Eastern Arc Montane Forests, Tanzania. Gerfaut 85:3-18.

Fjeldså J., and R. C. K. Bowie. 2008. New perspectives on the origin and diversification of Africa's forest avifauna. Afr. J. Ecol. 46:235-247.

Fjeldså J., R. C. K. Bowie, and C. Rahbek. 2012. The role of mountain ranges in the diversification of birds. Annual Review of Ecology, Evolution, and Systematics, Vol 43 43:249-265.

Fjeldså J., J. Kiure, N. Doggart, L. A. Hansen, and A. Perkin. 2010. Distribution of highland forest birds across a potential dispersal barrier in the Eastern Arc Mountains of Tanzania. Steenstrupia 32:1-43.

Goodwin G. H. 1997. Isolation of cDNAs encoding chicken homologues of the yeast SNF2 and Drosophila Brahma proteins. Gene 184:27-32.

Gowen F. C., J. M. Maley, C. Cicero, A. T. Peterson, B. C. Faircloth, T. C. Warr, and J. E. McCormack. 2014. Speciation in Western Scrub-Jays, Haldane's rule, and genetic clines in secondary contact. BMC evolutionary biology 14:1.

Grant B. R., and P. R. Grant. 1996. Cultural inheritance of song and its role in the evolution of Darwin's finches. Evolution 50:2471-2487.

This article is protected by copyright. All rights reserved.

Accepted Article

Griffiths R., and R. M. Korn. 1997. A CHD1 gene is Z chromosome linked in the chicken Gallus domesticus. Gene 197:225-229.

Gröning J., and A. Hochkirch. 2008. Reproductive interference between animal species. Q. Rev. Biol. 83:257-282.

Haavie J., T. Borge, S. Bures, L. Z. Garamszegi, H. M. Lampe, J. Moreno, A. Qvarnström, J. Torok, and G. -. Saetre. 2004. Flycatcher song in allopatry and sympatry: Convergence, divergence and reinforcement. J. Evol. Biol. 17:227-237.

Hahn B. A., and E. D. Silverman. 2006. Social cues facilitate habitat selection: American redstarts establish breeding territories in response to song. Biology Letters 2:337-340.

Hall B. P., and R. E. Moreau. 1970. An atlas of speciation in African passerine birds. British Museum (Natural History), London.

Helb H. W., F. Dowsettlemaire, H. H. Bergmann, and K. Conrads. 1985. Mixed singing in European songbirds - a review. Zeitschrift Fur Tierpsychologie-Journal of Comparative Ethology 69:27-41.

Hijmans R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25:1965-1978.

Hochkirch, A., Gröning, J., and A. Buecker. 2007. Sympatry with the devil: reproductive interference could hamper species coexistence. J. Anim. Ecol. 76:633-642.

Hubisz M. J., D. Falush, M. Stephens, and J. K. Pritchard. 2009. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources 9:1322-1332.

Hudson R. R., M. Slatkin, and W. P. Maddison. 1992. Estimation of levels of gene flow from DNA sequence data. Genetics 132:583-589.

Jiménez-Valverde A., Y. Nakazawa, A. Lira-Noriega, and A. Townsend Peterson. 2009. Environmental correlation structure and ecological niche model projections. Biodiversity Informatics 6:28-35.

Johnson B. B., T. A. White, C. A. Phillips, and K. R. Zamudio. 2015. Asymmetric introgression in a spotted salamander hybrid zone. J. Hered. 106:608-617.

Kaneshiro K. 1976. Ethological isolation and phylogeny in Planitibia subgroup of Hawaiian Drosophila. Evolution 30:740-745.

Katoh K., K. Kuma, H. Toh, and T. Miyata. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 33:511-518.

Kawata M., and J. Yoshimura. 2000. Speciation by sexual selection in hybridizing populations without viability selection. Evol. Ecol. Res. 2:897-909.

This article is protected by copyright. All rights reserved.

Accepted Article

Kimball R. T., E. L. Braun, F. K. Barker, R. C. K. Bowie, M. J. Braun, J. L. Chojnowski, S. J. Hackett, K. Han, J. Harshman, V. Heimer-Torres, W. Holznagel, C. J. Huddleston, B. D. Marks, K. J. Miglia, W. S. Moore, S. Reddy, F. H. Sheldon, J. V. Smith, C. C. Witt, and T. Yuri. 2009. A well-tested set of primers to amplify regions spread across the avian genome. Mol. Phylogenet. Evol. 50:654-660.

Kishi S., T. Nishida, and Y. Tsubaki. 2009. Reproductive interference determines persistence and exclusion in species interactions. J. Anim. Ecol. 78:1043-1049.

Krabbe N., and T. S. Schulenberg. 1997. Species limits and natural history of Scytalopus tapaculos (Rhinocryptidae), with descriptions of the Ecuadorian taxa, including three new species. Ornithological Monographs :46-47-88.

Lachlan R. 2007. Luscinia.

Lachlan R. F., and M. R. Servedio. 2004. Song learning accelerates allopatric speciation. Evolution 58:2049-2063.

Laiolo P. 2012. Interspecific interactions drive cultural co-evolution and acoustic convergence in syntopic species. J. Anim. Ecol. 81:594-604.

Lande R. 1981. Models of speciation by sexual selection on polygenic traits. Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences 78:3721-3725.

Librado P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

MacArthur R. H. 1972. Geographical Ecology. Princeton University Press, Princeton, New Jersey.

Marler P., and S. Peters. 1977. Selective vocal learning in a sparrow. Science 198:519-521.

Marler, P., and V. Sherman. 1985. Innate differences in singing behaviour of sparrows reared in isolation from adult conspecific song. Animal Behaviour 33:57-71.

McEntee J. P. 2013. Social selection, song evolution, and the ecology of parapatry in sunbirds. University of California, Berkeley, Berkeley, California.

McEntee J. P. 2014. Reciprocal territorial responses of parapatric African sunbirds: species-level asymmetry and intraspecific geographic variation. Behav. Ecol. 25:1380-1394.

M'Gonigle L. K., R. Mazzucco, S. P. Otto, and U. Dieckmann. 2012. Sexual selection enables long-term coexistence despite ecological equivalence. Nature 484:506-509.

Mila B., J. E. McCormack, G. Castaneda, R. K. Wayne, and T. B. Smith. 2007. Recent postglacial range expansion drives the rapid diversification of a songbird lineage in the genus Junco. Proceedings of the Royal Society B-Biological Sciences 274:2653-2660.

This article is protected by copyright. All rights reserved.

Accepted Article

Morton, E.S. 1975. Ecological sources of selection on avian sounds. Am. Nat. 109:17-34.

Mumbi C. T., R. Marchant, H. Hooghiemstra, and M. J. Wooller. 2008. Late Quaternary vegetation reconstruction from the Eastern Arc Mountains, Tanzania. Quatern. Res. 69:326-341.

Nottebohm, F. 1968. Auditory experience and song development in chaffinch Fringilla coelebs. Ibis 110:549-&.

Payne R. J. H., and D. C. Krakauer. 1997. Sexual selection, space, and speciation. Evolution 51:1-9.

Peterson A. T., J. Soberon, and V. Sanchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. Science 285:1265-1267.

Pfennig D. W., M. A. Wund, E. C. Snell-Rood, T. Cruickshank, C. D. Schlichting, and A. P. Moczek. 2010. Phenotypic plasticity's impacts on diversification and speciation. Trends in Ecology & Evolution 25:459-467.

Phillips S. J., R. P. Anderson, and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190:231-259.

Plummer M., N. Best, K. Cowles, and K. Vines. 2006. CODA: Convergence diagnosis and and output analysis for MCMC. R News 6:7-8-11.

Podos J. 2001. Correlated evolution of morphology and vocal signal structure in Darwin's finches. Nature (London) 409:185-188.

--- 1996. Motor constraints on vocal development in a songbird. Anim. Behav. 51:1061-1070.

Podos J., and P. S. Warren. 2007. The evolution of geographic variation in birdsong. Advances in the Study of Behavior :403-458.

Price T. D., and M. M. Bouvier. 2002. The evolution of F1 postzygotic incompatibilities in birds. Evolution 56:2083-2089.

Pyron R. A., G. C. Costa, M. A. Patten, and F. T. Burbrink. 2015. Phylogenetic niche conservatism and the evolutionary basis of ecological speciation. Biological Reviews 90:1248-1262.

Qvarnström A., J. Haavie, S. A. Saether, D. Eriksson, and T. Part. 2006. Song similarity predicts hybridization in flycatchers. J. Evol. Biol. 19:1202-1209.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Sanchez-Gonzalez L. A., A. G. Navarro-Sigueenza, N. K. Krabbe, J. Fjeldså, and J. Garcia-Moreno. 2015. Diversification in the Andes: the Atlapetes brush-finches. Zool. Scr. 44:135-152.

SAS Institute Inc. 2010. JMP 9.0.

This article is protected by copyright. All rights reserved.

Accepted Article

Schoener T. W. 1968. The Anolis lizards of Bimini: resource partitioning in a complex fauna. Ecology 49:704-726.

Secondi J., V. Bretagnolle, C. Compagnon, and B. Faivre. 2003. Species-specific song convergence in a moving hybrid zone between two passerines. Biol. J. Linn. Soc. 80:507-517.

Secondi J., P. Bordas, C. A. Hipsley, and S. Bensch. 2011. Bilateral song convergence in a Passerine hybrid zone: Genetics contribute in one species only. Evolutionary Biology 38:441-452.

Seddon N. 2005. Ecological adaptation and species recognition drives vocal evolution in neotropical suboscine birds. Evolution 59:200-215.

Seddon N., and J. A. Tobias. 2010. Character displacement from the receiver's perspective: species and mate recognition despite convergent signals in suboscine birds. Proceedings of the Royal Society B-Biological Sciences 277:2475-2483.

Seehausen O., and D. Schluter. 2004. Male-male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. Proceedings of the Royal Society B-Biological Sciences 271:1345-1353.

Slabbekoorn H., and T. B. Smith. 2002. Habitat-dependent song divergence in the little greenbul: An analysis of environmental selection pressures on acoustic signals. Evolution 56:1849-1858.

Soha J. A., and P. Marler. 2000. A species-specific acoustic cue for selective song learning in the white-crowned sparrow. Anim. Behav. 60:297-306.

Sorenson M. D., J. C. Ast, D. E. Dimcheff, T. Yuri, and D. P. Mindell. 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Mol. Phylogenet. Evol. 12:105-114.

Stamps J. A. 1988. Conspecific attraction and aggregation in territorial species. Am. Nat. 131:329-347.

Stelkens R. B., M. E. R. Pierotti, D. A. Joyce, A. M. Smith, I. van der Sluijs, and O. Seehausen. 2008. Disruptive sexual selection on male nuptial coloration in an experimental hybrid population of cichlid fish. Philosophical Transactions of the Royal Society B-Biological Sciences 363:2861-2870.

Stephens M., N. J. Smith, and P. Donnelly. 2001. A new statistical method for haplotype reconstruction from population data. Am. J. Hum. Genet. 68:978-989.

Stuart S. N., F. P. Jensen, and S. Brogger-Jensen. 1987. Altitudinal zonation of the avifauna in Mwanihana and Magombera Forests, Eastern Tanzania. Gerfaut 77:165-186.

This article is protected by copyright. All rights reserved.

Accepted Article

Szymura J. M., and N. H. Barton. 1991. The genetic structure of the hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegate*: Comparisons between transects and between loci. Evolution 45:237-261.

--- 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *Bombina variegata*, Near Cracow in Southern Poland. Evolution 40:1141-1159.

Thorpe W. H. 1958. The learning of song patterns by birds, with especial reference to the song of the chaffinch Fringilla coelebs. Ibis 100:535-570.

Thum R. A. 2007. Reproductive interference, priority effects and the maintenance of parapatry in Skistodiaptomus copepods. Oikos 116:759-768.

Uyeda J. C., S. J. Arnold, P. A. Hohenlohe, and L. S. Mead. 2009. Drift promotes speciation by sexual selection. Evolution 63:583-594.

van der Sluijs I., T. J. M. Van Dooren, K. D. Hofker, J. J. M. van Alphen, R. B. Stelkens, and O. Seehausen. 2008. Female mating preference functions predict sexual selection against hybrids between sibling species of cichlid fish. Philosophical Transactions of the Royal Society B-Biological Sciences 363:2871-2877.

Verzijden M. N., C. Ten Cate, M. R. Servedio, G. M. Kozak, J. W. Boughman, and E. I. Svensson. 2012. The impact of learning on sexual selection and speciation. Trends in Ecology & Evolution 27:511-519.

Vokurkova J., T. Petruskova, R. Reifova, A. Kozman, L. Morkovsky, S. Kipper, M. Weiss, J. Reif, P. T. Dolata, and A. Petrusek. 2013. The causes and evolutionary consequences of mixed singing in two hybridizing songbird species (*Luscinia* spp.). Plos One 8:e60172.

Warren D. L., R. E. Glor, and M. Turelli. 2010. ENMTools: a toolbox for comparative studies of environmental niche models. Ecography 33:607-611.

--- 2008. Environmental Niche Equivalency Versus Conservatism: Quantitative approaches to niche evolution. Evolution 62:2868-2883.

Werema C., J. P. McEntee, E. Mulungu, and M. Mbilinyi. 2014. Preliminary observations on the avifauna of Ikokoto Forest, Udzungwa Mountains, Tanzania. Scopus 32.1:19-20-26.

West-Eberhard M. J. 2005. Developmental plasticity and the origin of species differences. Proc. Natl. Acad. Sci. U. S. A. 102:6543-6549.

--- 2014. Darwin's forgotten idea: The social essence of sexual selection. Neurosci. Biobehav. Rev. 46:501-508.

--- 1983. Sexual Selection, Social Competition, and Speciation. Q. Rev. Biol. 58:155-183.

Wiens J. J., and C. H. Graham. 2005. Niche conservatism: Integrating evolution, ecology, and conservation biology. Annual Review of Ecology Evolution and Systematics 36:519-539.

This article is protected by copyright. All rights reserved.

Accepted Article

Wiley R. H., and D. G. Richards. 1982. Adaptations for acoustic communication in birds: sound transmission and signal detection. Acoustic communication in birds 1:131-181.

Wilkins M. R., N. Seddon, and R. J. Safran. 2013. Evolutionary divergence in acoustic signals: causes and consequences. Trends in Ecology & Evolution 28:156-166.

Yannic G., P. Basset, and J. Hausser. 2008. A hybrid zone with coincident clines for autosomal and sex-specific markers in the Sorex araneus group. J. Evol. Biol. 21:658-667.

Table 1: Cline models Center and width estimates with 95% confidence intervals for each of the five modeled cline architectures used to investigate each of the three examined traits. For each trait, preferred models (ΔAIC<2) are in bold.

Trait	Center	Width	ΔAIC	Tails	Parameters
Song PC1			3.88	none	7
Song PC1	**163.0 (161.3-165.0)**	**5.9 (0.8-9.5)**	**0**	**left**	**9**
Song PC1			**0.31**	**right**	**9**
Song PC1			**0.14**	**mirror**	**9**
Song PC1			5.07	both	11
Molecular index			15.95	none	7
Molecular index			17.30	left	9
Molecular index	**163.7 (159.1-164.8)**	**3.7 (0.3-5.5)**	**0**	**right**	**9**
Molecular index			11.57	mirror	9
Molecular index			5.21	both	11
Culmen length	**155.1 (140.4-158.8)**	**21.4 (8.6-54.0)**	**0**	**none**	**7**
Culmen length			4.54	left	9
Culmen length			5.24	right	9
Culmen length			9.78	mirror	9
Culmen length			9.80	both	11

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