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Author
McEntee, Jay Patrick

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Social Selection, Song Evolution, and the Ecology of Parapatry in Sunbirds

by

Jay Patrick McEntee

A dissertation submitted in partial satisfaction of the requirements

for the degree of Doctor of Philosophy in Integrative Biology

in the Graduate Division of the University of California, Berkeley

Committee in Charge:
Professor Rauri Bowie, Chair
Professor Craig Moritz
Professor Frederic Theunissen

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Abstract

Social Selection, Song Evolution, and the Ecology of Parapatry in Sunbirds

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Professor Rauri Bowie, Chair

Social trait evolution can play a critical role in diversification and speciation. In birds, and especially oscine songbirds, the lability, species-specificity, and behavioral functions of song suggest that song divergence may lead or sustain the speciation process. This series of studies focuses on the evolution of song and its consequences in the Eastern Afromontane sky island sunbird species complex, which comprises the Eastern Double-collared Sunbird *Nectarinia mediocris* and its close relatives.

In Chapter 1, I examine the structure of one vocalization type, here termed perched song, for the members of the species complex. I show that song divergence matches molecular phylogenetic reconstructions for six major lineages of the species complex, and that song divergence is multi-dimensional. I use both unsupervised and supervised grouping techniques to assess the distinctness of song phenotypes as measured, with similar results. Model selection after unsupervised clustering analysis suggests that six clusters exist, and these correspond to *a priori* species distinctions at 92.8%. Model-based discriminant function analysis also correctly identifies songs to the six species designated *a priori* at 92.8%.

The discovery and characterization of the contact zone between *Nectarinia moreaui* and *N. fuelleborni* are detailed in Chapter 2. Molecular analyses reveal that there is hybridization, with little or no introgression, between the two species where they meet. The absence of an apparent ecological gradient closely corresponding with the abrupt spatial replacement of one species with the other suggested that interactions between the two species play a strong role in limiting each others' distribution. To test whether bioclimatic niche divergence is evident between species, I used ecological niche models developed from climate variables associated with species occurrence points. I show that the niches of the two species as characterized are conserved relative to a null distribution as generated by sampling the space within which each species' distribution is embedded. This test supports the hypothesis that the presence of each species inhibits the expansion of the others' distribution. Further, I characterize individual genotypes and song and bill length phenotypes sampled in a transect across the contact zone to estimate and compare the width of character clines. Cline analyses show that the genotype and song phenotype clines are roughly coincident and concordant, and very narrow, with the estimated cline width for these two characters ~1.5 km. The bill length cline is offset from the other two clines, and wider. I discuss the many processes that could contribute to the stability and narrowness of the genotype and song clines, including conspecific clumping.

In Chapter 3, I present the results of two playback experiments performed to examine male territorial responses to divergent signals in the parapatrically distributed *N. moreaui* and *N. fuelleborni*. These two species have diverged greatly in song phenotype, and subtly in morphometric and plumage traits. The first of the two experiments examines responses to multimodal signaling by assessing reactions to mount/song playback combinations, which are crossed for the two species. This experiment resulted in asymmetric heterospecific response between the two species, with *N. moreaui* responding consistent with predictions based on the species recognition hypothesis, and *N. fuelleborni* failing to respond more frequently to its own song than to *N. moreaui* song. Duration of response by treatment did not correspond to expectations based on species recognition, as there was a trend for individuals to respond longer to heterospecific mounts. In the second experiment, I investigated geographic variation in territorial response to the sibling species compared to own species' song and an ecologically relevant control stimulus, in the form of the song of a sunbird that co-occurs with both species for all tested populations. Responses to the control species were infrequent. Geographic variation in response differences to heterospecific (sibling taxon) and conspecific songs occurs. Strong responses to heterospecifics were not limited to the area of contact in *N. fuelleborni*, suggesting a role for stochastic processes in the evolutionary loss of response to the sibling taxon's song.

To conclude, song divergence accompanies molecular divergence in the Eastern Afromontane sky island sunbird species complex. Its role in the speciation process might be quite complex, and not as simple as the correlated evolution of female preference with male phenotype as in a Fisherian runaway process. I suggest that one role of song divergence during allopatric or parapatric speciation may be to reduce co-occurrence of individuals with dissimilar song phenotypes through anisotropic dispersal.

To the people of eastern Africa, and especially the Tanzanian *wananchi*, for all they've taught me. Shukrani.

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To my dad, who put names to different kinds of birds when I was young.

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CHAPTER 1

Song structure and divergence in the Eastern Afromontane sky island sunbird complex

Introduction

Approximately 40% of the world's bird species occur as allospecies, geographic forms of species complexes that have been designated taxonomically as different species (Price 2008). Often this taxonomy has been specified based on plumage differences among otherwise morphologically similar geographically designated groups. Vocal characters are also sometimes distinct among species complex members (Alström and Ranft 2003; Martens et al. 2003). However, despite the utility of these traits for taxonomy, robust quantification of differences for both plumages and songs with strong spatial sampling is not all that frequently performed. The origin of character differences and the forces that drive divergent evolutionary trajectory have been a concern of ornithologists, and is of special interest in the study of speciation and diversification. With respect to vocal phenotypes, many stochastic and deterministic mechanisms have been hypothesized to account for the origin and maintenance of geographic variation (Podos and Warren 2007). To better study these processes, thorough description and quantification of these differences are necessary.

In this chapter I deal specifically with the quantification of song features across the Eastern Afromontane sky island sunbird complex. Thorough song quantification including within-individual and within-species spatial replication is rarely done. Previous efforts to quantify song phenotypes at this level are few (Irwin 2000; Packert et al. 2003; Martens et al. 2003; Packert et al. 2004; Paeckert et al. 2006). These studies have demonstrated that song variation in the focal groups is consistent with a role for song in diversification and speciation, in that song variants are geographically restricted, have low variation within taxa relative to among taxa, and that differences in song among populations tend to be correlated with genetic differences among populations. The existence of this geographic variation appears a common feature of oscine songbird evolution, and is comparatively uncommon in suboscines (Podos and Warren 2007; Catchpole and Slater 2008).

The goals of this chapter are to describe and quantify song variation across the Eastern Afromontane sky island sunbird complex, to examine this variation using clustering techniques and discriminant function analysis, and to discuss hypotheses that explain species-level differences.

Natural history of focal taxa

The Eastern Afromontane sky island sunbird complex comprises *Nectarina mediocris*, *N. usambarica*, *N. moreaui*, *N. loveridgei*, and *N. fuelleborni*. Molecular and song phenotype differences, the latter described here, suggest the recognition of a new taxon within *N. mediocris* sensu stricto, referred to here as *Nectarinia* sp nov. These birds' distributions cover sub-montane and montane environments spanning the northernmost population at Mt. Kulal in northern Kenya, through the central Kenyan highlands, the northern Tanzanian volcanic highlands, the igneous Eastern Arc Mountains, and the mountains of various geological origins that ring Lake Nyasa (Lake Malawi). The southernmost populations of the species complex are *N. fuelleborni* (*bensoni*) populations on Mt. Mulanje (Malawi) and Namuli (Mozambique). Ecologically and morphologically similar species appear to replace these species to the west, especially within and near the Albertine Rift. The westernmost populations, that come nearest central African highlands including the Albertine Rift, are found at Kakamega Forest (Kenya), the Ngorongoro Crater highlands (Tanzania), the Mbulu highlands (Tanzania), Mt. Hanang (Tanzania) and the

Ufipa Plateau (Tanzania). Contrary to previous reports of a far western Tanzanian population of *N. fuelleborni* (*Cinnyris mediocris fuelleborni* at the time of reports), similar-sized double-collared sunbirds from Mahale Mountains have been determined to be Regal Sunbird *N. regia* (RCK Bowie pers. comm.).

Phylogenetic analyses of concatenated sequence data from mitochondrial genes (ND2 and ND3) and nuclear intron loci recover two major clades within the species complex with high confidence (Bowie et al. 2004), RCK Bowie, pers. comm., see Figure 1). The northern clade comprises three distinct lineages in this analysis: Kenyan populations of *Nectarinia mediocris*, Tanzanian populations of *N. mediocris* (the aforementioned new taxon *N.* sp nov), and *N. usambarica*. The southern clade comprises three major lineages *N. moreaui*, *N. loveridgei*, and *N. fuelleborni*. *N. moreaui* may itself comprises two lineages, with the Nguru Mountains population representing a distinct lineage. No phenotypic differences are known between Nguru Mountains populations of *N. moreaui* and other populations.

Definitions

Song: In this chapter, I quantify and analyze variation in what I term perched song. Perched song is one of many vocal behaviors exhibited by members of this species complex. It is delivered from a perched position, usually from a relatively open perch in vegetation. The behavioral context of perched song is distinguished as a long-duration vocalization (>1s) from what I term chase song, which is associated with male-male antagonistic interactions. Perch heights for perched song vary from ~2 to 30m (JPM unpublished data). Perched song is delivered from a large variety of microhabitats: forest canopy, forest understory, forest edge, shrubland outside of the forest edge (up to ~50m from the forest edge), brackenfern, *Acacia* woodland, pine plantings, *etc*. It is typically delivered in bout form, such that consecutive songs are separated by a relatively short duration of silence or other calls. Songs sung during bouts appear to exhibit a lesser degree of among-song variation than songs sung as ‘one-offs’ (pers. obs.).

Introduction: The sequence of elements that initiates a song. I have defined the structure of these songs as having two parts, and the introduction is the first of the two parts. The elements of the introduction tend to be more widely spaced temporally than those of the body (Figure 2). Introductions vary greatly within individuals. All species are known to use introductions that involve singing broadband sounds at an accelerating rate up to the beginning of the body portion of the song (Figure 4).

Body: The second part of the song, in which notes are more closely spaced than in the introduction (see Figure 3). The structure of the body has variation within and among individuals, but is species-specific.

Element: An individual trace on the sonogram, representing a discrete portion of a song.

Micro-element: Extremely short elements. The rapid production of several micro-elements with extremely short intervals between them appears combined to produce composite units in some of the species addressed here. It is not clear whether sounds that register as continuous traces in sonograms but have multiple amplitude peaks are distinct biomechanically or perceptually from composite units that register on the sonogram as multiple distinct micro-elements. The seemingly continuous variation between micro-elements and elements results in a level of ambiguity in determining what the smallest discrete elements within a song should be for purposes of analyses. In practice, I relied on an automated approach to make initial determinations, and then reviewed segmentation of songs to check if (micro)elements that were visually distinct traces on the sonogram had been isolated as distinct elements.

Methods

The structure of the songs in this group presents a challenge for maximizing the power to detect differences. Songs generally consist of two parts: the first part, which I term introduction, is either a short series of 1-8 elements that are widely spaced relative to the elements in the second part, or a series of scratchy, relatively high-bandwidth elements that are delivered at an accelerating rate. Individuals use multiple different introductory sequences, and it appears all individuals are capable of using both of these two broad types of introduction. The acoustic properties of these two types of introduction are starkly different from one another, especially in species with short introductory sequences of the 1st type (compare Figures 2 and 4). In considering the similarities and differences among taxa in the presented analyses, it is important to keep this in mind. The presence of the similar, high-bandwidth trill-type introduction among taxa helps to establish homology of this vocal type among taxa and to establish homology of the general two-part structure of this vocal type among taxa. However, determining the precise inflection point where the introductory sequence ends and the second part of the song begins is not always trivial. For this reason, I have elected to analyze entire songs using 'summary statistics' over the elements of each song, as opposed to dividing the songs into two segments. The latter approach would likely maximize the power to detect differences among taxa in each of the two segments, but the segmentation process might not be adequately repeatable to justify this step without a quantitative basis, which could be quite complicated to develop.

Recordings

Song recordings were principally selected from the library of recordings made by the author from 2007-11 in Kenya, Tanzania, and Mozambique. Additional recordings were obtained from the Macaulay Library (http://macaulaylibrary.org), the British Natural History Museum natural sound recording archives, and the xeno-canto open access database (xeno-canto.org). Searches for high quality recordings were performed from these sources and among the author's field recordings. Recordings with high signal:noise ratio containing bouts of song were selected. Individual songs sung in isolation (i.e. not in bout form) were avoided for analysis, with the exception of a single *N. usambarica* recording, retained because of the limited sample size of high quality recordings of this species, which sings at lower amplitude relative to the other species in the species complex (JPM pers. obs.) and is therefore more challenging to record in the field.

Recordings were standardized for sampling to 44,100 Hz before analyses. Individual songs were extracted from longer recordings and filtered in Raven Pro 1.3 (Bioacoustics Research Program 2011). Each song was bandpass filtered at 2 to 10 kHz. Because of the impact of noise on the fine-scale structural analysis employed, recordings of *N. mediocris*, *N.* sp nov, and *N. usambarica* were further filtered at 2.5 to 9 kHz. The stricter filtering could not be applied to *N. fuelleborni* or *N. moreaui* because these species sometimes produce frequencies below 2.5 kHz during perched song. Additional noise was sometimes removed using spot filtering within sonograms e.g. for non-focal bird sounds. Examination of sound recordings was performed to check for the presence of non-avian environmental noise from animal sources. Insect sounds (probably cicadas) are evident from a limited subset of the recordings. Presence of insect sounds occurring as frequency bands in sonograms were noted, and the frequency range of these sounds was recorded. Pervasive, loud insect sounds in recordings from the Uluguru Mountains at frequencies ~2 to 4.5 kHz required additional low-frequency filtering of *N. loveridgei* recordings to enable fine structure analysis.

After filtering, individual songs were imported into the program Luscinia (Lachlan 2007). Sonagrams were produced in Luscinia using the following settings: Max. frequency: 10,000 Hz; Frame length: 5 ms; Time step: 1 ms; Spectrograph points: 221; Spectrograph overlap: 80%; Echo removal: 100%; Echo range: 100; Windowing function: Hann; and High Pass Threshold: 2000 Hz. Sonogram contrast was adjusted to achieve the highest visual signal:noise ratio. Signal within the sonogram was detected using Luscinia's built-in signal detection functionality. The mode was set to 'elements', and measurement settings were set to default values. Results of automatic signal detection were checked by eye and ear, with recordings slowed for playback to 1/8 speed. Inconsistencies in signal detection from were corrected using the *brush* tool.

From the extracted values for elements within songs as defined by Luscinia, I calculated 15 variables for each individual by devising 'summary statistics' for each variable for each song. Means of these summary statistics were calculated from the set of songs analyzed for each individual. Peak frequency is defined in Luscinia as the frequency with the highest amplitude for a given portion of the sonogram. Means were taken across the following per-song 'summary statistics': median gap duration between elements (ms), standard deviation (sd) of gap duration between elements (ms), median peak frequency, sd peak frequency, maximum peak frequency, minimum peak frequency, range peak frequency (song), number of elements, median bandwidth (Hz), sd bandwidth (Hz), per-element median frequency change (Hz), sd per-element frequency change (Hz), song duration (ms), element duration (ms), and median element frequency range (Hz). For downstream analyses, the number of elements, median bandwidth, and song duration measures were natural log-transformed to improve normality.

I performed MANOVA by species on the 15-variable data set using JMP 9.0 (SAS Institute Inc 2010), and individual ANOVA by species on each variable individually to assess statistical differences for each variable in isolation. To decrease dimensionality in the data set for use with mixture models, I then performed a principal components analysis, also in JMP 9.0. The first six components were retained for use in the modeling analyses.

Gaussian finite mixture modeling: Cluster and Discriminant Function Analyses

I used Gaussian finite mixture modeling to perform cluster analysis on individual song phenotype scores with the package Mclust (Fraley et al. 2012) in R 2.15.1 (R Core Team 2012). I used this package to develop multiple mixture models, which were then compared using the Bayesian Information Criterion (BIC). The best model by BIC score was then used to assess the correspondence of mixture components with species identity of the individual. Models varied by the number of mixture components (interpreted as clusters, varying from 1 to 9 (but see Baudry et al. 2010), and parameterization via the covariance matrix determining flexibility in the volume and shape of the different cluster distributions. I used six possible parameterizations, corresponding to either spherical distributions (with variable or equal volume but equal shape clusters) or diagonal distributions (variable or equal volume and/or shape). I assessed the 54 unique models generated by the mixture component number by covariance parameterization combinations using BIC, and present the array of BIC scores and the clustering results of the favored model.

I then performed discriminant function analysis (DFA) using the MclustDA algorithms of the Mclust package. MclustDA implements model-based DFA that fits Gaussian mixture models to each of the categorized groups in the training data. This approach has the additional flexibility that categorized group distributions can be modeled as mixtures of multiple components, but in this data set the sample size for some species is small (*loveridgei*=6, sp nov=9), such that inferring multiple components within them appears unwarranted. I therefore limit each category to a single mixture component. Limiting each category to a single component also allows for more clarity in displaying data with modeled parameters for 6 categories, such that each mixture component ellipse from the model represents a single category in visualizations.

Results

Song differences by species

Boxplots of each of the 15 measured song variables arrayed by species are shown in Figure 5. A MANOVA for these 15 variables for the six species as groups was significant (Table 1: Pillai's trace=3.03, F=12.53, df=75, p<.0001). Only the natural log of instantaneous frequency change is not significantly different among species after Bonferroni correction for 15 univariate ANOVA comparisons at an alpha level of .05 (p=.041, significance at p=0.0033). Values for the individual mean of frequency change for elements within songs were all <0.5, meaning that a greater proportion of sound production during songs occurs during downward inflecting frequency sweeps than during upward inflecting frequency sweeps (i.e. the slope of frequency change is more frequently negative than positive) for all species in these analyses.

Song descriptions

Nectarinia mediocris (née Cinnyris mediocris Shelley 1885*)* The songs of the first named species from the species complex are described in Cheke et al. (2001) as "Two tseep notes 0.5 seconds apart, followed by a downslurred ts-zsurr and a rapid rattle-like warble for 2-7 seconds, with much frequently modulation . . . Similar to song of *C. loveridgei* but slower and notes more discernible in the rattle." This description gives an impression of the two-part structure of *mediocris* song, where the 'tseep' notes and 'ts-zsurr' would form part of the introduction as defined here, and the 'rapid rattle-like warble' represents the body portion of the song. However, the introduction varies greatly within, and likely among, individuals, so it is not as stereotyped as the Cheke et al. (2001) description would suggest. Some within-individual variation in the introduction can be seen in Figure 2a, which shows three song exemplars from a single individual. The introduction can also consist of a series of more broadband sounds, increasing in rate as the bird approaches the body part of the song (similar to the other species shown in Figure 4). The body portion of the song consists of bursts of microelements, divided by gaps, which could perhaps be described as studdered.

Nectarinia sp nov The effect of evolutionary history (phylogenetic effect) is seen in structural similarities in the songs of this taxon and *N. mediocris*. These taxa emit similar body portions of the song, where micro-elements are rapidly delivered to form longer elements (~3-10 micro-elements per larger elements, see Figure 3g-h) in the 'studdered' pattern described for *mediocris*. Examinations of the fine-scale structure of these songs indicates that the gaps between composite elements in *N.* sp nov songs are ~15% longer on average than those in *mediocris* songs (Figure 5, 'median pause duration'). More obvious gross differences occur in song duration and the number of elements per song – *N.* sp nov songs average 3.2 times as long as *mediocris* songs and contain 3.2 times as many elements. *N.* sp nov songs have relatively stereotyped introductions consisting of widely spaced elements that are shorter and have higher bandwidth than typical introductory elements of *mediocris* songs (Figure 2a-b). However, they also use the accelerating rate, high-bandwidth element introduction found in the other members of the species complex.

Nectarinia usambarica (*née Cinnyris mediocris usambarica* Grote 1922) To a human observer, *usambarica* songs are quiet, and more delicate-sounding than *mediocris* and *N.* sp nov. They tend to sing at low amplitude, which also makes them more challenging to record in the field. This difference is, unfortunately, not quantifiable from field recordings. *Usambarica* males sing relatively lower bandwidth elements compared to both *mediocris* and *N.* sp nov (Figure 5: Ln median bandwidth), so the quality of their songs is less scratchy than these two species. They sometimes sing elements that are broken up into micro-elements as in *N.* sp nov and *mediocris*, and *usambarica* songs share somewhat the staggered pattern of pauses with these two species. This similarity appears to be reflected in measures of the variation in pause duration among species (Figure 5: SD pause duration). Introductory elements in this species have higher frequency and lower bandwidth than *N.* sp nov introductory elements, though this can be remarked only qualitatively as quantification of these elements was not independently performed.

Nectarinia loveridgei (*née Cinnyris loveridgei* Hartert 1922) Aptly described in Cheke et al. 2001 as "a short (3-4 seconds) warble of very high-pitched *tsi* notes delivered so fast that they recall an insect such as a grasshopper; sometimes preceded by a few double notes of *tsp-tee*, *tsp-tee*, *tsp-tee*," *loveridgei*'s song is surprising given its size. Though larger by mass than all other species in the complex (Cheke et al. 2001; Bowie et al. 2004), *loveridgei* exhibits by far the highest minimum peak frequency in songs (Figure 5). Consistent with the description from Cheke et al. (2001), the median pause duration between elements is lower in this species than in all the other members of the species complex. This combination of features indeed results in an insect-like sound. Comparison with *moreaui* songs reveals that *loveridgei* songs are like sped-up *moreaui* songs with median peak frequencies shifted higher ~22%. The body of *loveridgei* songs are comprised of jumbled trills, with a large portion of each song body made up of similar, rapidly delivered (micro)elements. Longer gaps between rapidly delivered spurts of micro-elements, as in *mediocris* and *N.* sp nov, are not typical of *loveridgei* (or *moreaui* – see Figure 5: SD pause duration). Instead, they deliver a fairly steady stream of very short elements or micro-elements.

Nectarinia moreaui (*née Cinnyris mediocris moreaui* Sclater 1933) *Moreaui*'s song is described in Cheke et al. 2001 as "a rapid, almost musical cascade of high-pitched notes similar to song of *C. mediocris*." It is, as explained above, most similar in structure to *loveridgei* song, with the body made up of a nearly steady stream of elements. Pause duration is slightly longer in *moreaui* than *loveridgei*, but like in *loveridgei* pauses are not so variable in duration (Figure 5: SD pause duration). In addition to the lower frequency of elements in *moreaui* song relative to loveridgei, median bandwidth of *moreaui* song is higher. As a consequence of this set of features, *moreaui* songs are more easily detected by the human ear than *loveridgei* songs in the field.

Nectarinia fuelleborni (nee Cinnyris mediocris fuelleborni Reichenow 1899*)* The songs of *fuelleborni* are the most distinct among the members of the species complex. Within the body of the song, both elements and pauses are long in duration. The acoustic properties of the elements themselves vary greatly, as reflected in the SD peak frequency (Figure 5), and as captured in their great peak frequency range at the level of song. Their songs have a thrush-like sing-song quality, with frequencies exploring higher and lower frequencies in an up-and-down pattern. They occasionally exhibit mimicry of other bird species, including Cinnamon Bracken Warbler *Bradypterus cinnamomeus* (pers. obs.). Their songs are also much longer (~2.5-3 times as long on average) as their sibling species *loveridgei* and *moreaui*.

Insect sounds as ambient noise

Examination of the recordings revealed loud insect sounds with relatively constant frequency bandwidths simultaneous with sunbird singing for a few of the sunbird populations recorded. The highest-amplitude insect sounds as visible in sonograms were low-frequency sounds (500-6000 Hz) in the Uluguru and high-frequency sounds in the Usambara (7500-12600 Hz, Figure 8).

Principal components analysis

Results of principal components analyses of the 15 quantified variables are depicted in Table 2. The first 6 principal components, describing 89.6% of the variation in the data, were used for cluster and discriminant function analyses.

Gaussian finite mixture cluster analysis

For clustering, the initial data set included 51 *fuelleborni*, 6 *loveridgei*, 9 sp nov, 17 *mediocris*, 42 *moreaui*, and 13 *usambarica* individuals. The best model by BIC had 6 components, and was a diagonal distribution model, with variable volume and equal shape among component distributions as specified by the covariance matrix (Figure 6). Classification broadly corresponded with taxonomic designations of the five named species and the yet-to-be-named sixth species. The error calculated from cluster classification using this model was 7.2%. Hence, the unsupervised clustering algorithm clustered the data into groups corresponding to the species as hypothesized by the author, and this clustering designation identified 92.8% of the individuals to correct species clusters under the author's *a priori* taxonomy.

The DFA model (n=138, df=92) with one cluster per component and a full covariance matrix for each component, had a training error of 1.4%. 10-fold cross validation yielded an error rate of 7.2% in assignment (similar to the clustering analysis error). Dimension reduction was performed for visualization, and plots of the first four of the six dimensions are depicted in Figure 7.

Discussion

Song divergence in the Eastern Afromontane sky island species complex is concordant with a taxonomic hypothesis based on a combination of molecular data (Figure 1), geography, and morphology. There are subtle morpholometric and/or plumage differences (Bowie et al. 2004) between all the entities designated as species in this chapter except possibly *N. mediocris* and the proposed new taxon *N.* sp nov. Although a robust morphological study has not been performed to examine differences between these two species, song divergence between these forms appears concordant with molecular differences as revealed by their separate placement in a phylogenetic tree based on concatenated nuclear and mtDNA sequences (RCK Bowie, unpublished data). The concordance of lineage divergence at a molecular level and divergence in song suggests either that 1) song can be a driver of speciation processes (West-Eberhard 1983), or 2) that song divergence results from other speciation processes (Oneal and Knowles 2013). As the *mediocris*-sp nov split appears to involve at most minimal morphometric or plumage differences, song divergence may play a divergence-promoting role at an earlier initial stage of speciation than those traits.

Because the songs themselves are distinguishable by human ear (pers. obs.), the mixture model statistics presented here might be viewed as a way to assess the applicability of these methods. An approach to characterizing songs by generating ‘summary statistics’ of multiple continuously-varying measures, in conjunction with Gaussian finite mixture modeling, resulted in relatively high power to discern songs of different species. The clustering approach, in which multiple models are compared by the Bayesian Information Criterion, resulted in a model with 6 components, which here is interpreted to mean there are six clusters. These six clusters were concordant with species designations as indicated by geography, or made by JPM in the field. These results suggest that the methods used may have utility in similar cases where differences among groups may not be salient to human perception. In this case, analyzed vocalizations are extremely complex and variable, but nonetheless recordings could be grouped by the taxonomic hypothesis for species in the species complex.

Examining the species-level song differences in the context of the phylogenetic relationships (Figure 1), it is evident that speciation events have been marked by song divergence in different dimensions of acoustic structure. Strong divergence in central tendencies for song frequency marks the divergence event only between *N. loveridgei* and the *N. moreaui-N. fuelleborni* clade. Because *loveridgei* sings at higher frequencies relative to all other species in this group, it is of interest to examine the likely drivers of this evolutionary change. Frequency tends to be negatively correlated with body mass (Ryan and Brenowitz 1985; Seddon 2005; Gillooly and Ophir 2010), but *loveridgei* has the greatest body mass in the species complex and sings at the highest frequencies. Because the trajectory of trait evolution under social selection can be arbitrary, social selection is a plausible mechanism for this directional change. However, much of the recent literature on acoustic communication in nature (Ryan and Brenowitz 1985; Slabbekoorn and Smith 2002a; Slabbekoorn and den Boer-Visser 2006; Kirschel et al. 2009; Luther and Derryberry 2012) has explored the role of noise as a driver of acoustic divergence. Geographic variation in noise across the distribution of the species complex is unlikely to be strongly influenced by vegetation differences, because 1) these sky island sunbirds occupy similar environments; and 2) there is likely to be a tremendous amount of variation in the acoustic environment experienced by a single male, because an individual of any of these species may sing from forest edge, forest canopy, shrubland outside of the forest, or the forest understory, at various points in its lifetime or within a day. I suggest here that a consistent source of geographic variation is more likely to result from differences in the insect communities (and perhaps variation in the phenology of acoustic communication of those insects) that co-exist with this species complex. If one argues that insect noise drives the frequency shift seen in *N. loveridgei*, one would make the prediction that there is a band of insect noise in the soundspace of the Ulugurus (where *loveridgei* is found) at or within ~ 2500 to 5000 Hz, which the sibling taxa of *loveridgei* exploit, but which it avoids. Indeed, persistent insect noise in the Uluguru occupies this frequency range, and can be even more extensive, covering ~500 to 6000 Hz at times. The hypothesis that insect noise drives the frequency differences between it and its sibling taxa merits further examination.

The evolution of frequency differences is also interesting in the case of *N. fuelleborni*. Frequency differences are part of a suite of characteristics that differentiate *fuelleborni* from its sibling taxa. However, differences are not in the central tendencies of frequency, but in the range of frequency used. *Fuelleborni* explores far more of the frequency soundscape than its sibling taxa. As discussed in the case of *loveridgei*, noise could play a role in the explanation for this pattern. It is possible that there is little insect noise in areas occupied by *fuelleborni*, such that constraints on its frequency spectrum are minimal. Alternately, because the spectro-temporal structure of *fuelleborni* elements differs greatly from its sibling taxa, insect noise may not have as great an effect on *fuelleborni* song transmission. The evolution of the element content of *fuelleborni* song could therefore have allowed for an increase in frequency range by a feedback mechanism, even in the presence of high-frequency or low-frequency noise. Regardless, the dramatic divergence in spectro-temporal properties in the *fuelleborni* lineage involves changes in many different dimensions. With this in mind, I propose a hypothesis of heterospecific copying to explain the radical divergence of *fuelleborni* song. *Fuelleborni* shares much of its distribution with the Yellow-browed Seedeater *Serinus whytii*. Their songs are similar in a number of respects, including the ‘sing-song’ quality of alternating higher and lower frequency portions of song, and similar gap duration between component elements of songs. It is plausible, especially given that *fuelleborni* occasionally mimic other species’ songs within the structure of their own songs, that the origin of their unique phenotype within the species complex is copying error followed by a cultural and social selection.

Song duration shows dramatic variation among the Eastern Afromontane sky island sunbird species. It is striking that both *fuelleborni* and sp nov possess songs that are ~3 times as long as their sister taxa. Weir and Wheatcroft (2010) conclude that song duration does not evolve as quickly in the tropics as it does in temperate zones based on sister taxa comparisons. These authors do not provide raw duration values or duration differences for their sister taxa, but threefold differences in song duration among sister pairs must have been rare among low-latitude taxa in their data set, or occurred only in sister taxa with long divergence times at low latitudes. The *mediocris-N.* sp nov split is recent (~2.7% sequence divergence in the ND2 gene; RCK Bowie, unpublished data), and their shared distribution crosses the equator. It is possible that fast divergence in song duration at low latitudes is rare and that this sister pair represent an unusual event, or perhaps the effect seen in Weir and Wheatcroft (2010) is stronger in New World oscines than it is in Old World oscines.

Finally, one of the unanticipated results of the multivariate analysis was the lack of statistically significant difference among the focal taxa in a variable that reflects the instantaneous slope of frequency through time within song elements. These values revealed a universal bias for down-sloping frequencies through time within this species complex. This result is consistent with the analyses of Tierney et al. 2005, who found a bias towards descending frequencies versus ascending frequencies within 'notes' across 80 songbird species. This pattern is suggestive of a shared biomechanical constraint, which may relate to peripheral vocal mechanisms and the interaction of airflow with the muscles of the vocal apparatus (Tierney et al. 2011).

Conclusions

The diversity of song phenotypes found within the Eastern Afromontane sky island sunbird species complex makes this group a compelling system for the study of song divergence. Of particular interest with respect to this group are examinations of the mechanisms leading to divergence in different dimensions of song phenotypes. Analyses controlling for relatedness of populations are a goal of future work. The description and quantification of aspects of these species' vocalizations as described in this chapter, however, should allow for future studies to be pursued along a number of different lines of inquiry: biomechanical constraints, syllable meme evolution, and perception of divergent song phenotypes.

Table 1: Results of MANOVA and corresponding univariate ANOVA for the 15 measured variables from songs of the six species that make up the Eastern Afromontane sky island sunbird species complex.

MANOVA	**Value**	**Approx F**	**Num DF**	**Den DF**	**p-value**
	3.03	12.5	75	569.4	<0.0001*

ANOVA	**Value**	**Exact F**	**Num DF**	**Den DF**	**p-value**
Median gap	2.33	64.5	5	132	<0.0001*
SD gap	0.84	22.1	5	132	<0.0001*
Median peak frequency	0.89	23.4	5	132	<0.0001*
SD peak frequency	4.99	131.7	5	132	<0.0001*
Max peak frequency	2.1	55.5	5	132	<0.0001*
Min peak frequency	1.87	49.3	5	132	<0.0001*
Range peak frequency (song)	2.64	69.8	5	132	<0.0001*
Ln number of elements	0.48	12.7	5	132	<0.0001*
Ln median bandwidth	0.93	24.4	5	132	<0.0001*
SD bandwidth	0.38	10.1	5	132	<0.0001*
Log median frequency change	0.09	2.4	5	132	0.04
SD frequency change	0.51	13.5	5	132	<0.0001*
Ln song duration	1.72	45.5	5	132	<0.0001*
Median element duration	2.43	64.2	5	132	<0.0001*
Median element frequency range	2.58	68	5	132	<0.0001*

Table 2: Statistics for principal components from a principal component analysis of the 15 measured song variables from individuals of the Eastern Afromontane sky island sunbird species complex.

Number	**Eigenvalue**	**Percent**	**Cum Percent**	**ChiSquare**	**DF**	**Prob>Chisq**
1	5.83	38.8	38.8	1716.0	119	<.0001
2	2.39	15.9	54.8	1146.6	104	<.0001
3	1.91	12.7	67.5	918.0	90	<.0001
4	1.40	9.3	76.8	684.7	77	<.0001
5	1.13	7.6	84.4	476.3	65	<.0001
6	.78	5.2	89.6	242.0	54	<.0001
7	.52	3.5	93.0	38.7	44	0.6983
8	.32	2.2	95.2		35	
9	.23	1.5	96.7		27	
10	.19	1.3	98.0		20	
11	.12	0.8	98.8		14	
12	.09	0.6	99.4		9	
13	.07	0.5	99.9		5	
14	.02	0.1	100		2	

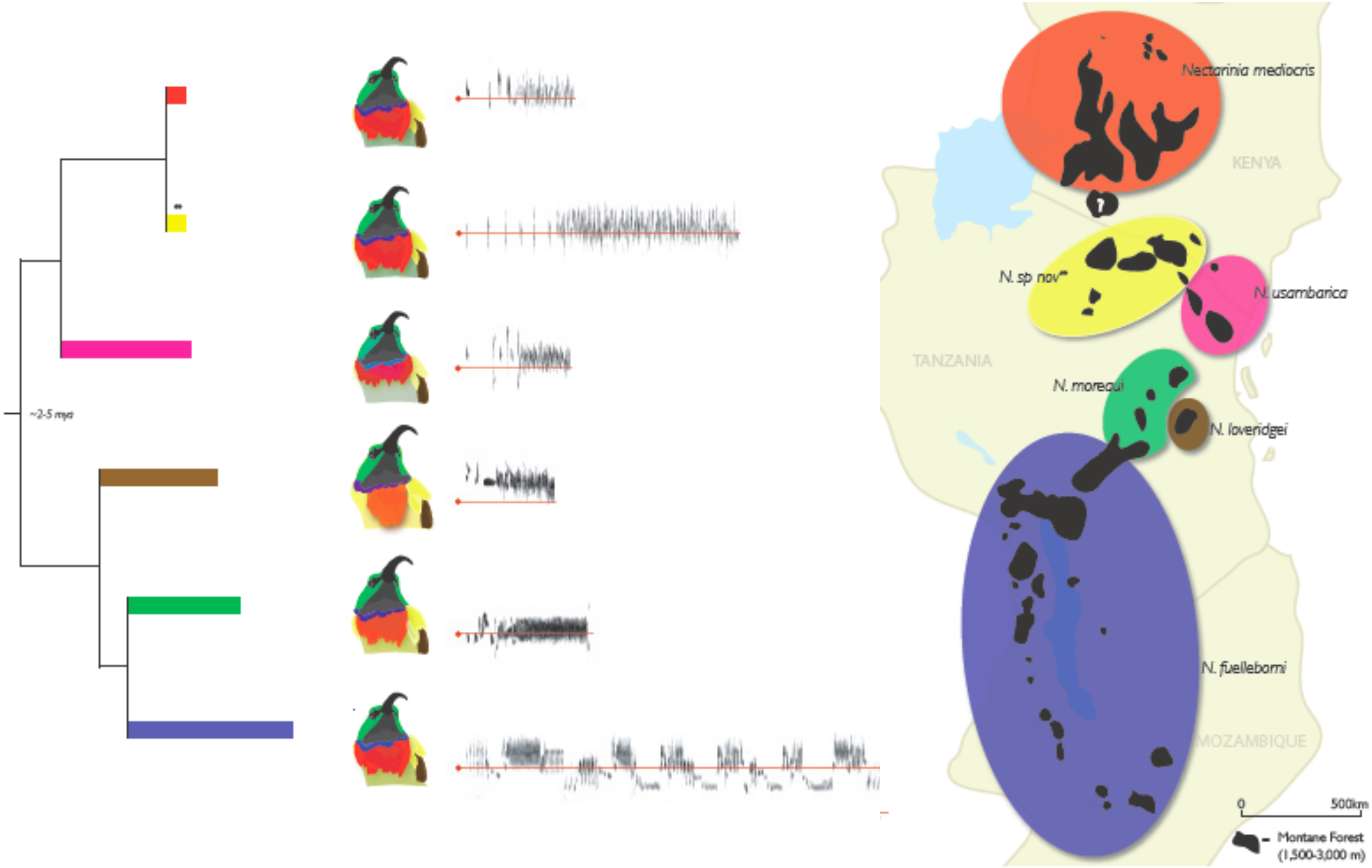


Figure 1: From left to right: Phylogeny, graphically represented males, representative sonograms, and distributions of the six species comprising the Eastern Afromontane sky island sunbird species complex. Color bars in the phylogeny match colored distribution bubbles on the distribution map. The red dot and line in the representative sonograms indicate 5 kHz frequency. The phylogenetic relationships and branch lengths represent those recovered in a phylogenetic analysis of concatenated DNA sequence data from nuclear and mitochondrial DNA sequences (RCK Bowie, unpublished data). Graphic designed by Kathleen Rudolph.

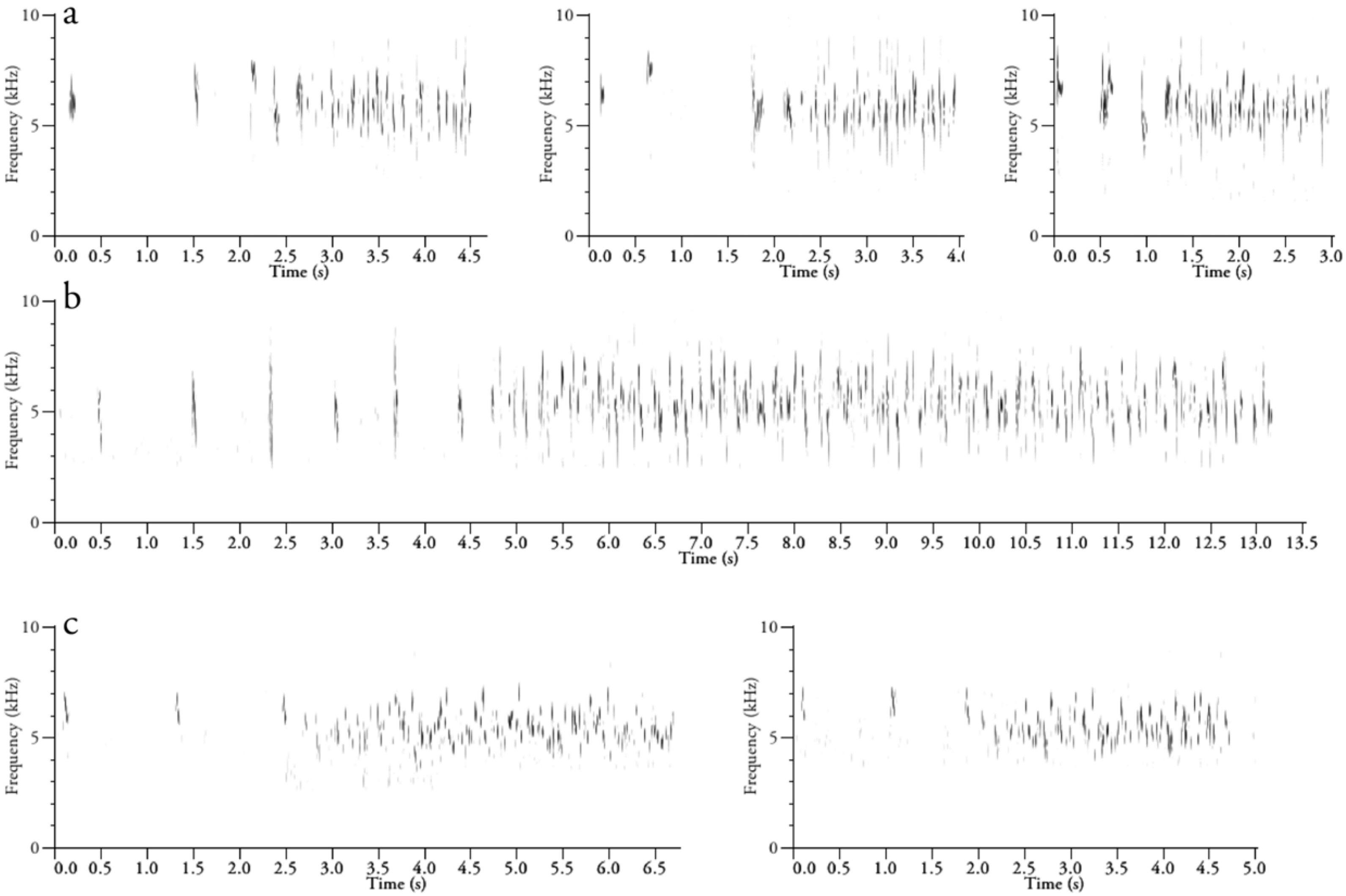


Figure 2: Representative sonograms of full songs of six species: a – *Nectarinia mediocris*; b – *N.* sp nov; c – *N. usambarica*; The time axis is standardized.

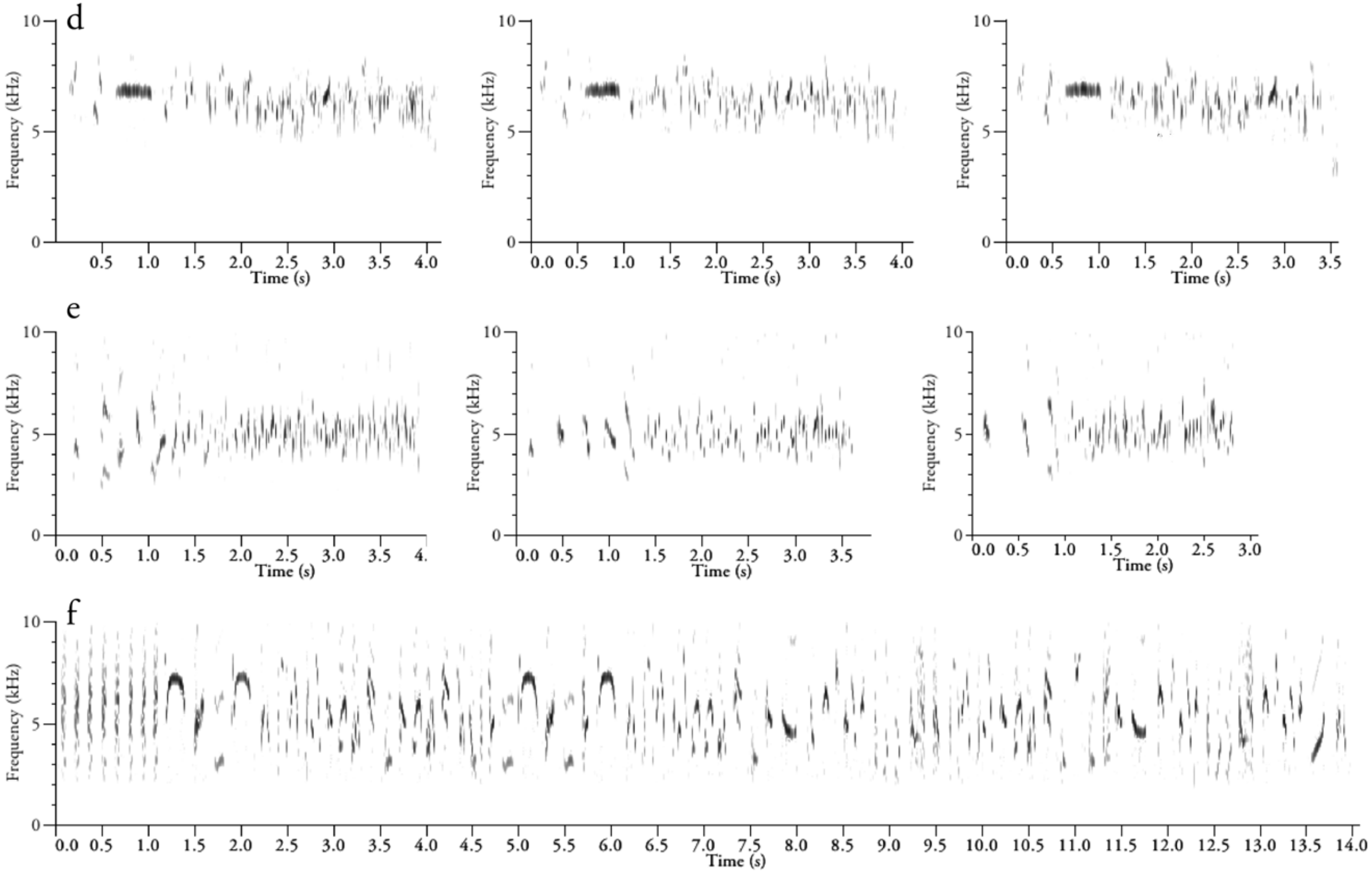


Figure 2 - continued: Representative sonograms of full songs of six species: d – *N. loveridgei*; e – *N. moreaui*; f – *N. fuelleborni*. The time axis is standardized.

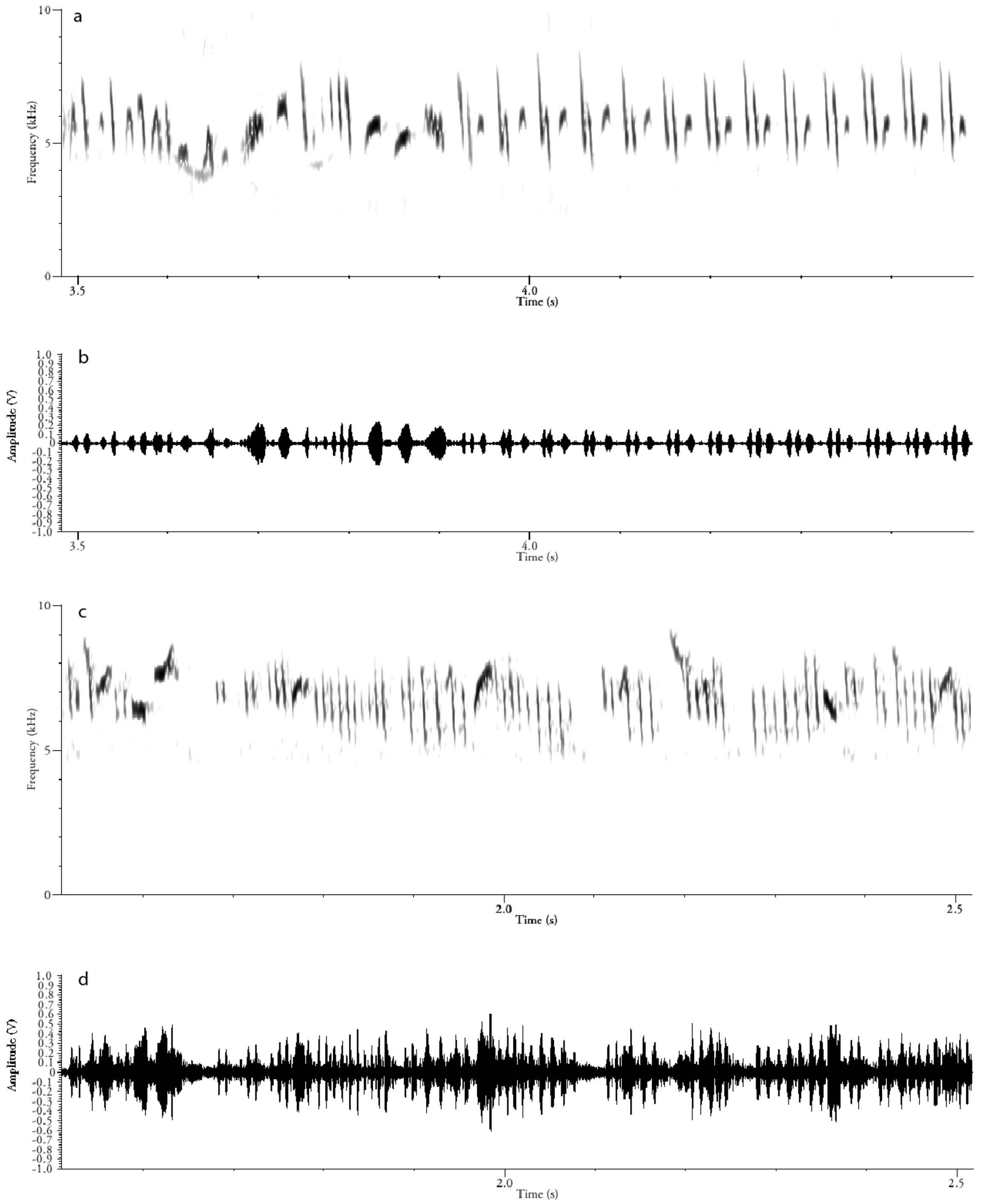


Figure 3: Sonograms and waveforms standardized to show 1 s of the body portion of song (see Definitions in text), generated in Luscinia (Lachlan 2007). a & b) *Nectarinia moreaui*; c & d) *N. loveridgei*; e & f) *N. mediocris*; g & h) *N.* sp nov; I & j) *N. usambarica*; k & l) *N. fuelleborni*. Differences in amplitude peaks among waveforms shown should not be interpreted as relevant to species-level differences, however the spacing of these amplitude peaks are relevant.

Figure 3e - 3h

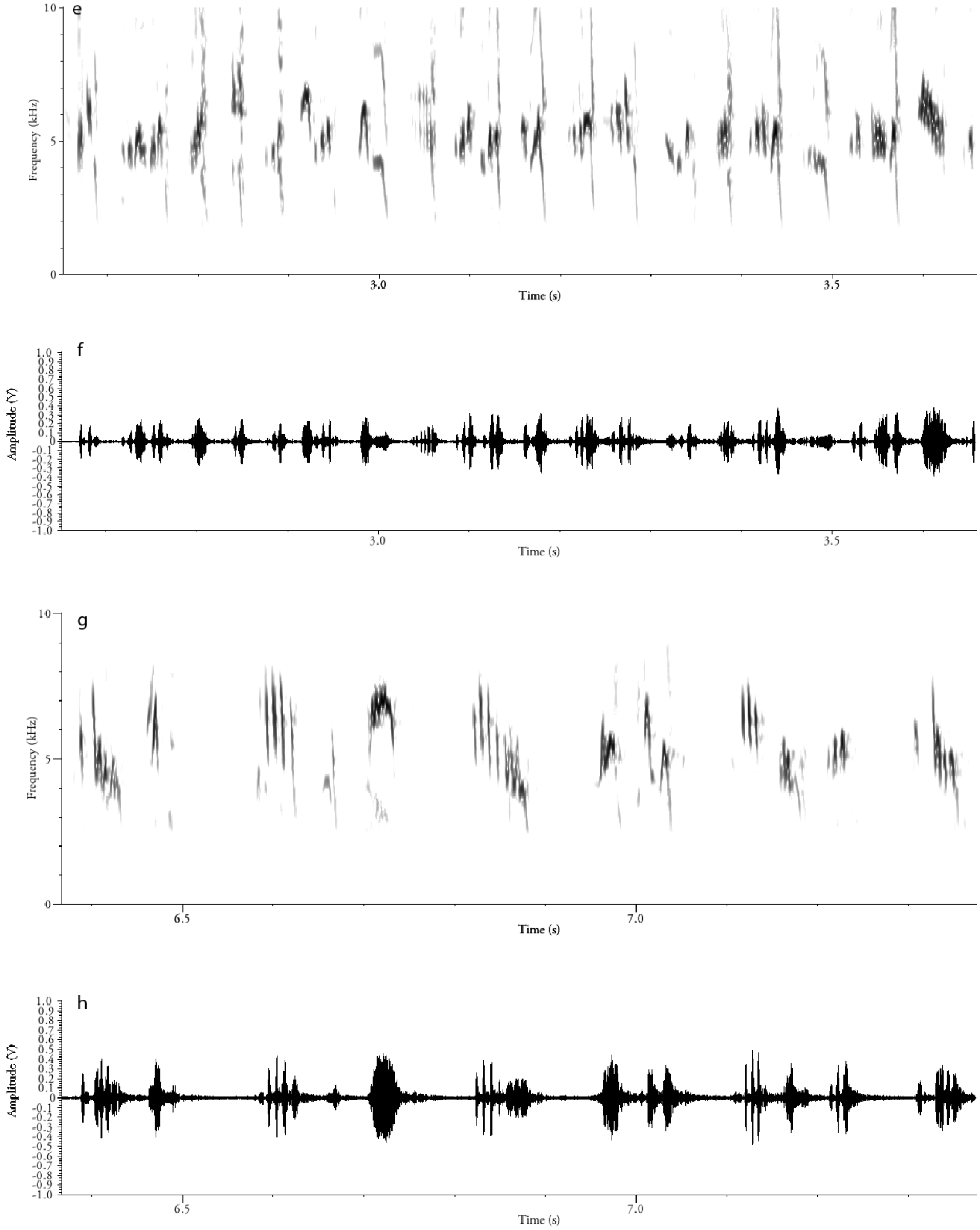
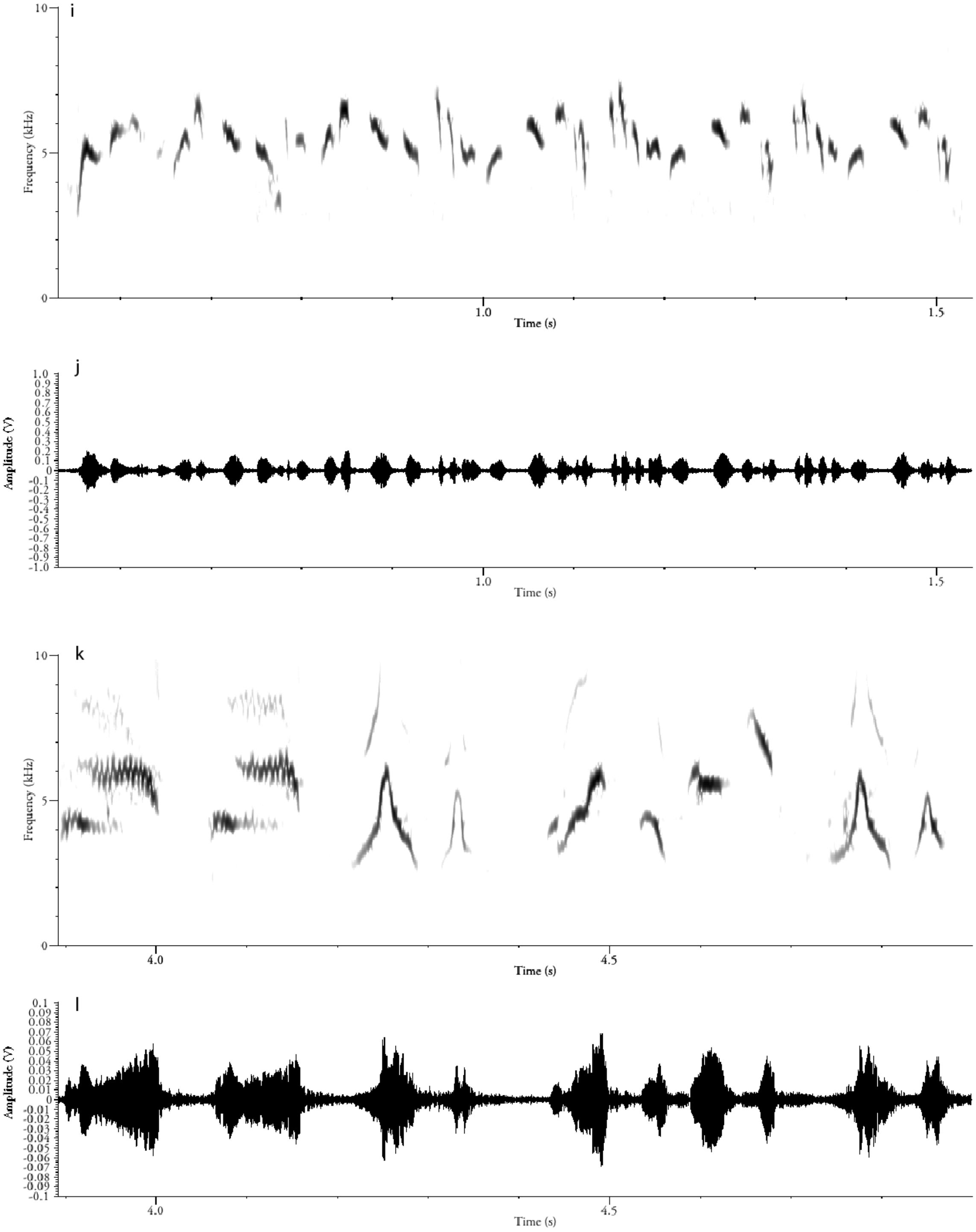

e
Frequency (kHz)
10
5
0
3.0
3.5
Time (s)
f
Amplitude (V)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
3.0
3.5
Time (s)
g
Frequency (kHz)
10
5
0
6.5
7.0
Time (s)
h
Amplitude (V)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
6.5
7.0
Time (s)

Figure 3i - 3l


i
Frequency (kHz)
10
5
0
1.0
1.5
Time (s)
j
Amplitude (V)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
1.0
1.5
Time (s)
k
Frequency (kHz)
10
5
0
4.0
4.5
Time (s)
l
Amplitude (V)
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
-0.01
-0.02
-0.03
-0.04
-0.05
-0.06
-0.07
-0.08
-0.09
-0.1
4.0
4.5
Time (s)

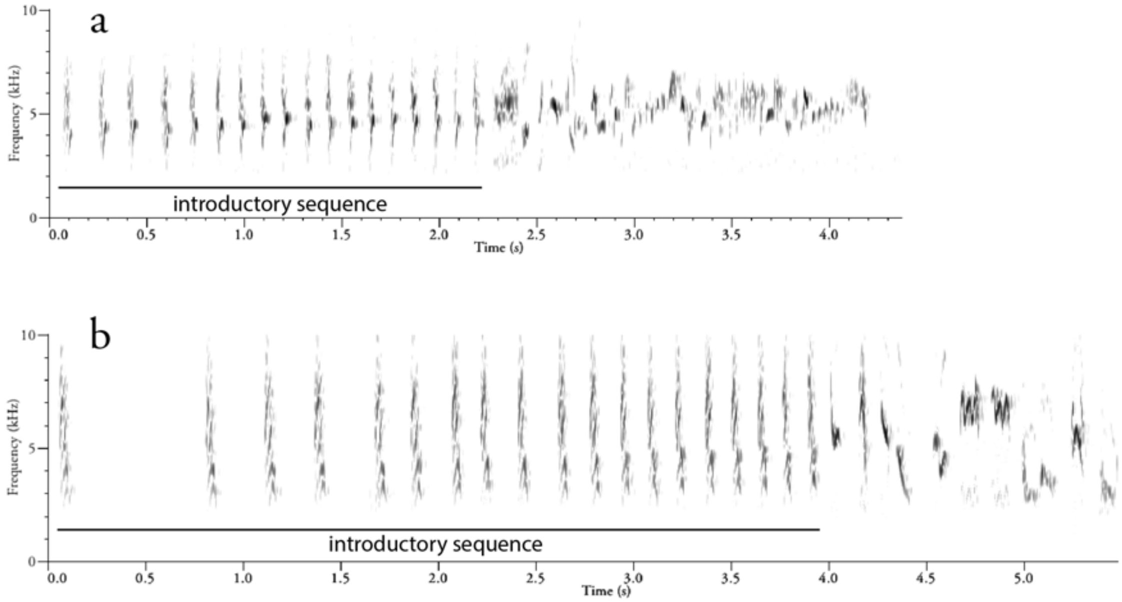


Figure 4: Sonograms of a) *Nectarinia moreaui* and b) *N. fuelleborni* (truncated) songs showing broadband introductory sequences with increasing rate of delivery. Similar introductory sequences are found in all six of the species from the Eastern Afromontane sky island sunbird species complex, consistent with homology of this song type (perched song) and homology of the two-partedness of these songs (introduction and body).

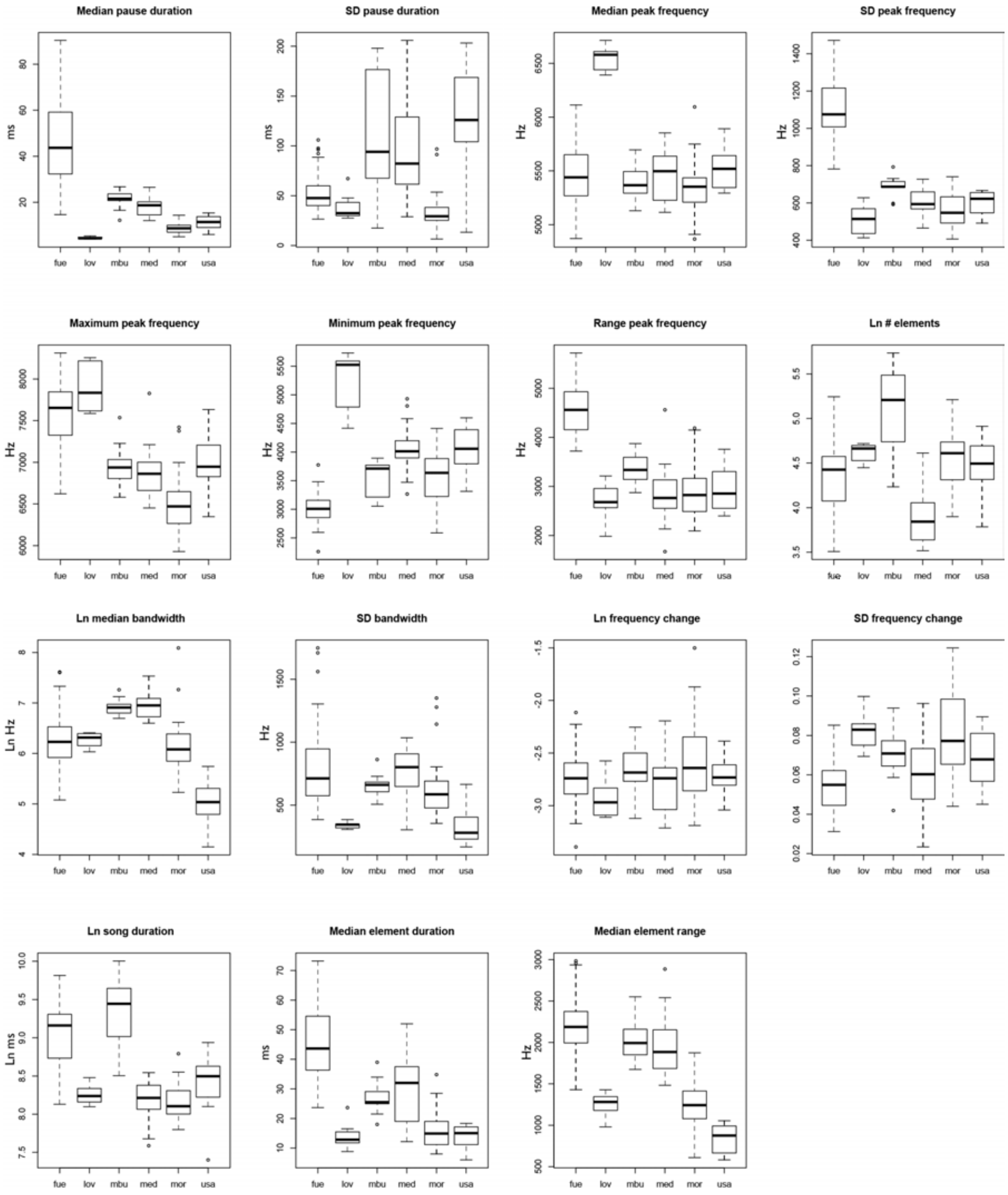


Figure 5: Boxplots of each of 15 measured song variables by species in the taxonomic hypothesis used by the author for the Eastern Afromontane sky island sunbird species complex. fue = *Nectarinia fuelleborni*, lov = *N. loveridgei*, mbu = *N.* sp nov, med = *N. mediocris*, mor = *N. moreaui*, usa = *N. usambarica*. All variables are significant at an alpha level of .05 after Bonferroni correction except the natural log of frequency change (Ln frequency change, p = .041, significance at p<.0033).

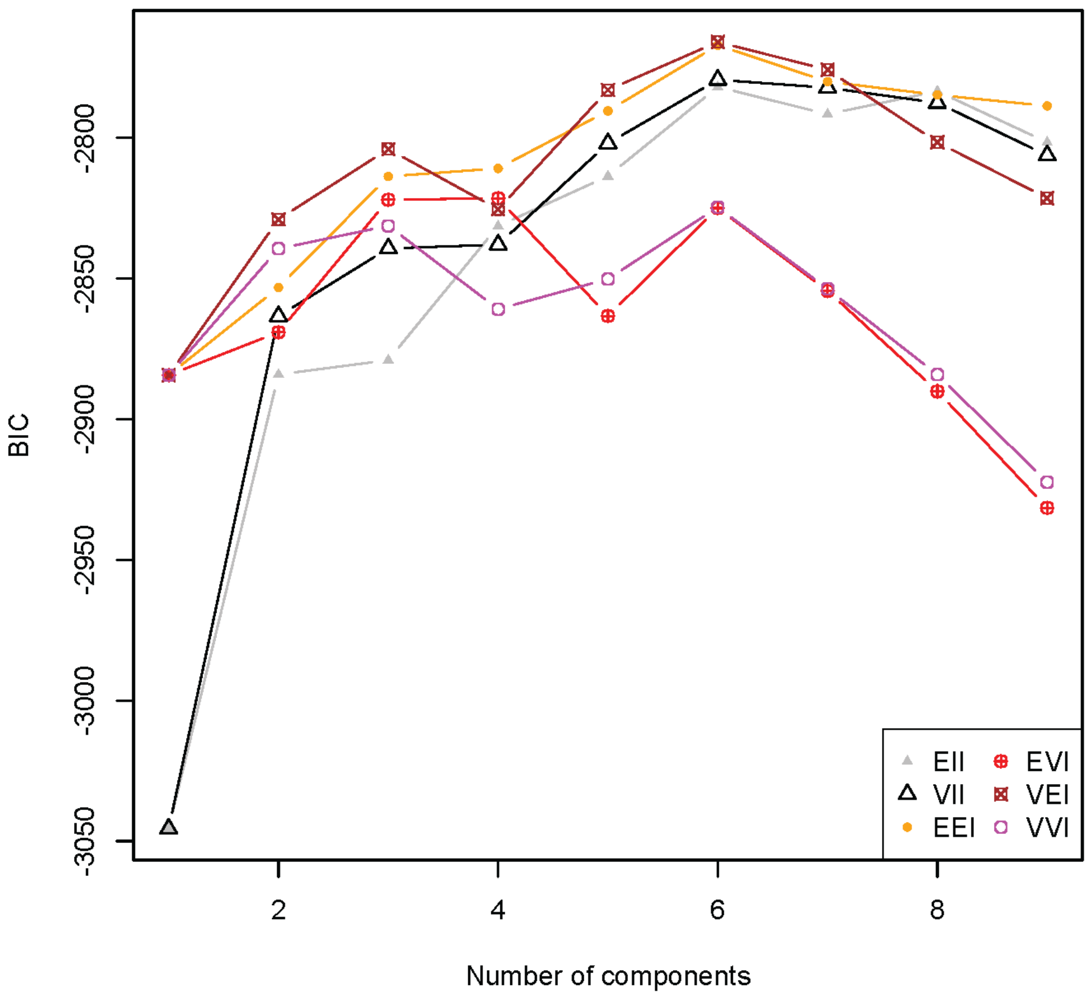


Figure 6: Bayesian Information Criterion for 54 Gaussian finite mixture models representing combinations of mixture component number and parameterization of the covariance matrix. Better models have higher BIC scores. In this case, the best model is a VEI model with 6 mixture components The six covariance parameterization types are: EII – equal volume, equal shape, spherical distribution; VII – variable volume, equal shape, spherical distribution; EEI – equal volume, equal shape, diagonal distribution; EVI – equal volume, variable shape, diagonal distribution; VEI – variable volume, equal shape, diagonal distribution; VVI – variable volume, variable shape, diagonal distribution.

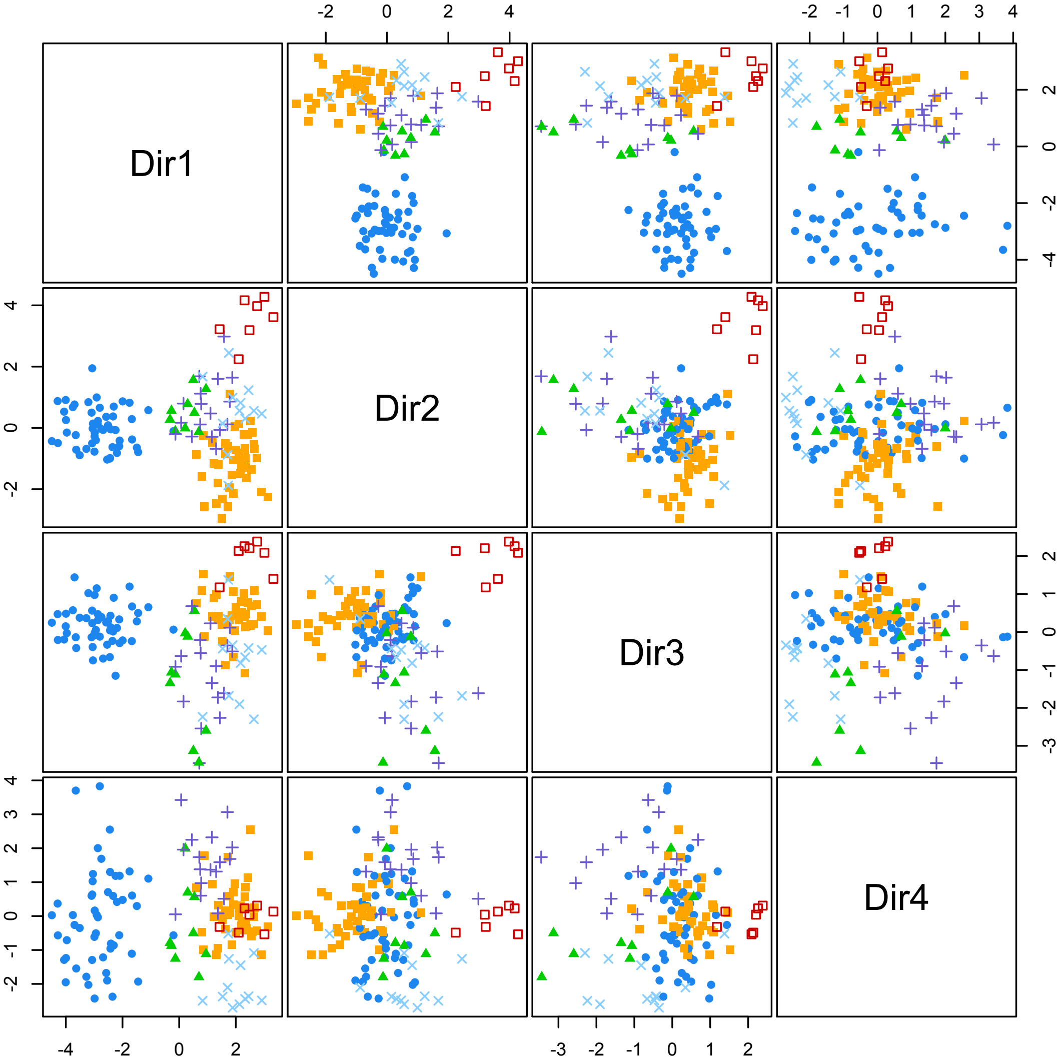


Figure 7: Plots of the first four dimensions of phenotypic space corresponding to a model-based Discriminant Function Analysis (DFA) performed using the Mclust package for R. The analyzed phenotype space was based on the first six principal components from a PCA of 15 measured variables from songs. Species coding: Blue circles = *fuelleborni*, red squares = *loveridgei*, yellow squares = *moreaui*, purple crosses = *mediocris*, green triangles = sp nov, light blue x's = *usambarica*.

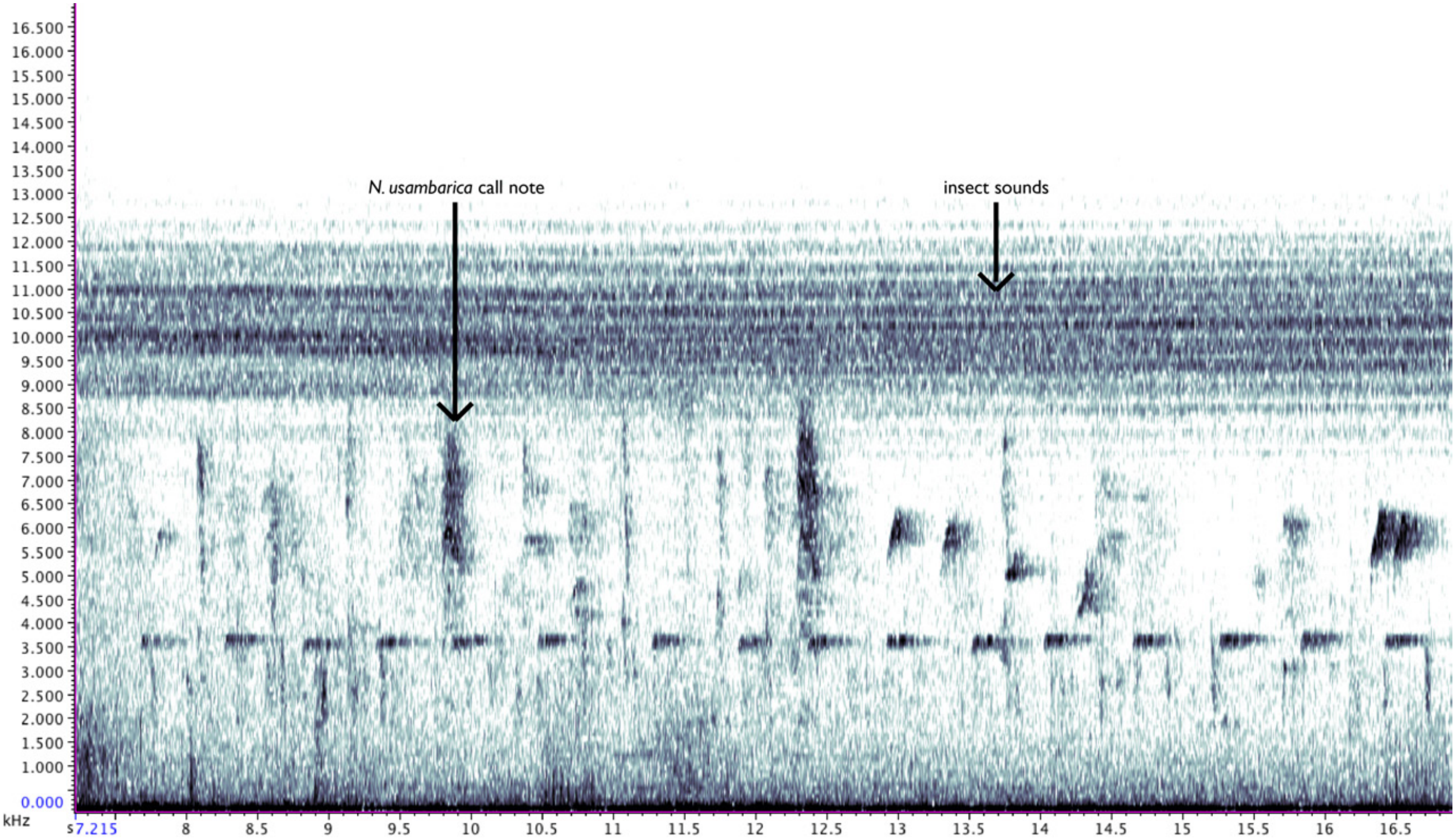


Figure 8: A sonogram of an unfiltered recording, generated in Raven Pro 1.3. The recording was made at the Mazumbai Forest Reserve in the West Usambara Mountains, Tanzania. The dark band at ~8700 to 12000 Hz is the result of insect (probably cicada) signaling. A stretch of the recording that shows two calls of *Nectarinia usambarica* was selected to show that these calls occur at lower frequencies than most of the cicada sound.

CHAPTER 2

Parapatry between avian sibling species with great song divergence and high niche conservatism

Abstract

Parapatric boundaries between incipient or recently diverged species present the opportunity to study myriad evolutionary and ecological processes, including the extent and mode of reproductive isolation, the role of interspecific interactions in setting range limits, and the impact of contact on traits. Here I present the discovery of a narrow parapatric boundary between two sunbird species with minimal ecological and plumage differences but great divergence in learned song. The sunbirds *Nectarinia moreaui* and *N. fuelleborni* meet in the Udzungwa Mountains, Tanzania, forming a contact zone where pure individuals outnumber all classes of hybrids at all sites with substantial sampling. The zone is narrow relative to likely dispersal distances – indeed, it is likely be the narrowest avian hybrid zone yet described. I present molecular evidence indicating that the two species produce viable hybrid offspring, but that individuals with mixed ancestry occur at low frequency over a limited area, and do not act as a conduit for introgression. Instead, selection against hybrids is evident from the lack of gene flow at the hybrid zone. Secondly, I examine species-level trait divergence in abiotic niche, bill morphology, and territorial songs. I find that the two species possess extremely similar abiotic niches, but subtly different mean bill lengths and radically different songs. In the third set of analyses, I test for concordance and coincidence of genotypic and phenotypic clines across space. Because the production of species-typical complex songs requires learning in oscine songbirds, heterospecific copying could cause the song cline to be shallow or offset relative to the molecular cline. Learned song instead behaves like a typical quantitative trait in the cline analysis: the song cline and a multi-locus molecular index based on one mtDNA and five nuclear intron markers are concordant and coincident. Finally, the cline in bill length is removed ~10 km from the song and genome clines. I interpret this geographic decoupling of species-level morphological differences and species-specific song traits, together with data showing that primary differences in song are most likely based on neural-mechanism differences and not performance-limitation, as indirect evidence that morphological evolution did not drive song divergence along the axis of greatest divergence between the two species. Moreover, niche conservatism and microhabitat generalism strongly suggest that differential transmission efficacy has also not driven song divergence. Instead, social selection driving neural-based song predispositions is the preferred hypothesis. I interpret the combined results of this set of analyses as suggestive that stabilized parapatry is due to a combination of behavioral and ecological mechanisms resulting in Allee effects. I further hypothesize that local song cultures dissuade heterospecific migrants from settling or establishing territories, resulting in conspecific aggregation, in turn elevating levels of assortative mating via local allopatry during breeding seasons, and resulting in extremely limited sympatry. These findings set the stage for future research in this contact zone on 1) the role of social traits in speciation; 2) geographic variation in the perception of heterospecific signals; and 3) the role of inter-specific interactions in setting range limits.

Introduction

" . . . the low degree of ecological divergence of socially selected sibling species may particularly dispose them to parapatric distributions. And the aggressiveness and territoriality of socially competitive individuals, as well as the tendency to aggregate preferentially with conspecifics (e.g., in mating or nesting sites), may sometimes contribute to the maintenance of clearcut boundaries between species."

- Mary Jane West-Eberhard (1983)

Contact zones between recently diverged or incipient species (hereafter 'diverging species') can serve as natural laboratories for the study of both speciation and ecological co-existence. Traits that have diverged in allopatry might persist, disappear, or become more exaggerated in sympatry or parapatry dependent on the plasticity of these traits and the evolutionary effects of ecological and/or reproductive interactions between the diverging species (Brown and Wilson 1956; Grant 1972). By examining geographic variation in traits among populations of recently diverged or incipient species in secondary contact, it is possible to understand which traits are most likely to prevent merging of incipient species in the face of interspecific interactions and gene flow (Dobzhansky 1940; Barton and Hewitt 1981; Lande 1981; Lande 1982; Barton and Hewitt 1985; Butlin 1987; Sanderson 1989; Liou and Price 1994). In this way, instances of secondary contact are essential for the study of both speciation and ecological interaction between divergent lineages.

Ecological and evolutionary outcomes of contact between incipient or recently diverged species take on a variety of forms, which can be summarized by the extent of reproductive isolation (Mayr 1963), the form and magnitude of trait changes resulting from interactions with a close evolutionary relative (Grant 1972), and the extent of coexistence (Macarthur 1958; MacArthur and Levins 1967). These outcomes are thought to result as a function of the degree and type of evolutionary divergence – habitat niche, ecomorphological, physiological, social, *etc.* – that has taken place prior to contact (Schluter 2000). Generally, substantial divergence along an ecological axis is thought to be necessary for substantial sympatry to be achieved upon secondary contact. Reproductive isolation, on the other hand, may result in the absence of substantial ecological divergence – it can evolve due to social divergence without ecological divergence (West-Eberhard 1983), or from mutation-order processes leading to selection against hybrids (Coyne and Orr 2004). Hence, substantial sympatry might be achieved without strong reproductive isolation if ecological divergence is strong, and reproductive isolation might be achieved while substantial sympatry is prevented by ecological similarity. The 'modality' terminology developed by (Harrison and Bogdanowicz 1997) for hybrid zones can be extended to cover the spectrum of possibilities: from 'unimodal' distributions of organisms where a single mode is prevalent in all populations, inclusive of areas predominantly comprised of individuals of mixed ancestry, to fully 'bimodal' distributions where widespread sympatry is achieved by two distinct entities without (successful) interbreeding.

Trait divergence and secondary contact

As stated above, the evolution of traits in allopatry is critical to the outcomes of secondary contact. In birds, as in most other taxa, it is evident that the early stages of speciation can involve changes in many different types of traits, and the outcomes of secondary contact are likely to vary correspondingly. Though there appears to be no general rule to which traits diverge initially in birds or other groups, numerous authors have commented on the relative commonness of paths to divergence. Here I focus on ecological and social traits. In an influential, broad taxonomic study that included birds, Peterson et al. (1999) found that ecological niche divergence is slow relative to speciation times for allopatric sister taxon pairs. Similarly, Kozak and Wiens (2006) suggested that allopatric divergence is driven by niche conservatism in some groups. Alternatively, many authors have suggested that differential adaptation along gradients and other forms of ecological divergence are common mechanisms for speciation (Endler 1977; Nosil 2012). Indeed, among the most famous speciation examples in birds are the divergence events of the Galapagos finches, for which niche shifts and ecological divergence play a primary, if not sufficient, role in speciation (Schluter and Grant 1984; Schluter and Nagel 1995; Schluter 2000). Ecological divergence is thought to be the most potent force in speciation, in part because ecological divergence can cause byproduct or subsequent change in other traits, e.g. cues used in species recognition, thereby contributing to reproductive isolation through a variety of processes (Podos 1996; Slabbekoorn and Smith 2002b; Seddon 2005). Social traits can be driven by habitat differences or eco-morphological divergence, but they need not be. In birds, there are many celebrated examples of species with similar ecological requirements distinguishable from one another by elaborate social traits – effectively geographic replacements of ecological equivalents (Price 1998; Price 2008). These examples suggest a strong role for social divergence under niche conservatism allopatry, although studies of secondary contact between such forms are few (but see e.g. (Brumfield et al. 2001)).

Songs of song-learning birds in particular have received a great deal of attention as a social trait with the potential to play a role in speciation in birds. Much recent attention has focused on the ways that song evolution may diverge through differential selection based on transmission efficacy (Morton 1975; Wiley 1991; Slabbekoorn and Smith 2002a; Tobias et al. 2010), or byproduct evolution as a consequence of morphological evolution (Podos 2001; Podos et al. 2004; Podos and Warren 2007; Derryberry 2009). However, social selection on song in the absence of consistent transmission differences or extensive morphological evolution is also likely to be pervasive. Cultural evolution provides an additional means for rapid divergence that does not rely on the generation of molecular variants. Extremely important for learned song to have a central role in speciation, however, is its robustness to convergence during secondary contact of divergent species. Numerous examples exist of convergence of song phenotypes in sympatry (Cody 1969; Qvarnstrom et al. 2006; Secondi et al. 2011), even in non-learning birds (Tobias et al. 2010). Song convergence is associated with interspecific territoriality, and thus appears to be driven by male-male interactions or through passive heterospecific copying errors, although the latter is not possible in the example from non-learning birds (*Hypocnemis*: Seddon and Tobias 2010). Song convergence can be detrimental to males when songs are used both in territoriality and in courtship. *Ficedula* flycatcher males exhibiting intermediate song attract heterospecific mates more often than males with songs more like the parental forms, and individuals with intermediate song phenotypes suffer fitness losses a result of increased hybridization rates (Qvarnstrom et al. 2006). Thus, song represents a signal used in functions with species recognition implications, and may be prone to rapid evolution (West-Eberhard 1983; Lachlan and Servedio 2004), but learned song especially might be prone to convergence during secondary contact. Song therefore represents an extremely interesting character for study during secondary contact.

Hypotheses regarding the outcome of secondary contact dependent on divergence modes can be developed based on ecological and evolutionary theory. West-Eberhard (1983) generated one such hypothesis to explain the commonness of narrow parapatric distributions without extensive hybridization, between socially diverged sibling species. West-Eberhard made the observations that 1) social trait divergence is a common pattern of geographically isolated populations (within and among species); 2) social traits can diverge "with or without ecological differences" between populations; 3) social traits can be used in mate choice and their divergence is a likely route to reproductive isolation; and 4) that closely related species tend to have similar ecological requirements generally, but that this might be especially so among species who are prone to rapid divergence in social traits under niche conservatism allopatry. From this set of observations, West-Eberhard hypothesized that socially divergent sister taxa should be especially subject to forming parapatric distributions, because their divergence does not depend on ecological differences, and during secondary contact they should exhibit strong ecological competition because of their extensive resource overlap while maintaining distinctness as biological entities because of reproductive isolation. Moreover, she hypothesized that two additional facets of social animal behavior are likely to play a role in generating narrow range boundaries: social animals can be intensely territorial and/or aggressive – increasing the importance of interference competition in interspecific interactions, and social animals tend to cluster preferentially with conspecifics (see also Stamps 1988).

The ecology of parapatry

Darwin (1859) conceptualized range boundaries as resulting from the interplay of abiotic and biotic factors, and chief among these biotic factors was competition from organisms with similar ecological requirements. Incidences of secondary contact or parapatric distributions may allow insights into the ecological processes that create range limits via interspecific interactions, especially among species with relatively strong reproductive isolation.

Parapatric distributions in which the width of contact zones are narrow compared to the distributions of the entities involved might be explained by recent contact between lineages, or alternatively relative stability of cline shapes through time owing to a variety of processes(Barton and Hewitt 1981; Case and Taper 2000; Case et al. 2005). Neutral diffusion models are used to describe situations where parapatric distributions occur as a result of recent contact, and in such cases parapatry is by definition ephemeral. Hypotheses of recent contact generally involve some ecological change or disturbance permitting a rapid advance by one or both entities (Anderson 1948; Swenson and Howard 2005). Additional predictions that stem from the recent contact hypothesis include that, for hybridizing taxa, advanced backcrosses and introgression should be absent (Moore 1977). It is possible to estimate the time since contact under a model of neutral diffusion to assess this hypothesis with respect to all the empirical observations of the contact area.

The stabilization or quasi-stabilization of parapatry, mutually exclusive of neutral diffusion, may be driven by any combination of a multitude of factors (Case and Taper 2000; Case et al. 2005). Under stabilized parapatry, a cline can be maintained while it moves across the landscape over time, thus stability refers to the relative position of alleles, genotypes, or phenotypes and not their specific geographic location. According to models, such movements should mostly be driven by changes in population structure over space among ecologically similar entities (Barton and Hewitt 1985). A common explanation for stabilized parapatry in the literature is dispersal/selection balance, in which a cline in alleles or genotypes is maintained either by an ecological gradient or ecotone (Endler 1977; Roughgarden 1979), selection against hybrids (the tension zone model, Barton and Hewitt 1981; Barton and Hewitt 1985), or a combination of these, and where dispersal is isotropic. Ecological gradients may also explain the shape of parapatric boundaries where dispersal is not isotropic, for example where there is differential habitat selection between entities (Shigesada et al. 1979; Pease et al. 1989; Holmes et al. 1994; Arnold 1997; Case and Taper 2000; Cicero 2004). Gradients need not be steep to generate a steep transition. For example, a gradual ecological gradient can interact with a dispersal barrier to shape a cline that is narrow compared to the relative specialization of the biological entities or species, resulting in a transition that is abrupt relative to the slope of the gradient (Case et al. 2005). Counter-intuitively, in a model that includes local adaptation in a quantitative trait, a parapatric boundary can be repelled by a dispersal barrier when local adaptation is inhibited by gene flow, resulting in a sharp boundary offset from a dispersal barrier (Goldberg and Lande 2007).

Additional factors beyond ecological gradients and selection against hybrids that can contribute to the stability of parapatry involve interspecific interactions, and have been modeled using individual-based models with Lotka-Volterra origins (Case et al. 2005). The factors are ecological in nature, but it is important to keep in mind that while they can occur in the presence of an ecological gradient – these factors need not be associated with a gradient to contribute to parapatry stabilization. One factor is the intensity of interspecific competition: stability is enforced when interspecific competition has stronger per-capita effects than intraspecific competition. The effects of strong interspecific competition in parapatry have been modeled as constants in non-evolutionary models (Case et al. 2005), and frequency-dependent effects from phenotype distributions in evolutionary models (Case and Taper 2000). Reproductive interference (Walker 1974; Groening and Hochkirch 2008) includes selection against hybrids as in selection/dispersal balance models, but also those costs borne of wasted time or effort in securing mating opportunities when heterospecific/conspecific density is high, or for females, harassment from heterospecific males (Wilson and Hedrick 1982). If there is assortative mating by locally rare migrant heterospecifics, a greater fraction of the costs of reproductive interference will result from search inefficiencies instead of hybridization/attempted hybridization (Wilson and Hedrick 1982). The combined effects of the multiple components of reproductive interference can produce priority effects (Case et al. 2005; Thum 2007). Habitat patchiness should increase the steepness of parapatric transitions enforced by any combination of competition, reproductive interference, and ecological gradients, and is likely the norm in nature (Bull and Possingham 1995).

Goals

By examining multiple aspects of divergence in the focal species, together with the outcomes of secondary contact, it is possible to gain insight into speciation processes and range limits, and to form hypotheses about their interactions. The goals for this chapter are to 1) examine lineage-level divergence versus conservatism in bioclimatic niche, bill morphology, and a learned song type; 2) assess the extent of reproductive isolation between taxa; 3) examine the comparative geographic distribution of genotypes and phenotypes across the area of contact; and 4) integrate the results from these studies to assess the applicability and explanatory power of speciation and range limit models to explain the divergence and distribution of *N. moreaui* and *fuelleborni*. Additionally, I address the taxonomic status of these two species, which have alternately been given subspecific status as *N. mediocris moreaui* and *N. mediocris fuelleborni*, and conclude that, as recommended by Bowie et al. (2004), both taxa merit species status by prevalent species concepts.

Focal taxa background

Nectarinia (née *Cinnyris*) *moreaui* and *N. fuelleborni* (alternately classified as the subspecies *N. mediocris fuelleborni*) are sunbirds from an Eastern Afromontane species complex whose taxonomy is in flux. Three to five species are presently accepted taxonomically for this complex (Bowie et al. 2004; Clements et al. 2011). The complex stretches from Mt. Kulal, Kenya, south to Mts. Namuli and Mulanje in Mozambique and Malawi, respectively. *N. fuelleborni* is the southernmost of these taxa (and is sometimes considered to be comprised of two subspecies, the nominate and *N. f. bensoni*, which have been separated based on belly color). *N. moreaui* and *N. fuelleborni*, like the other members of the species complex, breed in dense populations in montane forests, forest edge, montane shrubland with a brackenfern or grass understory, tea plantations, farms (especially those with banana plants), and gardens (Cheke et al. 2001, JPM pers. obs.). Bowie et al. (2004) found that *N. moreaui* and *N. fuelleborni* possessed divergent sequences at the mtDNA ND3 and CR1 genes, and each are reciprocally monophyletic for the concatenated ND3 and CR1 sequences with respect to the other members of the species complex in phylogenetic analyses. Furthermore, these authors found that the mtDNA of both species could be found in samples from the Udzungwa Mountains, Tanzania. The Udzungwa, much like the other Eastern Arc mountain blocks (e.g. Ukaguru, Usambara, Uluguru), had largely been considered as a single biogeographic unit for forest-dependent birds (Stuart et al. 1993; Fjeldså and Rabol 1995; Cordeiro 2000), such that the proximity of divergent mtDNA lineages in the northeastern Udzungwa was something of a surprise despite reports that individuals "within the concept" of *moreaui* had been captured in the northeastern Udzungwa and the nearby Uvidunda Mountains (Stuart et al. 1987). Plumage differences between the two species are subtle, and the principal difference that can be used to distinguish between them in the field is the presence of yellow on the sides of the breast in *N. moreaui*, which is absent in *N. fuelleborni*, although both species possess yellow pectoral tufts. Remaining differences are more subtle, but may include the spectral color properties of the red breast band, the iridescent blue or violet upper breast band, and the upper tail coverts. Field examination of these traits suggests that a spectrographic study of these color differences should be performed, as overlap between species may be extensive. Differences between females are even more subtle, and field differentiation in the area of contact was not attempted.

Both species breed from ~1400m to the upper extent of montane forest throughout their respective distributions (JPM pers obs; fuelleborni: Mt. Namuli, Njesi, Rungwe, Udzungwa highlands including Mufindi, Ikokoto, Kihulula, and Nyumbanitu; moreaui: Nguru Mountains (Nguru South Forest Reserve), Ukaguru Mountains (Mamirwa South Forest Reserve), Rubeho Mountains (Mafwemiro), and Udzungwa Mountains (Image, Selebu, Ndundulu). The upper elevation for breeding is in some places limited where forests and grasslands give way to granite domes supporting only sparse vegetation (Namuli, Nyumbanitu, Selebu). The limits for lower breeding elevations are not known. Like other members of the species complex (Cheke et al. 2001; Werema 2007), at least some populations or individuals of *moreaui* and *fuelleborni* undergo seasonal movements. In late June 2010 in the Luwala area of Ndundulu forest (7.77° S, 36.46° E) during a year where rains continued late in the year, individuals were not performing song at any elevation, and *N. moreaui* were regularly observed outside the forest edge at relatively low elevation (~1700m, JPM pers obs; RCK Bowie unpublished data). In a subsequent visit to Ndundulu in late July-early August 2010, individuals were singing at higher elevations (1800 - 2300 m), indicated breeding readiness, and only a single individual could be found outside the forest edge at ~1700m where they were regularly observed in June. This pattern indicates that the onset of the breeding season in 2010 occurred between late June and late July, and was accompanied by movements to higher elevations. More dramatically, six specimens of *N. moreaui* or *N. fuelleborni* were taken at the low-elevation Ukami forest in the non-breeding season on March 18, 2007, by Louis Hansen and Jacob Kiure at ~1100-1200 m, below the known elevational breeding range of both species. A subsequent breeding-season trip to Ukami made by JPM, Chacha Werema, Elia Mulungu, and Maneno Mbilinyi in July 2009 yielded no observations of either species at Ukami over 2 days of observation. Indeed, this trip included a long elevational transect on foot from a peak elevation of ~1800 at a ridge in Nyumbanitu forest, south to Ukami, and *fuelleborni* were present only at the highest elevations (~1650-1800) along this 8.6 km transect (map distance). Sequencing of mtDNA from three of the Ukami specimens revealed 1 *moreaui* and 2 *fuelleborni* haplotypes (RCK Bowie, unpublished data), indicating these individuals would likely have come from Nyumbanitu, or Nyumbanitu and Ndundulu, with either forest a substantial distance away. This evidence indicates that both species undertake substantial seasonal movement, both by elevation and map distance, in the area of parapatric contact (see Results).

The forest areas inhabited by both species are thought to have endured through climatic fluctuations during the Plio-Pleistocene, as indicated by study of Late Quaternary vegetation change (Mumbi et al. 2008). Indeed, these authors show that there is remarkable stability over the past ~39,000 years in the northeast Udzungwa. The build-up of avian paleo- and neo-endemic diversity in the Eastern Arc Mountains, but especially in the northeast Udzungwa, has served as further evidence that these mountains maintain extremely stable environments through time (Fjeldså and Lovett 1997a; Fjeldså and Lovett 1997b). Most forested areas in these mountains are currently well-protected by the Tanzanian government as conservation areas of varied legal status, and are not subject to intense conversion for agriculture at present, though they incur limited extractive activities (mostly hunting - JPM pers. obs.).

N. fuelleborni and N. moreaui exhibit several forms of sexual dimorphism. With respect to plumage dichromatism, males are strikingly colorful, while females are dull. Males exhibit iridescent green coloring on the back, head, throat, upper breast, and wing coverts; a bright red breast band with a violet or blue band above it, bordering the iridiescent green at the upper breast; bright yellow pectoral tufts (and yellow sides of breast in *moreaui*); and violet or blue iridescent upper tail coverts. Females are dull olive-green in both species over most of the body, with the stomach slightly lighter in color and sometimes intergraded with yellow. Males are larger than females in standard morphometric measurements, especially in bill length in *N. moreaui*, by ~15% (Bowie et al. 2004). Behavioral dimorphism includes that only males exhibit the long, complex territorial and courtship vocalization types. Females use a repertoire of short duration (<1s) calls, but do not sing.

Males defend breeding season territories, and may also defend foraging resources or mates during the non-breeding season. Territorial behavior is often intense, with high-speed male-male chases accompanied by complex vocalizations, and at times mechanical sounds produced either by bill clapping or wing snapping. Territorial battles sometimes involve multiple individuals, and as many as four males have been seen in extremely close proximity during antagonistic interactions. During the breeding season, perched song is performed often and throughout the day, without a sharply peaked distribution of singing activity in the morning or evening (pers. obs.).

Methods

Molecular methods – sampling, laboratory procedures, and molecular analyses

Tissue and blood samples were acquired from field samples and museum specimens. Field sampling took place in Tanzania via mist-netting, with nets placed in the forest understory or edge. Additionally, territorial males individuals were occasionally target netted, aided by song playback from either a Marantz PMD670 recorder or an mp3 player connected to an Anchor Minivox portable speaker. Blood samples were preserved at ambient temperature in Seutin lysis buffer (Seutin et al. 1991). Tissue samples were taken and preserved at ambient temperature in the field using either 70-90% ethanol or RNALater. Blood and tissue samples were later frozen. Individuals to be released after blood sampling were fitted with an aluminum band imprinted with an individual identifying number (provided by the Nairobi National Museum via Neil and Liz Baker) and three colored bands. On rare occasions when an aluminum ring was unavailable, individuals were fitted with colored bands only. Of 132 individuals in the molecular data set, 76 samples came from field efforts by JPM from 2008-10. The remaining 57 samples come from blood and tissue collected in Tanzania and Malawi by Jacob Kiure, Louis Hansen, Rauri Bowie, and Jon Fjeldså, during the 1990's and early 2000's (Appendix 1). In total, these samples represent collections from 14 different populations from the Ukaguru Mountains in central Tanzania to the Misuku Hills in northern Malawi.

DNA extraction was performed using Qiagen DNEasy blood and tissue kits (Qiagen, Valencia, CA). DNA concentrations were measured spectrally post-extraction by NanoDrop, and in some cases were standardized to 10 ng/µl. Sanger sequencing was performed for the mtDNA gene ND2 (Sorenson et al. 1999), the Z-linked intron loci CHDZ (Griffiths and Korn 1997), MUSK (Kimball et al. 2009), and BRM (Goodwin 1997), and the autosomal introns 11836 and 18142 (Backstroem et al. 2008). Sequences were checked for quality, then aligned by the MAFFT alignment algorithm (Katoh et al. 2005) within Geneious Pro 5.1.6 (Biomatters, http://www.geneious.com). The alignment was then checked by eye. Three introns, MUSK, BRM, and 11836, had multiple indels. Some individuals were heterozygous for deletions, precluding simple alignment. Sequences for these individuals were inferred using the program OLFinder (Dixon 2010). Nuclear introns were subsequently phased probabilistically into haplotypes using the program PHASE (Stephens et al. 2001) after formatting with seqphase (http://www.mnhn.fr/jfflot/seqphase/).

Once nuclear haplotypes had been inferred in PHASE, population structure and individual population membership were investigated using the Bayesian population assignment algorithms of the program STRUCTURE (Pritchard et al. 2000; Falush et al. 2003; Falush et al. 2007; Hubisz et al. 2009). Generally, STRUCTURE uses a two-step analysis to: 1) calculate likelihood scores for different numbers of populations (*k*) represented in a dataset consisting of individual multi-locus genotypes, and then 2) to calculate the probability of assignment of each individual to each of the *k* populations. After the first step, support for different values of *k* is compared by likelihood score. The algorithm is then run again using the preferred value of *k*. In the analyses for this chapter, I set *k*=2 *a priori* based on previous results (JPM, unpublished data). Results of Bayesian population assignment are depicted from a) a STRUCTURE run where only nuclear intron haplotypes were included, compared against mtDNA haplotypes scored as either *fuelleborni* or *moreaui* in origin; and b) a STRUCTURE run where both nuclear intron and mtDNA haplotypes were both included for each individual in the data set. For cline analyses (see below), probability of assignment to different populations is based on a run of STRUCTURE that treats the dichotomously scored mtDNA as a single known haplotype for a diploid locus. As a consequence, individuals with high probabilities of assignment to one species based on the nuclear genome alone (Figure 1a) had a lower probability of assignment to that species in the cline analysis if they exhibit cytonuclear discordance (only 4 of 132 individuals had cytonuclear discordance). Scores used for cline analyses are analogous to 'hybrid index scores' in previous hybrid zone work (Jiggins and Mallet 2000).

Based on the results of the STRUCTURE output and other analyses, it was desirable to make an indirect estimate of within-species dispersal kernels from the multi-locus molecular data (Rousset 1997). As a starting point, I used the haplotype sequences of ND2 and the nuclear introns as inferred from PHASE to calculate within-species population genetic statistics and K_{st} indices (Hudson et al. 1992) using DNAsp (Librado and Rozas 2009). Individuals with mixed ancestry were excluded from these analyses. Only populations with sampling of 5 or more individuals were included in this analysis, and only those from within the cline fitting area were included (Misuku Hills samples were excluded). Because of the limited structure observed within species, it is evident that migration, and hence dispersal, is substantial across the populations included in this study. Absence of an isolation-by-distance pattern or the markers and populations used prevents a dispersal estimate using the methods of Rousset (1997).

Niche similarity test

To test the hypothesis that *N. moreaui* and *N. fuelleborni* have diverged under niche conservatism, I performed a one-tailed background similarity test of modeled bioclimatic niche (Warren et al. 2008). The background similarity method tests the hypothesis that projected ecological niche models developed from the spatial distribution of two biological entities exhibit a level of similarity that differs from the similarity of projected niches modeled from randomly sampled points within an area that the focal organisms are likely to encounter through time (hereafter, 'buffer'). To generate the null distribution, a series of randomly sampled points from a buffer area surrounding species A's occurrences are used to generate a niche model that is compared to the niche model generated for the actual occurrence points for species B. The selection of an appropriate area as the buffer from which to randomly sample points for this test is a matter of debate (see e.g. Soberón and Peterson 2005). For this test I used a buffer zone of 100km around the set of distribution points for each species. Occurrence samples included 19 occurrence points for *N. moreaui* and 25 occurrence points for *N. fuelleborni*. Correspondingly, as recommended by Warren et al. (2008), randomly sampled background points were grouped into 19 observations from the 100-km buffer around *N. fuelleborni* occurrences and 25 observations from the 100-km buffer around *N. moreaui* occurrences. At each point from the actual occurrence data and the randomly sampled background sets, 7 BIOCLIM variables (BIO1, BIO2, BIO5, BIO6, BIO12, BIO13, BIO14) were extracted at a resolution of 2.5 arc-minutes. 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 (Jimenez-Valverde et al. 2009).

Niche models from both the true occurrences and 100 sets of background sample points from the *fuelleborni* and *moreaui* buffers were generated using the maximum entropy distribution modeling software Maxent (Phillips et al. 2006). Maxent uses presence data and background samples (distinct from points sampled from the buffer area for the background similarity test) to produce predictive niche models by machine-learning maximum entropy modeling. The generated model is the maximum entropy distribution constrained only by the relationships of the features (the BIOCLIM variables) in predicting the occurrence data against the environmental background (Phillips et al. 2006). Examination of the response curves in Maxent revealed that the seven BIOCLIM variables used were still correlated to some degree as predictors of niche suitability for the two species. Specifically, temperature variables that are correlated with elevation might co-vary in this data set, and because the focal taxa occur only at higher elevations, the features used as predictors exhibit correlated responses for suitability. Because the goal of this comparison is to compare modeled niches and not to finely analyze suitability responses to individual predictors, I use the results of niche models with all seven initial variables included (modeling procedures using Maxent often use all 19 BIOCLIM variables instead of a reduced set).

The apparent mutual exclusion of these species in extremely similar habitat and their minimal morphological differentiation suggested a hypothesis of strong ecological similarity, hence I made the *a priori* prediction for the background similarity test that *moreaui* and *fuelleborni* would exhibit greater niche similarity than the background similarity distributions.

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. There appeared to be substantial between-year error for similar measurements taken in the field on live individuals, and these measurements were therefore not included in the data set. Mandible length (length from the distal tip of the mandible to the distal end of the nares) was also measured, and error was similar for both measurements. I use culmen length here because the entire culmen length may be under selection related to the exploitation of flower corollas, and could conceivably track local differences in the array of nectar-providing plants not easily noticed in the field. I measured a total of 141 *N. moreaui* and *N. fuelleborni* specimens. 122 of these measurements are incorporated in the clinal analysis (see below).

Song analyses

Recording efforts determined that both *N. moreaui* and *N. fuelleborni* have extensive vocal repertoires consisting of ~11 unique vocal signal types (McEntee, unpublished data). The analyzed male songs represent one of these unique signal types. They are interpreted as homologous vocalizations between the two species because they share structural similarities and are used in similar contexts. This structural similarity is further conserved across the Eastern Double-collared Sunbird species complex: *Nectarinia mediocris*, *N. usambarica*, *N. loveridgei*, *N. moreaui*, and *N. fuelleborni*. (Bioacoustics Research Program 2008), which improves confidence in the homology inference. Contextually, territorial males sing bouts of these songs from exposed perches throughout the day, often but not always from a perch that is high relative to the surrounding vegetation. It is the only signal type with duration >1s that is not exclusively associated with close-proximity interactions. Instead, it tends to be sung while the individual is at a substantial distance (>5m) from any conspecific.

Sound recordings were made in the field in 2008-10 using Sennheiser ME-67 shotgun microphones and Marantz solid state recorders (PMD660 and PMD670, 16 bit precision, 44.1 or 48 kHz sampling rate). Two additional *N. fuelleborni* recordings by David Moyer were obtained from the Macaulay Library, Cornell University Lab of Ornithology. Before analysis, recordings were standardized at 44.1 kHz sampling rate using the software GoldWave 5.25 (Goldwave Inc. 2005). High quality song recordings were selected for analysis, made into separate files, then bandpass filtered between 2 and 10 kHz in Raven Pro (Bioacoustics Research Program 2008). Subsequent sonogram production and sound analysis was performed in Luscinia (Lachlan 2007), which allows extraction of data from each individual component element of a song (see Figure 2). Luscinia sonograms were created with the following settings: Max. frequency: 10,000 Hz; Frame length: 5 ms; Time step: 1 ms; Spectrograph points: 240; Spectrograph overlap: 80%; Echo removal: 100%; Echo range: 100; Windowing function: Hann; and High Pass Threshold: 2000 Hz. I then adjusted sonogram contrast with the Dynamic range and Dynamic equalization settings to maximize the visual signal:noise ratio. Signals were extracted from files automatically using the Select All button in the Measurements tab, with the Mode set to elements and measurement settings set to default values. Results of automatic signal detection were checked by eye and ear by the author, with recordings slowed for playback to 1/8 speed. Some recordings were discarded based on an insufficient signal:noise ratio at this step. Minor inconsistencies in signal detection from better recordings were corrected ‘by hand’ using Luscinia’s *brush* tool. Values for song variables were obtained for each of 14 measurements from calculations based on data extracted for each unique element in Luscinia. Mean values for the set of songs for each individual were then calculated. Subsequently, each of these values was scaled using the *scale* function in R (R Core Team 2012). The scaled variables were then analyzed by principal components analysis in JMP 9 (SAS Institute Inc 2010).

Sampling strategy included a search for at least three high quality songs for each individual in the data set. For a small number of individuals, recordings captured a bout of ≥3 songs but fewer than 3 of these songs could be used for analysis. These individuals were retained in the analysis. For individuals with obviously aberrant phenotypes, greater numbers of songs were used. Song number per individual varied from 1 – 30 (mean=3.3, SD=3.2). As the unit of analysis in this study is ultimately the individual, and because it was logistically intractable to take blood samples or specimens of every recorded individual, it is pertinent to discuss how it was established that individuals are not represented more than once in the data set. In a few instances, individual identity of the singer was established in the field through the use of color bands. For other individuals, recording location was used as a proxy for individual identity. For territories near to one another, the presence of multiple individuals was established by simultaneous visual observation or the occurrence of overlapping singing bouts on adjacent territories. Otherwise, a minimum distance of 60m between recording localities was used to establish separation between individuals. This minimum distance threshold was selected based on extensive observation of individual territorial behavior and from observations of the typical densities of territorial males.

Cline fitting

To analyze individual- and population-level geographic variation in genotypes and phenotypes, I reduced the dimensionality of the geographic data by associating each population to a distance along a single curve. Sampled populations occur in a curvilinear arc along the southern Eastern Arc Mountains and into the volcanic highlands at the northern end of Lake Malawi. For the cline analyses, the northernmost population is in the Ukaguru Mountains and the southwestern-most sampled population was at Mt. Rungwe. I fixed the sampling points at these two locations as the vertices of a monotonic curve, and then adjusted the degree of curvature by hand in ArcMap 10 (ESRI 2011) such that the curve evenly split the set of populations at the center of the contact zone (Figure 3a). Distances along this curve were then calculated by finding the nearest point along the curve for each sampled population. All populations are represented at a single distance along the cline (individuals within populations occur at the same distance along the cline). The curve is 504.5 km in total length.

Cline fitting was performed with the software Cfit (Gay et al. 2008), in which sigmoidal clines were simultaneously fit to three quantitative traits: genotype, song, and bill length. Continuous genotype values for all molecular samples were represented by the probability of assignment to *N. moreaui* from a run of the STRUCTURE algorithm with *k* set to 2 and using haplotypes for all 6 loci, including the ND2 haplotype. Because the results of the principal component analysis on song phenotypes resulted in a PC1 that corresponded to the axis differentiating species (see Results), PC1 values were used to model the cline for song. Lastly, scaled culmen length values were fit to clines.

All clines were fit using three variance parameters, corresponding to a single variance parameter per parental type and a variance parameter for intermediates (corresponding to the ‘unimodal’ model of Gay et al. 2008). I used two cline architectures: simple sigmoid clines, and sigmoid clines flanked by symmetric exponential tails. These two architectures were modeled for each additional combination of parameters. I performed a search for a shared center and slope for the three trait clines by comparing center-constrained and slope-constrained models to models without these constraints using model selection. Various combinations of constraints among the traits were attempted. I began by modeling all three clines with the slope and center constrained to be identical respectively, and then allowed the slope and center to be free parameters for all traits. Based on the results of these two initial models, I proceeded to vary the combination of constraints (see list of models, Table 4). Model comparisons were made using AIC values, with the best model chosen under the criterion that a ΔAIC>2 indicates a superior model.

Results

Molecular sequence data

Summary statistics and the results of analyses of sequence polymorphisms are found in Table 1. Included are estimates of K_{st} indices among populations within species. Of the six molecular loci in the study, *moreaui* exhibited a statistically significant K_{st} (with Bonferroni correction) only for MUSK. This was surprising given that included populations are from different mountain blocks isolated by unsuitable low-elevation environments, and that other bird species from these different areas typically exhibit strong structure among mountain blocks. It indicates that dispersal has been important in the population history of *N. moreaui*. *N. fuelleborni* exhibited statistically significant K_{st} only for ND2 and MUSK. In *N. fuelleborni*, structure for these loci was driven by differences between the Rungwe and Udzungwa populations, which are separated by the Makambako Gap, a known biogeographic break.

Population assignment in STRUCTURE

Population assignment in structure for individuals at *k*=2 closely matched *a priori* expectations for species identity of individuals based on morphology, geography, and the previous mtDNA phylogeography study (Bowie et al. 2004). I interpret these results to mean that species differences are reflected in population assignments when *k*=2, and present this result graphically (Figure 1a). I use a cutoff of .90 posterior probability of assignment to designate pure individuals of either species. ND2 sequences were designated as *fuelleborni* or *moreaui* in origin by examining the alignment. The 4-6% sequence divergence between species creates a distinctive pattern of differences reflecting species origin in the alignment, and individuals were dichotomously scored by inspection (Figure 1a). In the combined mitochondrial and nuclear sequence data set, eight of 132 individuals show mixed ancestry. Four individuals have recombinant nuclear genomes, and four individuals exhibit cyto-nuclear discordance. All individuals with mixed ancestry come from the northeast Udzungwa mountains populations known as Kihulula, Nyumbanitu, Image, and Ndundulu forests (Figure 3b). Consistent with identifications based on male phenotypes, syntopy of pure individuals is recovered from the molecular analysis at only two localities: Nyumbanitu and Ikokoto (see cline analyses).

Species-level phenotypic comparisons

Ecological niche

Species distribution models from Maxent had high prediction value for the training set for both species (*fuelleborni* AUC: .966; moreaui AUC: .988). Temperature variables contributed more than precipitation variables to the ecological niche models for both species. In both the relativized contribution and permutation tests performed by 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, and was flat beyond minimal values of precipitation for the driest month. Predictions projected into the range of the sister species resulted in remarkably high concordance of the projection with sister species' localities. These predictions also appear to have high specificity, as the spatial extent of patches of predicted suitability in the sister species' range tends to be small (Figure 4).

Results of the background similarity test, performed in ENMTools (Warren et al. 2010), are shown in Figure 5. Compared to the distribution of niche similarity scores generated from comparing background point models of species A with the actual distribution model for species B, niche models for *fuelleborni* and *moreaui* had higher similarity scores for both ecological indices calculated: Schoener's *D* and *I*. These tests indicate abiotic niche conservatism through speciation, with niche similarity greater relative to background similarity (p=.01) for all four comparisons.

Bill length

Male bill lengths were significantly different by species in a t-test (n_1= 51 *fuelleborni*, n_2= 92 *moreaui*, t=-8.8566, df=97.861, p=3.692×10^{-14}). Mean±SE for bill lengths were *fuelleborni*=23.29±0.15 and *moreaui*=24.89±0.11.

Song analyses

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, 1 degree of freedom, $p<2.2 \times 10^{-16}$ – performed in R using the manova command). Nine of the 14 tested variables were significantly different at the species level by individual ANOVA, corrected for multiple comparisons. Results of tests for individual variables are found in Table 2. I assume in using MANOVA that it is robust to both bimodality in some of the variable distributions and strong correlations between some variables. Regardless, establishing that there is a statistically significant difference in songs of these two species is trivial given that differences between them are readily salient to humans – here I focus on which aspects in particular are different.

I present representative sonograms of the two species (Figure 2) and visualize the data further after principal component analysis (PCA) in JMP 9.0.0 (SAS Institute Inc 2010). For the PCA, I included songs from areas across the distribution of the two species, including in the contact zone area. In the principal component analysis, the first six principal components described 90.6% of the variation. The first principal component corresponded strongly with species differences. Loadings for PC1 for all variables with statistically significant differences in the individual ANOVAs (above, Table 2) had absolute value >.45, indicating that there is correlation between these variables along the axis of species differences. Individuals with intermediate values along PC1 were recorded in the contact zone. The loadings for all principal components are presented in Table 3. A biplot showing the variation in the first two principal components, encompassing 59.4% of the variation in the data, is shown in Figure 6.

Clines

The preferred model included sigmoid clines with symmetric exponential tails, constrained such that the genotype and song clines were concordant and coincident, with the culmen length center and slope independently estimated (Table 4). In the preferred model, the culmen length cline is removed ~10 km N from the other clines, and is shallower (culmen cline slope=0.15; genotype and song cline slope = 2.67 in the preferred model). The estimated cline width (4/w, as defined in Gay et al. 2008) for both the song and genotype clines is 1.5 km. This value should not be interpreted as a highly precise estimate because of the limitations of the methodology and the patchiness of the sampled populations. However, even at an order of magnitude, this estimate represents the narrowest estimated cline for an avian hybrid zone that I know of.

The center of the genotype and song clines corresponds closely to the positions of Nyumbanitu and Kihulula forests along the transect. There is true syntopy at Nyumbanitu, where pure individuals of both species can be found interacting with one another, males of each species battle each other for territories, and hybrids occur. There are only two samples from Kihulula forest, and one exhibits mixed ancestry. The center of the song cline is positioned between Selebu and Kihulula in the northwestern ‘arm’ of the cline, and between Ndundulu and Nyumbanitu on the southeastern ‘arm’ of the cline. Most of the rapid turnover occurs *between* breeding populations, corresponding well in both position and slope to the abrupt turnover observed for male song and plumage phenotypes during fieldwork.

The coincidence and concordance of genotype and song clines suggest that song behaves much as a quantitative trait across the contact zone, and indeed suggests that heterospecific copying has not broadened the song cline as phenotypes become merged in parapatry. It is necessary to point out, though, that not all intermediate values for learned song come from individuals with mixed ancestry. At least one of the intermediate values for song in the contact zone was exhibited by a pure *moreaui* individual that was recorded after having its blood sampled. Indeed, there are a number of individuals from Nyumbanitu whose songs have intermediate values for the first principal component. Those individuals with intermediate song phenotypes likely represent a combination of hybrids and pure individuals. Though I did not obtain recordings for any individuals whose genotypes reveal they are hybrids, The pure *moreaui* individual exhibiting intermediate song was one of ~5 *moreaui*-plumaged territory-holding males at Nyumbanitu whose songs were recorded. With the exception of the genotyped individual, these *moreaui* individuals infrequently sang songs at adequate amplitude that the recordings could be analyzed. Examination of the genotyped individual's songs reveals that one commonly used element (or syllable) is an element widely used by *fuelleborni*, but nowhere else used by *moreaui*. The intermediate phenotype of this individual and its use of a *fuelleborni* element type indicate that heterospecific copying, or phenotypic adjustment towards *fuelleborni*-like song, partly explains the presence of intermediate song phenotypes at Nyumbanitu. It also may explain the absence of typical *moreaui* songs at Nyumbanitu despite the presence of genotypically pure *moreaui* males. Thus, at a geographic scale, song behaves much like a typical quantitative trait, but heterospecific copying is apparent within one contact zone population. If the heterospecific copying is disadvantageous, selection against heterospecific migrants might complement selection against hybrids in reinforcing the stability of the cline.

Discussion

The ecology and biogeography of N. moreaui-N. fuelleborni parapatry

The parapatric boundary described herein represents the hybrid zone with the narrowest estimated width among bird examples, at 1.5km. Although I caution against interpreting the estimated genotype and song width as more than an order-of-magnitude estimate, it is strikingly narrow. The narrowness of the boundary further surprises in that it occurs in two species with extremely similar niche requirements. Effectively, the boundary occurs between socially divergent species that are ecological replacements across space (Price 1998). This is not to say that the two species are ecologically *identical*, but that the weight of evidence from multiple data types (background similarity test of niche similarity, the near-complete mutual exclusion, the non-ecotonal position of the boundary, the non-coincidence of an ecological character with the genotypic cline) indicates that ecological divergence is minimal in these two species. This accumulated evidence strongly suggests differential adaptation along an ecological gradient on its own (i.e. without an interaction with a dispersal barrier) is unlikely to explain the narrowness of the boundary. Neutral diffusion after recent contact also cannot explain the abrupt boundary, as the presence of advanced-stage backcrosses indicates that the contact zone has existed for multiple generations. The daily wanderings of an individual bird during the non-breeding season are likely to be on the same order as the width of the cline. The sharp, mirrored drop-off in abundance of both species must therefore be the result of interspecific interactions – both ecological and reproductive.

Where there is sympatry at Nyumbanitu, males exhibit interspecific territoriality, including the use of particular complex vocalizations used in aggressive interactions, different from the songs analyzed in this chapter. These aggressive vocalizations are restricted to communications with conspecifics elsewhere in the ranges of the two species (pers. obs.).

Parapatry is the ecological outcome of evolutionary and ecological processes and their feedbacks. In her seminal review on social selection and speciation, West-Eberhard (1983) suggested that a combination of ecological niche conservatism and behavioral phenomena in social organisms predispose ‘socially selected sibling species’ to the formation of parapatric boundaries. The first of these factors – ecological niche conservatism – is a central aspect of the speciation literature, and is key to one version of the commonly used ‘tension zone’ model for hybrid zone analysis (Key 1968; Barton and Hewitt 1985), where hybrid zones are a subset of those circumstances known as parapatry. Niche conservatism might be viewed as the flipside to ecological divergence – stabilizing selection during niche conservatism should prevent evolutionary changes in traits intimately connected to interspecific niche relationships, and any would-be cascading effects of these changes. What makes social traits so essential to study for animal speciation is that, despite the influence that ecological divergence can have on social traits, profound social trait divergence does not depend on ecological divergence and can proceed despite strong ecological niche conservatism (West-Eberhard 1983). I contend that the *fuelleborni-moreaui* contact zone is the type of boundary described by West-Eberhard – parapatry driven by interactions between ecologically similar taxa whose primary divergence is social. It is evident that hybridization plays some role in stabilizing the boundary between these species, but the number of hybrids is small and in no place where adequate sampling has been performed is hybrid density high relative to pure types. Thus, the *moreaui-fuelleborni* contact zone represents a hybrid zone, but one without ‘major overlap or massive hybridization’ (Mayr 1982). Consequently, the extraordinarily narrow width of the boundary is unlikely solely attributable to selection against hybrids, as the tension zone model presupposes. Additional likely contributing factors include sources of reproductive interference beyond hybridization, interspecific competition with anisotropic dispersal, and habitat patchiness, but not differential specialization along an ecological gradient. The potential contributing factors are discussed in this section.

Differential ecological specialization by species is unlikely to be a main contributing factor to the *N. fuelleborni-N. moreaui* boundary. The results presented from the background similarity test (Figures 4, 5) indicate a high degree of bioclimatic niche conservatism through the speciation event that separated *moreaui* and *fuelleborni*. Given the shared habitat associations and elevational distributions of these species across their distributions, this result is not surprising. The two species are in effect ecological replacements across space, using similar sets of food plants (e.g. *Tecoma capensis*, *Leonotis* spp., *Dombeya* sp.; pers. obs.), and sharing similar ecological roles within avian communities (e.g. both species compete with the common, widespread species Olive Sunbird, Amethyst Sunbird, Collared Sunbird, and Variable Sunbird for nectar). The avian communities with which these species interact are remarkably constant across the contact zone. Within the Udzungwa Mountains, both species interact with a near-identical complement of forest-dependent avian taxa – even species with extremely restricted ranges like the Rufous-winged Sunbird *Nectarinia rufipennis* occur on both sides of the parapatric boundary shared by *fuelleborni* and *moreaui* (Fjeldså et al. 2010). All of this evidence is consistent with the hypothesis that species-specific ecological advantages on either side of the boundary cannot explain such a narrow boundary. However, the species-level bill length differences suggest that adaptation has driven change in the mean value of an ecologically relevant trait. It is unlikely that this difference reflects an ecological transition at the exact position of the boundary because the bill length cline center is positioned 10km from the molecular cline center. I explore the possibility below that this discordance is related to a balance between local adaptation, gene flow, and geography (Goldberg and Lande 2007).

Given the ecological similarity of these species, the position of the parapatric boundary remains perplexing. There are two biogeographic breaks for forest-dependent organisms in the vicinity of this boundary, at the southwestern and northeastern ends of the Udzungwa Mountains: the Makambako Gap and the Great Ruaha River, respectively. It had been supposed prior to field work in the northeastern Udzungwa by Stuart, Jensen, and Brøgger-Jensen (1987) that *N. fuelleborni* was found throughout the Udzungwa Mountains, and that *N. moreaui* would only be found north of the Great Ruaha River. But, as confirmed here and previously reported based on visual surveys (Fjeldså et al. 2010) and mtDNA analysis (Bowie et al. 2004), this is not the case. *N. moreaui* occupies the Image-Selebu ridge, Ndundulu, Mwanihana, Gologolo (Elia Mulungu, pers. comm.), and Luhombero within the northeast Udzungwas (Fjeldså et al. 2010), with a handful of individuals extending into Nyumbanitu and a single specimen taken at Ikokoto. Consequently, the parapatric boundary occurs in an unexpected position, along the Udzungwa highland plateau partway between the Great Ruaha River and the Makambako Gap. Not only does the boundary fail to occur at previously hypothesized biogeographic breaks, it also fails to occur at the widest gaps between forest patches within the Udzungwa. The transition occurs without an apparent ecological gradient at a location that does not represent an intuitive geographical breaking point. These results support the hypothesis that the competitive and reproductive interactions between these two species are responsible for the positioning of the current shared range boundary, and not a hard dispersal boundary or differentiated abiotic niches. Furthermore, the surprising position of the boundary raises the possibility that it is not in an equilibrial position. As is proposed for circumstances like tension zones where parapatric species have similar ecological requirements (Barton and Hewitt 1985), the geographic position of this boundary need not be stable over time, and instead is likely to move either northeast or southwest as a consequence of variation in local density, until it encounters a boundary that is more restrictive for dispersal. Movement as a function of local densities is in turn likely to vary with the patch sizes of available habitat. Several of the forests occupied by *N. moreaui* in the northeast Udzungwas are extensive (Ndundulu, Luhombero, Image), and given the high densities of this species encountered in the field, are likely to contain extremely large numbers of individuals. If the contact zone is moving towards the northeast to the disadvantage of *N. moreaui*, it is unlikely that this movement will proceed at high velocity. The contact zone area populations of *N. fuelleborni* are conversely quite small. Nyumbanitu, Kihulula, and especially Ikokoto are small or tiny forest fragments where, though *N. fuelleborni* is likely the most abundant forest bird species present (pers. obs.), population sizes cannot compare to *N. moreaui* population sizes in extensive forests like Ndundulu. This disparity in regional population totals suggests that any on-going displacement of *N. moreaui* would have to be very slow, and perhaps that should current movement be taking place, it is more likely to be in the opposite direction, in favor of *N. moreaui* and to the detriment of *N. fuelleborni*. Estimates of population densities, population sizes, and continuous monitoring of the shared range boundary are desirable for the understanding of the population dynamics associated with it, and whether it is moving at a rate appreciable by survey methods. Alternatively, the boundary position could be stable. Bill size differences between *fuelleborni* and *moreaui* and among *moreaui* populations suggest that a shallow, subtle ecological gradient could result in different bill length optima across space. If such a gradient occurs, then the position of the boundary could result from the advantageous restriction of gene flow for northeastern Udzungwa *moreaui* populations (Goldberg and Lande 2007). That is, local adaptation could elevate local carrying capacity in the absence of gene flow from populations to the north of the Great Ruaha River. The Great Ruaha River might then serve to prevent swamping of advantageous alleles by gene flow from the Rubeho and Uvidunda Mountains. Goldberg and Lande (2007) find that areas of reduced dispersal can repel species boundaries when a shallow gradient with linearly varying optima exists. The position of the bill cline is consistent with the hypothesis that local adaptation for bill length occurs for *moreaui* in the northeast Udzungwa, and that this process is facilitated by reduced gene flow across the Great Ruaha River. This hypothesis merits further consideration.

There are at least six observations that suggest that the 5 and 7 km gaps that mark the transition between *moreaui*- and *fuelleborni*-dominated forest patches should frequently be crossed by *moreaui* and *fuelleborni* individuals, despite the low frequencies of heterospecific migrants. That is to say, so little sympatry is achieved despite much evidence that the potential for dispersal across forest gaps is high. First, K_{st} estimates within species indicate little structure among populations for the molecular markers used, despite the presence of long distances between sampling locations, habitat patchiness, and even previously identified biogeographic breaks between populations. Second, despite that there have not been efforts to quantify long-distance dispersal in either species, a banded *N. fuelleborni* female was once subdued 9.5 km from its initial capture point near Mufindi in the Udzungwa Mountains (Liz Baker, pers. comm.). Thirdly, a single *N. moreaui* male was taken as a specimen within the otherwise pure *fuelleborni* population at Ikokoto, a set of two small forest patches which a team of 4 individuals surveyed exhaustively looking for and failing to find any additional *moreaui* individuals, and which is 15 km from the nearest breeding population of *moreaui* at Selebu. Fourth, distances between many conspecific populations in the contact zone area are greater than the aforementioned 5 and 7 km distances. The Image-Selebu ridge *moreaui* population provides an especially compelling example: the nearest *fuelleborni* population is 5km away along the same mountain ridge, at Kihulula, while the nearest conspecific *moreaui* population is at Mt. Bismarck, 27 km away and across the deep Great Ruaha River gorge. Fifth, long-distance dispersal must have been achieved for these species to have occupied extremely isolated highlands like Mt. Namuli in central Mozambique – indeed these sunbirds appear to occupy all the most remote highland forests in their portion of the Eastern Afromontane. As this is the case, even if *mean* dispersal distance of these sunbirds is low for passerines (e.g. 1 km), a long dispersal tail should mean that a 5- to 7-km gap should nonetheless fail to prevent frequent dispersal events. Lastly, these species are elevational migrants, and in the case of Nyumbanitu and Ndundulu, individuals have been observed between the two sites at elevations lower than 1400m, bringing these individuals closer than the 7 km estimated distance between forests on an annual basis. Given these observations and the low density of hybrids found in molecular analyses, extremely limited co-occurrence suggests mechanisms outside of hybridization are contributing to the stability of the parapatric boundary. While many recent hybrid zone studies calculate selection against hybrids using the tension zone model (Devitt et al. 2011; Singhal and Moritz 2012), the distribution of individuals of the two species in this case suggest that various ecological mechanisms of mutual exclusion could contribute to the steepness of the clines, and therefore that estimates of selection against hybrids using tension zone models would be skewed.

Here I discuss potential ecological mechanisms of mutual exclusion other than ecological advantage that could stabilize narrow parapatry between *fuelleborni* and *moreaui*, in light of the results presented in this chapter. I have argued that an ecological gradient is unlikely to explain the narrowness of the boundary, and that selection against hybrids likely contributes but does not explain the near-complete mutual exclusion at the contact zone. Numerous potential remaining ecological mechanisms and factors could drive or enhance exclusion and have support from individual-based models, including reproductive interference components outside of selection against hybrids, habitat patchiness, interspecific competition with stronger per capita effects than intraspecific competition, variation in density, and anisotropic dispersal. Most of these mechanisms could act synergistically.

Selection against hybrids is one of the various forms of reproductive interference that can occur in parapatry. Additional forms of reproductive interference include individual costs borne of wasted effort or time from assessing potential but ultimately unsuitable mates, attempting to mate with incompatible individuals, or from harassment or injury by unsuitable or incompatible mates (Case et al. 2005). Costs from reproductive interference are likely to be borne mostly by females. In parapatric distributions at the species level, individuals that disperse across the boundary incur these costs during interactions with opposite sex heterospecifics. Such costs should be higher with increasing relative density of heterospecifics versus conspecifics, i.e. they should be highest in circumstances like the *fuelleborni-moreaui* contact zone, where there is near-complete turnover between habitat patches. Thus reproductive interference contributes to, but is not the only mechanism that could lead to Allee effects. If assortative mating by species is strong, Allee effects in an abrupt transition zone like this one can arise from costs of mate-searching. Because of the apparent low success of hybrids however, migrants faced with high heterospecific/conspecific density are only likely to have comparable fecundity to individuals in pure populations if mate-searching and pairing are extremely efficient despite the numerical dominance of heterospecifics.

For individuals of either species, these circumstances suggest a benefit to non-random (anisotropic) dispersal across the landscape. Individuals that remain on the conspecific side of the parapatric boundary will not suffer these reproductive interference effects. I am not suggesting that dispersal should be reduced via selection at the contact zone, although that remains a possibility. Instead, adaptive behavior generally is considered to include accurate assessment of local resource availability during dispersal. In this case, the divergent signals of these two species (songs and the subtle plumage differences) provide a mechanism for discrimination that heterospecific migrants could use to recognize where conspecific mates are at low density (females) or where territory establishment should be more challenging because it requires signaling to heterospecifics (males), meaning mates and territorial resources should be detectably low. If individuals are capable of making this assessment, and after ‘sampling’ heterospecific-dominated areas are prone to continue searching for conspecifics or returning to their source population, then the parapatric boundary should be further stabilized. This type of anisotropic dispersal, with settling rates varying inversely with heterospecific versus conspecific density, would explain the abrupt spatial transition between *moreaui*-dominated and *fuelleborni*-dominated habitat patches. Of the ecological mechanisms that could contribute to the stability of parapatry, anisotropic dispersal as specified here is the only mechanism that explains the extremely low frequencies of heterospecifics (0 *fuelleborni* haplotypes from 5 individuals at Selebu, 3 *fuelleborni* haplotypes and 0 pure *fuelleborni* individuals among 22 individuals at

Ndundulu, 2 pure *moreaui* of 15 individuals at Nyumbanitu, 1 pure *moreaui* from 17 individuals combined at Ikokoto and Kihulula) at patches close to heterospecific source populations.

As further detailed below, one territory-holding male with a pure *moreaui* genotype at the *fuelleborni*-dominated Nyumbanitu sings songs intermediate between *moreaui* and *fuelleborni* songs along the first principal component, and these songs contain elements borrowed from *fuelleborni* song culture. This phenotypic shift suggests that pure *moreaui* individuals incur a mismatch between song predispositions and local song culture at Nyumbanitu, which could disadvantage these individuals by making territory acquisition and defense more challenging and by reducing their appeal in mate choice (Qvarnstrom et al. 2006).

Costs of defending a territory without possessing local song could be interpreted either as reproductive interference or as interspecific competition with higher per-capita effect than intraspecific competition for heterospecific migrants (Roughgarden 1979; Goldberg and Lande 2007). Though interspecific and intraspecific competition parameters are generally modeled as constants, ecological disadvantages that may be experienced by heterospecific migrants (increased costs of defending a territory when not equipped with local song) would depend on heterospecific/conspecific densities and are therefore frequency-dependent. This type of disadvantage would again, then, represent a type of Allee effect for heterospecific migrants.

I note that all the processes I've outlined in this section are consistent with West-Eberhard's (1983) notion that social organisms are predisposed to parapatric boundaries after speciation events because they are simultaneously (and perhaps paradoxically) prone both to aggressive social interactions and aggregation with conspecifics. Social organisms use signals to preferentially aggregate with other conspecifics, and this influences the distribution of individuals across a landscape, causing clumping (Stamps 1988; Ray et al. 1991; Stamps 1991; Reed and Dobson 1993; Muller et al. 1997; Valone and Templeton 2002; Doligez et al. 2004; Ward and Schlossberg 2004; Fletcher 2006; Nocera et al. 2006; Hahn and Silverman 2006; Toews and Irwin 2008). This use of signals is rarely discussed in relation to speciation, however it has relevance, likely underappreciated, in the formation of parapatric boundaries. Locally uncommon heterospecific migrants suffer disadvantages in communicating territorial intent to aggressive males because they possess a divergent communication system, and the locally common species aggregates preferentially with conspecifics that share a communication system. Aggregation of *moreaui*, who are uncommon in Nyumbanitu, occurs there despite their small numbers. Though I captured only two individuals with pure *moreaui* genotypes there, additional individuals with *moreaui*-type plumage were observed, photographed, and sound recorded in the same vicinity as captured individuals. The tendency for individuals to aggregate based on shared signals or signal systems is a form of anisotropic dispersal that should sharpen parapatric boundaries (West-Eberhard 1983; Payne and Krakauer 1997; Garcia-Ramos et al. 2000). With respect to the *moreaui-fuelleborni* boundary, there are a variety of processes that could enhance aggregation at the species level. Females of each species may rely on conspecific signals to determine where they settle and/or breed. Female dispersal varying positively with conspecific density could be viewed as a component of assortative mating, where mating is partly assortative by proximity of conspecific versus heterospecific males. Male dispersal may also vary positively with conspecific versus heterospecific density. The social system is likely to play a role both in the dispersal and the success of males dependent on conspecific versus heterospecific density. Both *N. moreaui* and *N. fuelleborni* males establish and defend territories. These territories are sometimes jointly defended by a male and a female, but the males do the great bulk of the signaling work (see Chapter 3). Males take advantage of visual and auditory modes, with the auditory modes used in both short- and long-distance communication. As in other oscine songbirds (Catchpole and Slater 2008), *N. moreaui* and *fuelleborni* males use vocal signals to defend territories, and the learned song type analyzed in this chapter is an important component of the vocal repertoire with this function. Territory establishment is facilitated by successful communication of resource-holding capacity by males. In this contact zone, the great disparity between typical *moreaui* and *fuelleborni* song signals suggests a challenge for males in communicating territorial intent among heterospecifics. This scenario plays out at Nyumbanitu, where *moreaui* individuals successfully hold territories among *fuelleborni* males. None of these *moreaui* males, however, exhibits *moreaui*-like songs, instead delivering songs that appear intermediate along the first principal component in the song analysis in this chapter, indicating an incomplete phenotypic adjustment towards local (i.e. *fuelleborni*) songs. This pattern suggests that *moreaui* males exhibiting typical *moreaui* songs may not be able to establish and defend territories. Within species, immigrants who exhibit non-local dialects have been shown to suffer reduced fitness (MacDougall-Shackleton et al. 2002). In a situation more analogous to this contact zone, *Ficedula* flycatchers who sing 'mixed' songs suffer reduced fitness by attracting heterospecific matings (Qvarnstrom et al. 2006). Hence, both male dispersal and male success might be reduced in the *moreaui-fuelleborni* contact zone because of divergent social systems. When divergence at a molecular level takes place in conjunction with divergence in learned song, especially when there is a molecular change that narrows the range of phenotypes relative to the shared variation among divergent populations, reduced sympatry might result. Thus the role of song in the speciation of birds might sometimes be to reduce the frequency of sympatry of dissimilar forms. Reduction of sympatry of dissimilar forms reduces the number of opportunities for hybridization, increasing apparent prezygotic reproductive isolation.

Divergence and speciation

Reproductive isolation

The low frequency of individuals with mixed ancestry and the limited area of introgression together indicate an advanced stage in the speciation process in *fuelleborni* and *moreaui*. Though the structure analysis indicates that the two species can still produce viable offspring, F1 hybrids are rarely produced. The rarity of hybrid production is indicative of assortative mating within the contact zone. However, despite the regional proximity of pure parentals within the contact zone area, a component of this assortative mating, appears to be local geographic isolation. That is to say, one component of pre-zygotic reproductive isolation in this contact zone may be spatial clumping of conspecifics driven by ecological and/or behavioral mechanisms, as discussed above. In the only area where substantial sympatry is known to occur, a limited portion of Nyumbanitu forest, individuals of mixed ancestry represented 4 of 11 samples (3 of these had *fuelleborni* haplotypes for nuclear markers but a *moreaui*-type mtDNA, only one had a recombinant nuclear genome). Hence, while the overall paucity of individuals with mixed ancestry suggests strong behavioral isolation via mate choice, there is only one small site known where females have the opportunity to choose locally between multiple pure *moreaui* and multiple pure *fuelleborni*, and at this site individuals with mixed ancestry are found. Thus, while the results strongly suggest that assortative mating occurs, conspecific clumping may reduce the opportunity for hybridization. Song, and song culture, could play a role in conspecific clumping. Females may use song as a cue to select a breeding territory, and males may enforce conspecific clumping by preventing most heterospecific males from establishing territories.

Though partly explained by assortative mating, the extremely limited introgression seen at the contact zone is also indicative of selection against hybrids. Because hybrids themselves are low in frequency, most hybrid matings are likely to be backcrosses, and the absence of introgression in the molecular data set presented here indicates that at a minimum, half of an F1's nuclear genetic material will not persist through several generations in the parental populations it has backcrossed to. Many recent hybrid zone studies use the tension zone model and estimates of dispersal and linkage disequilibrium to calculate selection against hybrids (Szymura and Barton 1986). Given that the assumptions of the tension zone model appear to be violated in this contact zone (effectively, steepness of the clines is not solely due to selection against hybrids under random dispersal, Arnold 1997), such calculations are unjustified and not pursued further here. Nevertheless, I infer that selection against hybrids is substantial, as otherwise some amount of introgression would be evident in all contact zone populations. In addition to selection against hybrids, selection against heterospecific migrants across the parapatric boundary must be investigated in this contact zone. At the level of minimally diverged populations exhibiting song dialects in white-crowned sparrows *Zonotrichia leucophrys*, migrant males possessing non-local song have reduced reproductive fitness measured by number of sired offspring (MacDougall-Shackleton et al. 2002). In the *fuelleborni-moreaui* contact zone where song differences are far greater and most likely partly innate, numerous additional complications should present themselves to heterospecific migrants (e.g. for males, difficulty establishing a territory when possessed with radically different song and different song predispositions; for females, low densities of compatible males).

Divergence between *fuelleborni* and *moreaui* is characterized by great divergence in song traits and minimal ecological niche divergence. As above, the low frequency of individuals with mixed ancestry sampled at the contact zone is more likely a consequence of pre-zygotic reproductive isolation and not early-life inviability of offspring because birds maintain the ability to produce viable offspring long after divergence (Prager and Wilson 1975). As this is the case, the possibility that song plays a role as a behavioral isolating mechanism in these species is raised. However, as song is learned in oscine songbirds, which includes sunbirds, heterospecific copying is also a possibility, which could reduce the effectiveness of song as an isolating barrier. Thus there are many possibilities for the trajectory of song trait evolution where there is contact. The dramatic species-level differences in learned songs between *fuelleborni* and *moreaui* make it possible to easily detect intermediate phenotypes that fall between typical songs from allopatric populations.

I predicted that heterospecific copying (cultural inheritance in the presence of heterospecifics) would cause song differences to be reduced in the contact zone, resulting in a song trait cline that is less steep than molecular clines. Alternately, if heterospecific copying does not take place (innate predispositions prevent cultural inheritance from heterospecifics) then the song trait cline should be concordant with the molecular cline, behaving like a composite quantitative genetic trait. This study suggests that song behaves like a quantitative genetic trait, despite the potential for heterospecific learning. The integrity of this trait, as such, when subjected to interspecific interactions, affirms that it could play a role in speciation. However, as explained in the Results section, the discovery of an individual pure *moreaui* at Nyumbanitu that exhibited intermediate song indicates that heterospecific copying can take place. This individual can be viewed as something of an experiment – what is the song of a territorial *moreaui* male when it is outnumbered locally by territorial *fuelleborni* males? Its phenotype had an intermediate PC1 score, and it incorporates an element within songs known only from *fuelleborni* songs, but widespread in *fuelleborni* (the high-frequency, long element ending in a rapid downslur, as seen in Figure 2b at 2.0 s). Together, this evidence suggests that this individual has arrived at an intermediate phenotype partly through cultural inheritance of heterospecific song culture. This evidence then confirms that heterospecific copying is possible at the contact zone. Moreover, though there are pure *moreaui* genotypes at Nyumbanitu and Ikokoto forests, there are no typical *moreaui* song phenotypes from these populations. All song phenotypes from Ikokoto fall within the distribution of typical values for *fuelleborni* for the first principal component. The song phenotypes at Nyumbanitu are more variable, including intermediate phenotypes but critically, no values for the first principal component typical of pure *moreaui* populations, despite the presence of pure *moreaui* males. Local song culture seems to be determined more by the locally more abundant species instead of reflecting individual genotypes per se. A genotype-environment interaction results in intermediate phenotypes, though, as innate predispositions (or a combination of predisposition and earlier learning) preclude *moreaui* from exhibiting songs like pure *fuelleborni*.

Song and morphological divergence

One of the prominent hypotheses regarding divergence of acoustic signals is that morphology constrains acoustic signals (Podos et al. 2004), and that signals evolve as a byproduct consequence of morphological evolution. Simultaneous divergence in an ecomorphological trait and a signal involved in species recognition could cause rapid speciation (Podos 2001; Servedio et al. 2011). Because *fuelleborni* and *moreaui* differ at a species level in bill length, it is possible that this morphological shift has influenced the divergence of song signals. A prediction of this mechanism, sometimes called the byproduct mechanism for signal evolution, is that signals should co-vary with morphology. The cline analysis presented here allows a test of this prediction in relation to species-level differences via assessment of coincidence of bill length and song clines. The failure of these clines to be coincident indicates that morphological evolution did not drive the evolution of the song traits that exhibit the most variation among *moreaui* and *fuelleborni*. The failure of the byproduct mechanism to explain major species-level differences is, however, not surprising on at least two counts. Assuming that craniofacial musculature and its associated power is largely conserved among *fuelleborni* and *moreaui*, *moreaui*'s longer bills should limit the rate at which it can open and close its bill to modulate frequencies, which has been hypothesized to limit trill rate under the byproduct mechanism hypothesis (Podos 2001). *N. moreaui* averages longer bills, but exhibits faster maximal trill rates than does *fuelleborni*, opposite the prediction (see e.g. Figure 2). Secondly, though *moreaui* averages longer bills than *fuelleborni*, there is considerable overlap in bill length for pure populations of both species, whereas there is minimal or no overlap between species in values for the first principal component of song. Hence, while bill morphology evolution may contribute to the evolution of songs in these species, bill length divergence is not closely related to the principal axis of species differences in songs. Among the remaining mechanisms that could explain the dramatic song divergence between *fuelleborni* and *moreaui* are drift, social selection, and differential selection for transmission efficacy (via social selection, natural seletion, or cultural selection). The combination of strong abiotic niche conservatism between species and the wide variation in acoustic environments used by singers of both species even within a single territory (middle and low forest edge, mid-forest canopy, forest understory, on shrub perches in grassland/brackenfern habitats) suggest that differential selection for transmission efficacy (acoustic adaptation hypothesis) is unlikely to explain divergence. Social selection (Crook 1972; West-Eberhard 1979; West-Eberhard 1983) or a combination of social selection and drift (Uyeda et al. 2009) remain plausible hypotheses for *moreaui-fuelleborni* song divergence. Many of the song trait differences – song duration, element duration, pause duration between elements - are indicative of underlying evolutionary changes in perceptual or production predispositions (Soha and Marler 2000; Podos et al. 2004) at a neural level.

Conclusions

The non-ecotonal parapatric boundary between *N. moreaui* and *N. fuelleborni* represents the narrowest avian hybrid zone yet estimated, but the abruptness of this boundary is unlikely to represent a dispersal/selection balance. Ecological mechanisms that generate priority effects likely act in concert with reduced dispersal to areas with high heterospecific/conspecific ratios to stabilize the parapatric boundary. The coincidence and concordance of genotypic and learned song clines suggest both that song could play a role in maintaining species identities despite contact with heterospecifics, and that innate predispositions may be sufficient to prevent complete convergence of song in sympatry. The contact zone between these two species would be a suitable place to study the contribution of quantitative song differences both to speciation and to population-level processes like dispersal and demography.

Table 1. Summary statistics and polymorphism from DNA sequences at 5 nuclear loci and the mtDNA gene ND2. [I] Two nested indels, a 6bp and a 31 bp, occur within BRM sequences, and this variation is not included in the segregating site or haplotype diversity values. * Significant at p<.05 with Bonferroni correction.

Marker type	Locus(n)	# sites (bp)	R	LISB	S	H	Hd	π	K_{st}
moreaui									
mtDNA	ND2 (53)	882			66	17	0.844	.008	-.003
autosomal	11836 (106)	461	3	106	5	7	0.694	.009	.018
	18142 (108)	365	1	142	3	4	0.483	.004	.021
Z-linked	BRM (95)	241	0	241	3[I]	4[I]	0.233	.001	.047
	CHDZ (97)	416	0	416	12	7	0.773	.003	.015
	MUSK (97)	499	0	499	8	7	0.64	.003	.073*
fuelleborni									
mtDNA	ND2 (63)	882			71	19	0.845	.005	.066*
autosomal	11836 (126)	461	0	312	7	9	0.689	.004	.028
	18142 (126)	365	1	365	4	5	0.399	.002	.027
Z-linked	BRM (107)	241	0	241	2[I]	3[I]	0.073	.000	.001
	CHDZ (105)	416	0	416	8	6	0.147	.001	.023
	MUSK (107)	499	0	499	5	6	0.624	.002	.110*

Table 2. Song variables by species, with statistical test p-values from individual ANOVAs (significant differences in bold at p<.05 following Bonferroni correction).

	fuelleborni		*moreaui*		
	mean	SD	mean	SD	p-value
Mean gap after elements (s)	**60.230**	**14.239**	**17.943**	**8.000**	**2.2×10^{-16}**
CV gaps after elements	**96.045**	**34.349**	**191.793**	**50.582**	**4.1×10^{-15}**
Mean peak frequency (hz)	5370.897	193.292	5276.281	199.592	.03574
CV peak frequency	**21.025**	**3.014**	**10.638**	**1.869**	**2.2×10^{-16}**
Max peak frequency (hz)	**7615.83**	**338.7123**	**6491.505**	**346.619**	**2.2×10^{-16}**
Min peak frequency (hz)	**3020.911**	**238.838**	**3621.423**	**450.148**	**2.0×10^{-10}**
Range peak frequency (hz)	**4594.919**	**456.291**	**2870.082**	**505.342**	**2.2×10^{-16}**
Log mean # elements	4.391	0.388	4.510	0.362	.1629
Log mean bandwidth (hz)	6.551	0.418	6.427	0.449	.21
CV bandwidth	105.561	25.747	100.056	20.676	.2963
Log mean freq change (hz)	**-2.551**	**0.245**	**-2.322**	**0.303**	**4.4×10^{-4}**
CV frequency change	68.145	10.399	79.932	10.244	2.6×10^{-6}
Log duration (s)	**9.085**	**0.363**	**8.168**	**0.311**	**2.2×10^{-16}**
Median element duration (s)	**44.621**	**11.017**	**16.331**	**6.410**	**2.2×10^{-16}**

Table 3. Loadings on Principal Components from PCA on song variables, n= 101 individuals (variables are identical to those in Table 2), and cumulative variance explained. Note how closely the variables with heavy loadings on PC1 match variables with significant species differences from MANOVA/ANOVA in Table 2.

Variable/PC	1	2	3	4	5	6	7	8	9	10	11	12	13
Mean gap after	.887	-.053	-.176	-.021	-.100	-.101	.104	-.164	.323	.048	-.027	-.109	.054
CV gap after	-.763	.276	-.187	.015	.267	.163	.179	-.359	-.070	.196	.061	.026	.003
Mean peak freq	.148	-.256	.344	.852	.043	.015	.081	-.152	.045	-.170	.039	.058	.001
CV peak freq	.873	.233	-.219	-.029	.004	.166	-.149	.071	.068	.022	.261	.086	.010
Max peak freq	.835	.075	.157	.396	.117	.123	-.084	.094	-.130	.214	-.037	-.083	.004
Min peak freq	-.661	-.506	.184	.260	-.133	-.300	-.028	.142	.069	.262	.069	.000	.001
Range peak freq	.891	.299	.016	.141	.144	.230	-.043	-.006	-.122	.017	-.059	-.056	.002
Log element #	-.060	.621	.634	-.170	.263	-.312	.040	.045	-.043	-.022	.030	.023	.078
Log mean BW	.307	-.639	.288	-.266	.237	.354	.363	.170	.022	.007	.036	.005	.004
CV BW	.024	.702	-.392	.294	-.286	-.109	.356	.209	-.027	.011	-.012	.031	-.003
Log mean freq Δ	-.466	.503	.434	.010	-.243	.452	-.085	.035	.221	.084	-.086	.076	-.008
CV freq Δ	-.481	.099	-.397	.211	.694	-.014	-.082	.176	.179	-.019	-.061	.022	-.001
Log duration	.801	.285	.304	-.150	.181	-.310	.077	-.066	.099	.047	.004	.018	-.107
Med element duration	.868	-.346	-.167	-.073	-.007	-.129	-.007	-.056	-.048	.094	-.134	.211	.030
Cumulative % variance explained	**43.0**	**59.4**	**69.4**	**78.2**	**84.7**	**90.3**	**92.8**	**95.2**	**97.0**	**98.4**	**99.2**	**99.8**	**100.0**

Table 4: Cline fitting model selection by Akaike Information Criteria. Models were constrained by forcing the centers and slopes of different trait clines to be equal to each other. Subscript lowercase letters stand for the three different traits: g=genotype, s=song, cl=culmen length. Capital letters stand for cline parameters: C=center, S=slope, W=width. The preferred model is shown in bold.

Model constraints	C_g	C_s	C_{cl}	W_g	W_s	W_{cl}	AIC	ΔAIC	# parameters
Simple sigmoid									
$C_g = C_s = C_{cl}$; $S_g = S_s = S_{cl}$	165.6	165.6	165.6	10.5	10.5	10.5	39.6	61.9	17
None	167.4	162.2	155	7.7	7.3	12.9	-13.9	8.4	21
$S_g=S_s=S_{cl}$	168.8	162.4	149.9	7.7	7.7	7.7	69.1	91.4	19
$C_g = C_s = C_{cl}$	165.1	165.1	165.1	12.1	0.8	0.5	18.0	40.3	19
$C_g=C_s$	165.1	165.1	155	12.1	0.8	12.9	5.1	27.4	20
$C_g=C_s$; $S_g=S_s=S_{cl}$	164.2	164.2	142.6	3.1	3.1	3.1	242.8	265.1	18
$C_g=C_s$; $S_g=S_s$	164.2	164.2	155	3.1	3.1	12.9	233.2	255.5	19
Sigmoid with symmetric exponential tails									
$C_g = C_s = C_{cl}$; $S_g = S_s = S_{cl}$	164.1	164.1	164.1	3.3	3.3	3.3	250.7	273.1	23
None	166.2	162.2	155	11.1	7.3	12.9	-18.1	4.3	27
$S_g=S_s=S_{cl}$	166.7	162	155.4	9.1	9.1	9.1	-17.5	4.8	25
$C_g = C_s = C_{cl}$	165	165	165	12.9	0.7	1.1	-2.4	19.9	25
$C_g=C_s$	163.4	163.4	155	4.7	4.8	12.9	-16.5	5.8	26
$C_g=C_s$; $S_g=S_s=S_{cl}$	164.8	164.8	146.3	1.4	1.4	1.4	-17.0	5.3	24
$C_g=C_s$; $S_g=S_s$	**164.7**	**164.7**	**154**	**1.5**	**1.5**	**26.7**	**-22.3**	**0**	**25**

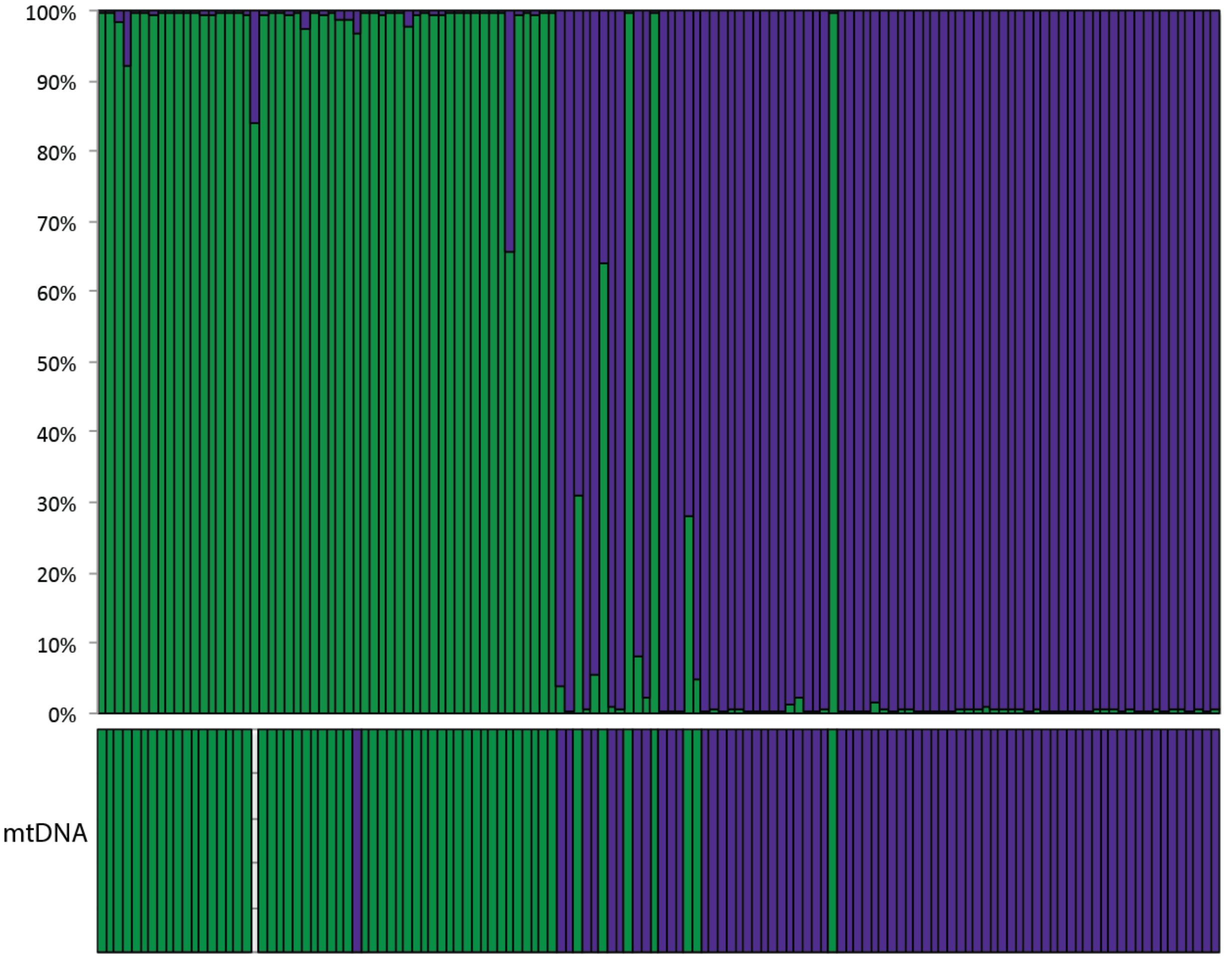


Figure 1a: The upper part of the plot is the probability of assignment to *Nectarinia moreaui* (green) or *N. fuelleborni* (purple) genotypic clusters for 132 individuals, from a single run of the structure algorithm with *k*=2 (linkage model with distance between Z-linked loci estimated from distances from the zebra finch *Taenopygia guttata*). Those individuals with probability of assignment >90% can be considered to be pure individuals of either species. The lower part of the plot indicates which of the mtDNA haplotype each individual has (the white signifies a missing sequence).

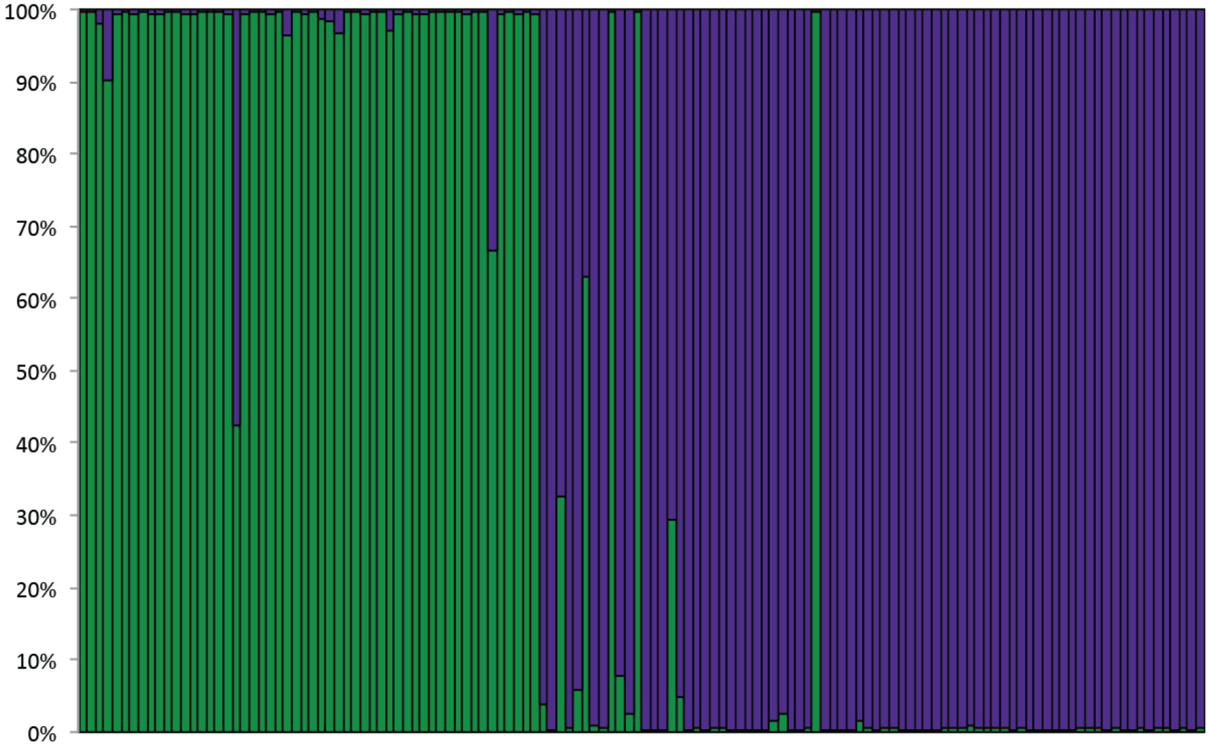


Figure 1b: Results of a single run of the structure algorithm for 132 individuals, with *k*=2 (linkage model, as in Figure 1a). In this run, mtDNA haplotype identity was included as one of the loci for each individual, coded similarly to Z-linked loci for females, but the mtDNA locus was specified as coming from a different parent as the Z chromosome. Note that there is generally very high correspondence between this and the nuclear-only analysis.

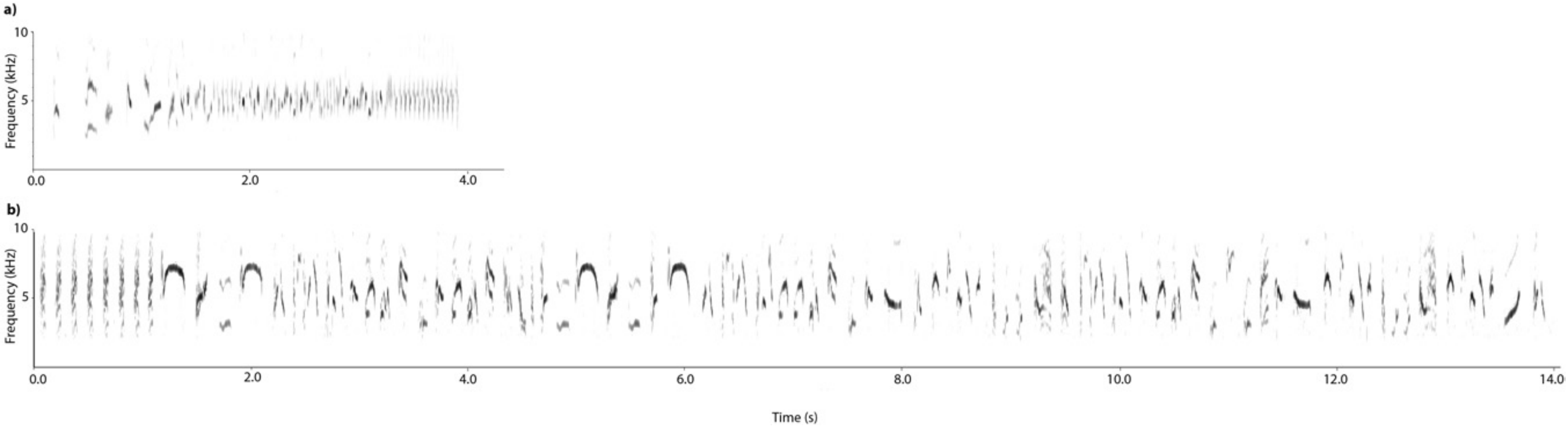


Figure 2: Representative sonograms of the territorial male songs of a) *Nectarinia moreaui* and b) *N. fuelleborni*.

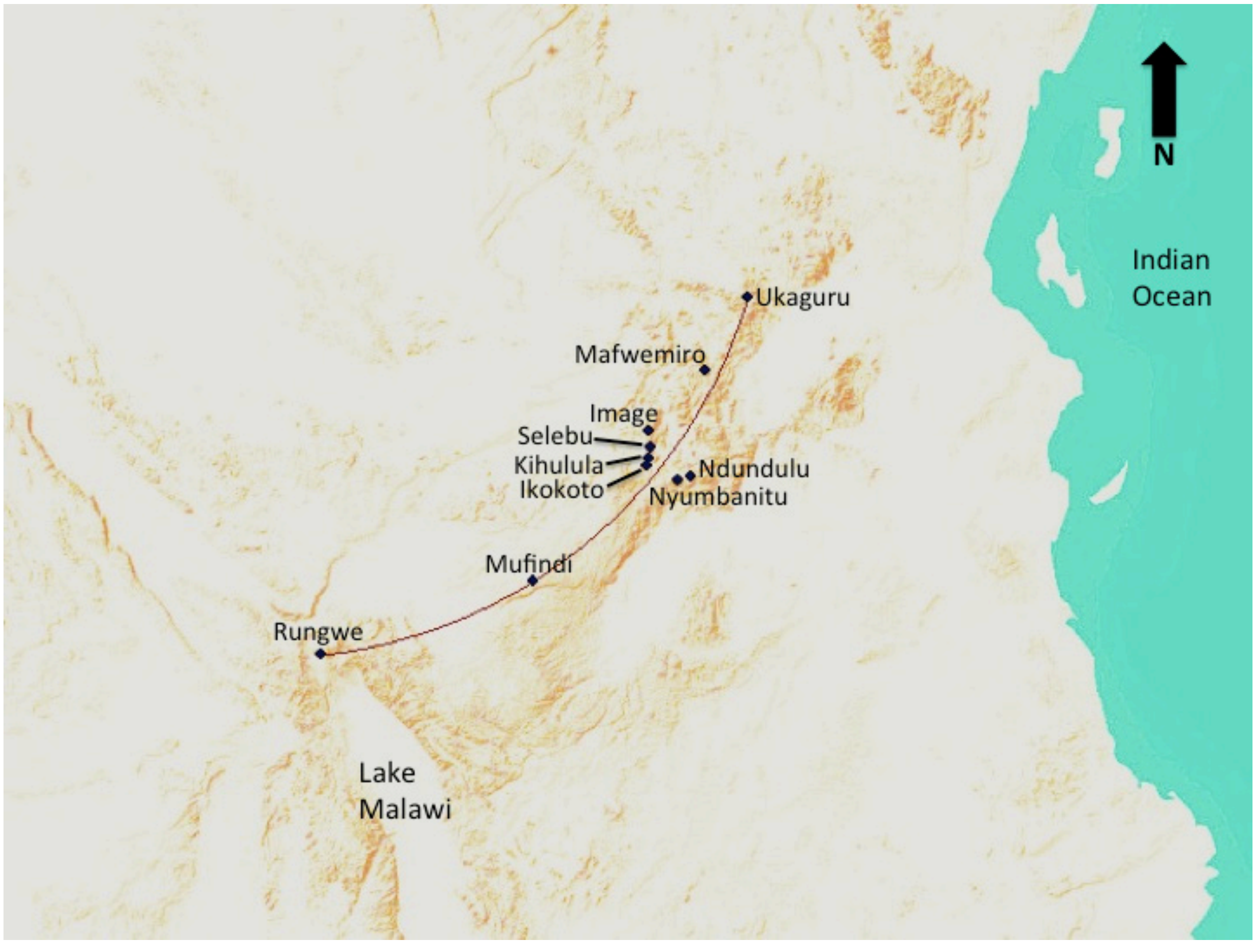


Figure 3a: Sampling populations (diamond shapes) in southern Tanzania and the cline used to associate population samples to one-dimensional space.

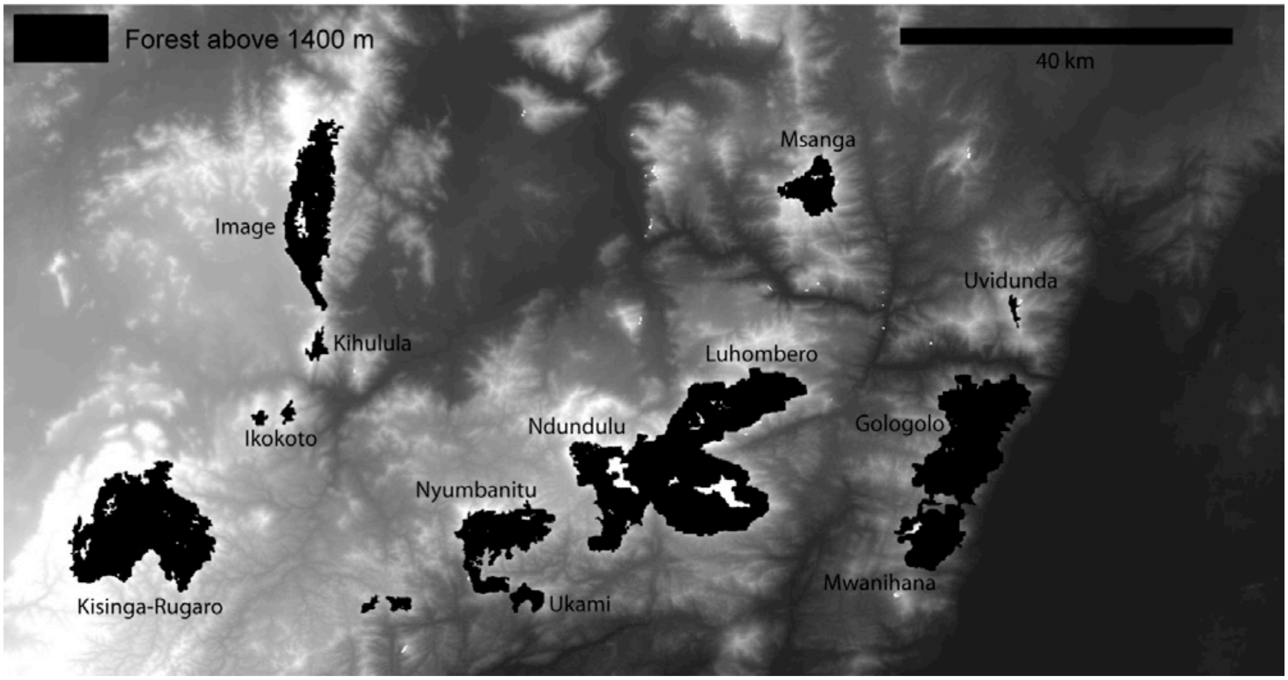


Figure 3b: Forest patches in the contact zone area, as referenced in the text. The entirety of the forest patch called Ukami, which is limited to the contiguous patch to the left of its name in the map, is below 1400m, but is included in the map as it is mentioned in the text. Outside of the forest patches, lighter color indicates higher elevation. Selebu, not found in the map, is at the southern end of the patch named Image, just across the gap between Kihulula and contiguous Image patch. Abrupt transitions between moreaui-dominated and fuelleborni-dominated forest patches occur between Kihulula and Selebu-Image and between Nyumbanitu and Ndundulu forests.

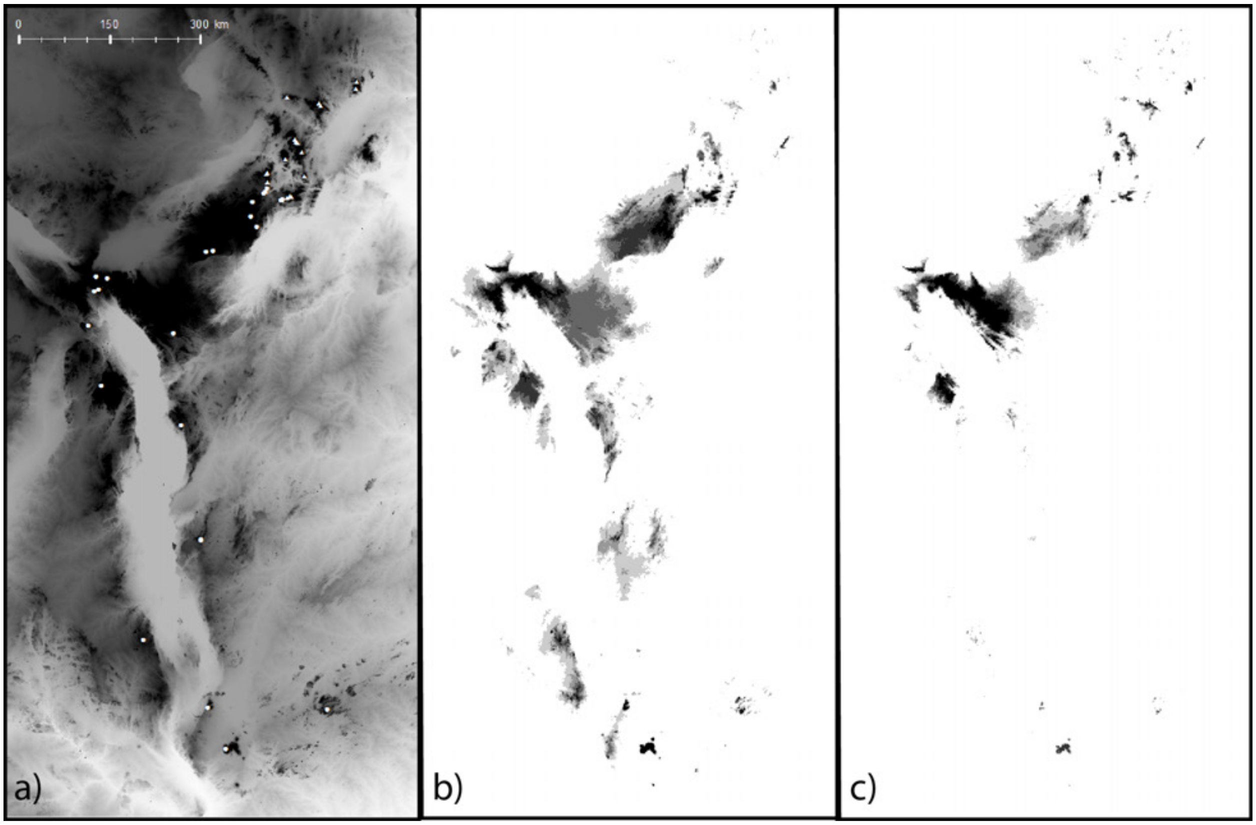


Figure 4: a) Species occurrence points for *Nectarinia fuelleborni* (circles) and *N. moreaui* (triangles) as recorded during the author's field work, and from museum specimens. b) A map of suitability generated from Maxent ecological niche modeling, with darker colors indicating greater suitability, based on occurrence points of *N. fuelleborni*. c) Same as b, but for *N. moreaui*. Note the high specificity predictions of suitability in the far south of the distribution of *N. fuelleborni* in the *N. moreaui* niche model output.

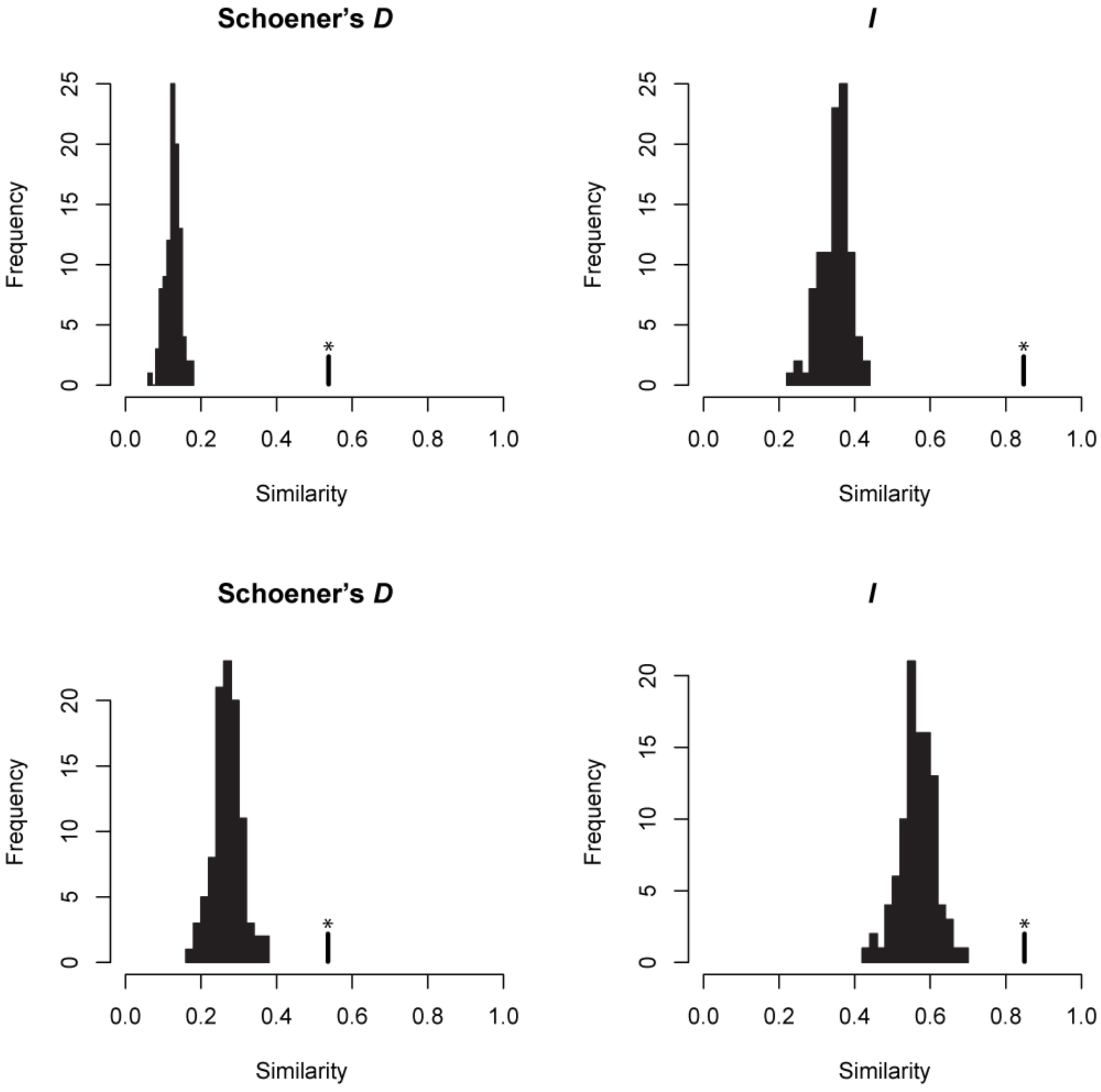


Figure 5: Results of 'background similarity' tests (Warren et al. 2008) for the niche similarity indices Schoener's *D* and *I* compared to null distributions. Observations of similarity between modeled niches of *Nectarinia fuelleborni* and *N. moreaui* are the lines with asterisks above them. The distributions are generated from comparing ecological niche models of randomly selected points from a buffer of 100km around the occurrences of each of the two species to the niche model of the other species. The top row compares observed similarity of niche models from randomly generated points within the 'buffer' area around *N. fuelleborni* occurrences to the *N. moreaui* niche model generated from real occurrences. The bottom row shows the opposite.

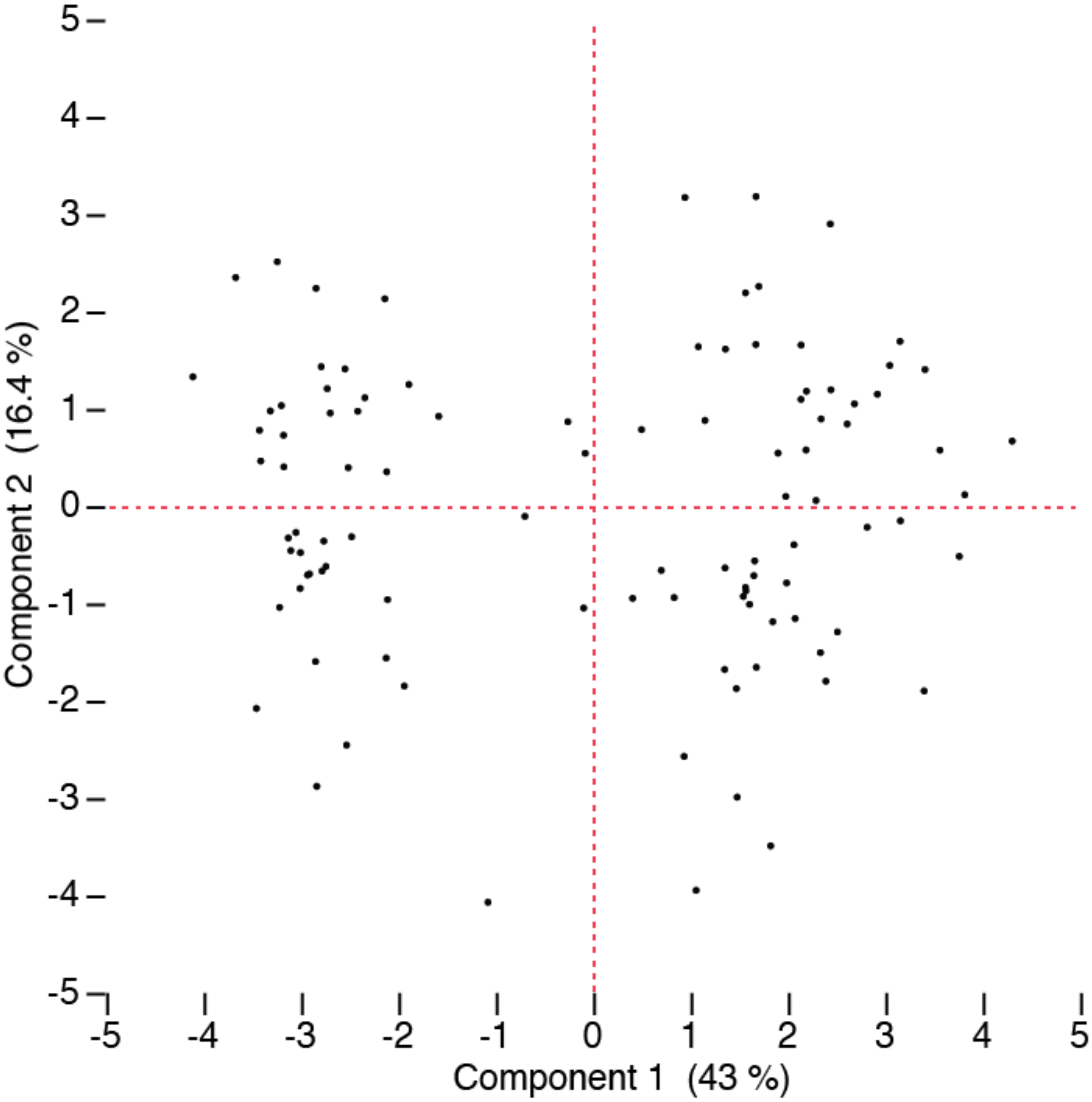


Figure 6: Biplot of PC1 and PC2 scores for principal component analysis of individual scores for song phenotypes. The cloud of points in positive space for PC1 represent *N. fuelleborni*, while negative scores are mostly *N. moreaui*. All intermediates along the first principal component come from the contact zone area.

CHAPTER 3

Species recognition' fails to explain territorial responses to sibling species in sunbirds despite strong song divergence

Abstract

Signals mediate the interactions between animal species. For individuals exhibiting territoriality, determination of the level of threat represented by intruders begins with perception of the intruder's signals. Territorial responses to such signals should ultimately be modulated by selection, as a tradeoff between the costs and benefits of mounting defensive behaviors, which depends critically on the type of intruder (e.g. male versus female, heterospecific versus conspecific). Researchers frequently apply the species recognition concept in empirical tests of territorial responses, especially in tests examining responses to recently diverged taxa in birds. Based on the assumption that conspecifics represent the most threatening category of intruder, a hypothesis based on species recognition predicts that individuals should exhibit stronger responses to conspecific signals versus heterospecific signals as long as these signals are differentiable to the territory holder. Predictions in such circumstances can also be made based on the resource overlap hypothesis, which predicts that the level of territorial response should be positively correlated with resource overlap between the territory holder and intruder. This hypothesis predicts that when interspecific resource overlap is especially high, responses can be equally strong to heterospecifics and conspecifics. Here I use a set of two territorial intrusion experiments to examine territorial responses to heterospecific and conspecific signals, focusing on responses between the sky island sunbird sibling species *Nectarinia moreaui* and *N. fuelleborni*. These two species are ecologically similar and share an extremely narrow parapatric boundary in southern Tanzania, with limited hybridization. They have subtly different plumages and dramatically different songs. I examine three aspects of territorial response to the sibling species: multimodal signaling (morphology/plumage and song), response to sibling species song relative to response to the song of an ubiquitous competitor for food resources, and geographic variation in response to sibling species song with spatial replication of 'allopatric' populations of *N. fuelleborni*. Over the two experiments, just one of four *fuelleborni* populations exhibited preferential response to conspecifics when making initial approaches, whereas two of three *moreaui* populations exhibited discrimination at this level. Magnitude of response, as indicated by duration of approach within 8m, did not depend on mount or song species in the multimodal experiment. In the song-only geographic variation experiment, responses to sibling species were far more frequent than responses to the ubiquitous ecological competitor, even distant from contact. Relative magnitude of response in *fuelleborni* varied geographically, with the allopatric population most distant from the parapatric boundary unexpectedly exhibiting strong responses to *moreaui* song. Generally, the results do not meet *a priori* expectations based on the species recognition hypothesis, and the evidence for the resource overlap hypothesis is mixed. The results of this experiment call into question the use of territorial intrusion experiments with male subjects as a substitute for mate choice experiments, but show that the evolution of territorial response is itself an extremely complex subject. The results also suggest that models for the evolution of territorial response should include two separate components: a psycho-perceptual model to explain the perception and significance of threats, and a response 'calibration' model to explain variation in response magnitude (e.g. duration or intensity).

Introduction

"Understanding species-specific responses to signals in nature is likely to require . . . *consideration of the potential costs and benefits of responding or failing to respond to particular signals* . . ."

- Terry Ord and Judy Stamps (2009)

"Territorial behaviour can therefore only be adaptive if maintenance of ownership is profitable, i.e. if the defence of a territory is less expensive in terms of *fitness* than the potential damage done by competitors in the absence of defence."

- Martin Hinsch and Jan Komdeur (2010)

Signal evolution is a nearly ubiquitous feature of animal speciation (West-Eberhard 1983). When animals diverge, many of the signals they use in intra- and interspecific communication tend also to diverge to the point of becoming specifically distinct (Darwin 1871). This signal divergence plays a widely appreciated role in the speciation process, in that divergent signals may prevent hybridization through pre-zygotic isolation (Mendelson and Shaw 2012). More generally, the species distinctiveness that often results from signal divergence accompanying diversification allows individuals to prioritize activities, including *but not limited to* mate choice, as signals are processed. A subset of this idea is generally termed the 'species recognition hypothesis' – that individuals use signals to distinguish conspecifics from non-conspecifics to prioritize activities including *but not limited to* mate choice (contra Mendelson and Shaw 2012). However, it is intuitive that individuals also successfully prioritize activities and produce behavioral responses based on correctly associating non-conspecific signals with corresponding ecological relevance (e.g. to identify and subsequently avoid or harass their predators or competitors), and there is abundant evidence for this process (chickadee studies). From an adaptationist perspective, individuals should minimize energy expenditure or time expenditure invested in interactions with organisms that neither represent potential threats nor sources of benefit, and prioritize fitness-consequential responses to ecologically or reproductively relevant individuals, including both conspecifics and heterospecifics. Costs to receivers from failing to respond behaviorally to the signals of other individuals that can greatly impact the fitness of the receiver should be high (Ord and Stamps 2009). Examples of these consequences are not hard to conceptualize. The failure to be vigilant when a predator is nearby, the failure to engage in courtship when a potential mate is available, and the failure to defend readily limiting resources from a competitor all have the potential to be extremely costly.

Recently diverged taxa present interesting scenarios for the study of signal evolution, perception, and behavioral response. Recently diverged taxa have shared the great bulk of their ancestry over the long history of evolution. For most species, just a tiny fraction of their evolutionary history is independent of closest living relatives, and because of phylogenetic effects, the general consequences include that recently diverged taxa tend to share many biological traits of import (Wiens and Graham 2005), relevantly including signaling modes, signal types within modes, sensory capabilities, perceptual mechanisms, morphology, and ecological niches. The result of conservatism in aspects of signaling traits and sensory systems is that pairs of closely related taxa frequently possess signals that are similar in multiple dimensions over multiple modes, and within each others' perceptual limits, even if these signals are differentiable. Niche conservatism in a very broad sense means that the signals of closely related taxa are often biologically relevant, because closely related organisms with similar abiotic niches often have the opportunity to occur in sympatry via secondary contact or *in situ* speciation. Furthermore, recently diverged taxa tend to share similar resource requirements, meaning they may compete with one another for resources in sympatry, including competition for access to mates when there is hybridization or attempted hybridization (Groening and Hochkirch 2008). Thus, closely related taxa regularly perceive and process each others' signals, and must make decisions on how to respond to these signals.

One realm of animal biology where these processes are manifest is resource defense, which frequently takes the form of territoriality. Territoriality occurs when individuals or groups defend resources over a limited area, a process mediated by social signals (Noble 1939; Nice 1941). While territorial defense is most often considered in an intraspecific context, interspecific territoriality is common and taxonomically widespread (Peiman and Robinson 2010). However, as not all other animals can be excluded from a territory, territorial holders must make decisions regarding which species or individuals they will attempt to exclude from their territories. This process can be adaptive when investment in defense activities is commensurate with marginal fitness benefits of an attempt at intruder exclusion (Hinsch and Komdeur 2010). Existing theoretical work generally moves forward from the assumption that organisms behave adaptively. Plasticity plays an important role here. Given the empirical evidence that learning often plays a role in associating signals with the threats or benefits represented by signal senders, theory on territorial aggression must accommodate adaptive plasticity through associative learning of signal content with signal senders (Irwin and Price 1999).

One prominent ecologically-motivated hypothesis that could explain variation in territorial aggression is the resource overlap hypothesis, which posits that resource overlap between individuals (or groups of individuals, as in social organisms or when mates jointly defend territories) determines levels of territorial aggression, which evolve adaptively. Under this hypothesis, resource overlap and territorial aggression should be positively correlated. In most cases, the resource overlap hypothesis predicts high levels of territorial aggression between same-sex conspecifics, and generally less aggression towards heterospecifics, as heterospecifics share at least one fewer resource axis: mates. When a heterospecific is from a closely related species with high ecological similarity and where hybridization is possible, heterospecific aggression could be equal to conspecific aggression under the resource overlap hypothesis. But for closely related species, or even within species, an alternate hypothesis is often used to make predictions: species recognition. Under the hypothesis of species recognition, signal similarity of the intruder to the territory holder or to the territory holder's conspecific recognition function predicts variation in territorial response (Ord and Stamps 2009). Though this hypothesis is in some ways motivated by receiver psychology, it is possible to view it as a sub-hypothesis of the resource overlap hypothesis. If conspecifics always have higher resource overlap with a territory holder than heterospecifics, then the two hypotheses make similar predictions, with one exception. When heterospecifics cannot be distinguished from conspecifics because of signal similarity, the resource overlap hypothesis fails (i.e. high heterospecific signal similarity with lower resource overlap than conspecifics results in recognition mistakes). Conversely, the species recognition hypothesis fails to predict territorial aggression when heterospecifics with differentiable signals represent a threat that is equivalent or greater than the threat posed by a conspecific – high levels of adaptive territorial aggression subsequent to heterospecific recognition are exhibited in this case.

Evidence consistent with both the resource overlap and species recognition hypotheses have been found in birds. Ord and Stamps (2009) found that, across the twelve studies that met the criteria for their meta-analysis, birds exhibited territorial responses of greater intensity or magnitude to conspecifics versus heterospecifics in all twelve. As above, this evidence might be taken as support for either the species recognition or the resource overlap hypothesis. It is of interest, however, that numerous studies have found that individuals often exhibit strong territorial responses to heterospecifics. Comparisons of heterospecific responses in allopatric and sympatric populations are informative in assessing the degree to which heterospecific aggression represents treatment as a conspecific (i.e. recognition mistakes) versus aggression aimed at heterospecifics after successful discrimination. At least three studies ((Gill and Murray 1972; Morrison 1982; Nuechterlein and Buitron 1998); as cited in Irwin and Price 1999) have found that species responded more to heterospecifics in populations where the two species were allopatric than they did in sympatry. The similarity of the signals of these species pairs suggests that individuals in allopatric populations are treating signals that are unfamiliar, or at least not characteristic of their breeding environments, as conspecifics in the absence of the opportunity to associate the signals with additional aspects of the heterospecifics' biology (see also Ord and Stamps 2009, (Nelson 1989)). Conversely, many studies have found greater magnitude or higher intensity responses to heterospecifics in sympatry, suggesting that heterospecific aggression in these cases must be triggered by the presence of heterospecifics that represent territorial threats. This pattern could indicate either a broadening of the psycho-perceptual concept of conspecifics or successful heterospecific recognition with the response calibrated to suit the threat represented by the heterospecific, likely through associative learning (discussed in Price 2008). The elegant experiment of Matyjasiak (2005) demonstrated that birds can associate vocal signals with visual signals when responding to heterospecific territorial intrusions, indicating heterospecific recognition occurs even when vocal signals are similar. In some instances though, the signals themselves can converge for heterospecifics in sympatry (e.g. (Rohwer 1973; Grether et al. 2009; Seddon and Tobias 2010)), in which case the likelihood that individuals treat heterospecifics as indistinct from conspecifics (i.e. without discrimination) increases.

Here, I examine male responses to simulated territorial intrusions of heterospecifics and conspecifics in the ecologically similar bird species Moreau's Sunbird *Nectarinia moreaui* and Fuelleborn's Sunbird *N. fuelleborni*, which share a narrow parapatric boundary with limited hybridization in southern Tanzania. The two species exhibit extremely different song phenotypes (Chapter 2) readily distinguished by humans, but strong similarity in plumage phenotypes and only subtly different morphology (Bowie et al. 2004). They are also similar by both bioclimatic niche analysis (Chapter 2) and autecology as indicated through natural history observation. The geographic context of their distributions provides the possibility to examine responses in the vicinity of contact between the species and far from this area. I report the results of two types of simulated territorial intrusion experiment that investigate understudied aspects of social signal perception: multimodal signaling and geographic variation in response to a recently diverged sibling species. The first experiment uses multimodal mount and song presentations, with mount and song species crossed (Uy et al. 2009), performed to a single population each of *moreaui* and *fuelleborni*, in the vicinity of the parapatric boundary (~22 and ~16 km from the nearest heterospecific populations, respectively). The second experiment is a song playback experiment that explores geographic variation in response to the sibling species heterospecific as compared to an ecologically relevant control stimulus: an alternate heterospecific congener. The goals of this study are to examine whether striking differences in song between the two species yield differential response in territorial subjects, and to test which of the hypotheses outlined above, if any, predicts geographic variation in territorial responses.

The song/mount experiment was conducted first, in the 2009 breeding season. Based on the species recognition hypothesis, I predicted that male territory holders would exhibit responses of greater magnitude to conspecific signals. Given the obvious, multidimensional differences in song phenotypes between species and their minimal morphometric and plumage differences, I predicted that the magnitude of differences in responses by species would be greater for song signals than mount signals. The most striking result of this first experiment was that *fuelleborni* territory holders responded equally or more frequently to *moreaui* song than *fuelleborni* song, whereas *moreaui* individuals responded more frequently to conspecific songs. The design of the subsequent year's experiment reflects this result, as this experiment is intended to examine whether these responses were representative of species-level differences, or whether geographic variation exists in response to disparate songs. I examine support for the species recognition, resource overlap, and uncertainty hypotheses with the results of the song-alone territorial intrusion experiment. Furthermore, I use this assessment of geographic variation in response to heterospecifics to address the implications for the evolution of territorial aggression towards heterospecifics (or heterotypics) through divergence.

Methods

Natural history of focal taxa

Nectarinia fuelleborni (née *N. mediocris fuelleborni*) and *N. moreaui* are nectar-feeding sunbirds (Nectariniidae) that occupy montane forest and forest edge habitats in eastern and southeastern Africa. These two taxa are sibling species, and could be sister species, depending on the placement of the other member, *N. loveridgei*, of their shared clade (Bowie et al. 2004). Both species have 'sky-island' distributions, where component populations occur as geographical isolates separated by inhospitable lowlands. Their breeding distributions encompass areas at ~1400 – 2400 m elevation, but both species are elevational migrants that descend to lower elevations during the non-breeding season in those places where suitable habitat exists. *N. moreaui* is a Tanzanian endemic geographically restricted to the central Eastern Arc Mountains, a chain of ancient, isolated mountain blocks created through punctuated fault-block action beginning in the early Tertiary (30 million ya) and subsiding during the Miocene (6 million ya, (Griffiths 1993)). Within the Eastern Arc Mountains, *N. moreaui* is limited to the Nguru, Ukaguru, Wota, Rubeho, and northeast Udzungwa Mountains (Bowie et al. 2004). *N. fuelleborni* (including *N. fuelleborni bensoni*) has a broader distribution, spanning southern Tanzania to southern Malawi/central Mozambique, and it thrives across the mosaic of volcanic and igneous mountains in this region. The distributions of *N. fuelleborni* and *N. moreaui* meet in the northeast Udzungwa Mountains (Figure 1, and Chapter 2: Figure 4a).

The position of the boundary between species is surprising because it occurs within the Udzungwa highlands, unassociated with any known ecological cline and between two known biogeographic breaks (Chapter 2). Either the Makambako gap to the south (Lovett and Wasser 1993), which isolates the igneous Udzungwa Mountains from their volcanic southern neighbor Mt. Rungwe, or the dry valleys and Great Ruaha River to the north that divide the Udzungwa from the Rubeho Mountains (Fjeldså et al. 2010), would seem far more probable places to find a transition between the two species. Instead, the position of the boundary makes evident that the two species mutually exclude one another from suitable habitat; i.e. their abundances are completely co-regulated (Connell 1961). Furthermore, examination of bioclimatic niches of the two species indicates that they possess extremely similar niches (Chapter 2): they are effectively ecological replacements across space in montane forests (Price 1998). Extensive field observations of both taxa, moreover, have revealed no apparent differences in autecology. Though both species thrive in forest and forest edge, within these environments they are generalists in microhabitat preferences for both foraging and nest sites (JPM unpublished data).

Rainfall peaks from January to May in the montane forests of central and southern Tanzania. In this region, both *fuelleborni* and *moreaui* breed from June to September, during the dry, cold season that follows these rains ((Cheke et al. 2001); pers. obs.). All the simulated territorial intrusion experiments were carried out during this breeding season, with the breeding season confirmed by the discovery of active nests (building, egg-laying, or nestlings present) at all seven tested populations during playback execution. Outside of the breeding season, both species exhibit elevational movements that bring them to lower elevations. These movements likely result in annual increases in interspecific contact between the forests known as Nyumbanitu and Ndundulu (see Chapter 2, Figure 3).

Molecular evidence indicates that F1 hybrids are rarely produced at the contact zone, and individuals of mixed ancestry are at low frequency in all well-sampled populations (Chapter 2). The two species possess slightly different morphology (Chapter 2, Bowie et al. 2004) and plumage (Bowie et al. 2004), but males sing starkly different, complex, long-distance territorial songs. These songs appear to have the dual functionality generally attributed to passerine bird songs: mate attraction (intersexual) and territorial signaling (intrasexual, (Catchpole and Slater 2008)). The two species are strikingly sexually dichromatic and sexually dimorphic in meristic measurements (Bowie et al. 2004), indicative of sex-specific behaviors and the likelihood of a strong impact of social selection in their evolution.

The territoriality of these two species has not previously been well characterized. Males sing and defend territories during the breeding season, while outside the breeding season there is little singing and, presumably, reduced territoriality. It is also possible that non-breeding season, food resource-based territories are maintained (Davies 1982), but observations of spatial clumping of males outside the breeding season support the hypothesis that territoriality is reduced in the non-breeding season relative to the breeding season. Natural history observations and mistnetting efforts have revealed that floaters are extremely numerous in breeding populations of both focal species. Erecting a mistnet in the understory of a known territory often results in the capture of numerous males who are not the territory holder. This observational evidence suggests that territories are fewer than the number of males occurring in a population (Brown 1969). It also reveals that the holders of territories, considered 2-dimensionally, are not rewarded with exclusive use of resources on their territories. Males using acoustic signals within the territory of another male often elicit territorial responses, including vigorous chases frequently preceded by soft song of the song form termed “perched song”, or an alternate song form termed “chase song” used exclusively (or nearly exclusively) in intra-specific, intra-sexual interactions (with the exception that they are used in *N. moreaui-N. fuelleborni* male-male interactions). Chase song can persist through chases, including during flights, while perched song is not sung in flight. Because exclusive use of food resources is not always observed, males may principally defend space suitable for mate attraction and nesting. Antagonistic interactions seem in some instances to be pursued between territory neighbors to settle territory boundaries, at times instigated by singing in the vicinity of the boundary between territories. In one instance in *N. fuelleborni* at Mufindi, neighboring males tolerated each others' use of singing perches within a single large tree, with the tree apparently divided so that each individual sang from a different section of the tree.

Simulated territorial intrusion experiments

Experiment 1: Song playback/mount presentation

The first experiment was carried out in 2009 at the forest localities locally known as Ikokoto (7.69°S, 36.14°E; elevation ~1790-1910m) and Image (7.41°S, 36.15°E; elevation ~2000-2260m), in the northeast Udzungwa Mountains, Tanzania. Ikokoto is dominated by *N. fuelleborni* (15 of 16 genetic samples from mistnet captures classified as *N. fuelleborni* – see Chapter 2; no *N. moreaui* were further observed during sound recording efforts and informal surveys (Werema et al. in rev). Image is dominated by *moreaui* (14 genetic samples classified as pure *N. moreaui* and a single individual as having mixed ancestry– see Chapter 2; no *N. fuelleborni* phenotypes were otherwise observed during sound recording and informal surveys at Image).

I employed a mount presentation/song playback design, with the specific identity of the mount and song fully crossed, similar to the design used by Uy et al. (2009)but without a 'negative' control. Seven *N. moreaui* and eight *N. fuelleborni* tracks with ~60 consecutive seconds of high signal-to-noise ratio bout singing were chosen for playback from digital recordings made by JPM in 2008-09 (16 bits, 44.1kHz sampling). Noise below 1.5 kHz and above 11 kHz was then removed by bandpass filtering (Raven Pro 1.3) for all recordings. Each song playback sequence began with 90 seconds of silence, followed by 2 minutes of song, then five minutes of silence. A 2-minute song period was created in most cases by repeating the ~1 minute bout initially chosen. In those instances where the initial recording was substantially less than 1 minute (e.g. 50 s), the 2-minute period was filled out with a portion of the same initial recording. As in Uy et al. (2009), two male mounts were used to represent each species. The limited number of mounts may introduce a pseudoreplication issue (Kroodsma 1989), but because morphological differences between the two species are multi-dimensional (e.g. bill length, color of red breast patch, presence of yellow at sides of breast; see Bowie et al. (2004)), a small number of mounts could adequately represent species-level differences. I further controlled for the possibility of pseudo-replication by including mount identity as a random effect in the general linear mixed modeling approach used in analysis. From the 15 song tracks and 4 mounts available, I created 32 stimulus combinations to represent four classes: *fuelleborni* song-*fuelleborni* mount, *fuelleborni* song-*moreaui* mount, *moreaui* mount-*fuelleborni* song, and *moreaui* mount-*moreaui* song. Each subject was tested in a single trial, where it responded to a single stimulus type. The order of presentation of the 32 stimulus combinations was randomized.

The field research team sought subjects for this experiment by walking forest roads, trails, and forest edges, while listening for vocalizing male sunbirds. Males of both species vocalize throughout daylight hours, but I avoided performing trials early in the morning when ambient temperatures were low, as I discovered in preliminary trials that birds rarely responded in these conditions. Experiments were thus initiated between 0933 and 1705, and were run from July 15 to August 1, 2009. When a vocalizing male was found, the speaker and mount were placed at a distance ~10 – 30 m either from the individual or from a perch recently used by the subject for vocalization. The speaker (Anchor Minivox; Anchor Audio Portable Sound Systems, Carlsbad, CA) and mp3 player (3^{rd} generation Apple iPod Nano; Apple Inc., Cupertino, CA) used for song playback were placed on the ground, and mounts were affixed in an upright position to vegetation above the speaker (height 0.5 to 2 m). As much as possible, I performed trials with subjects who were either singing or had sung immediately before the trial was initiated. 48 of the 62 subjects analyzed sang within the 5 minutes prior to trial initiation. The remainder exhibited behaviors indicative territory defense – i.e. extensive calling, chasing another individual, close male-female interaction, or exclusively using an area with multiple nectar sources – during the pre-trial period. Three observers were positioned in different directions from the speaker and mount, with one (JPM) recording observations of subject responses into a digital voice recorder. A second observer made an audio recording of the trial using a Sennheiser ME67/K6 power module microphone combination and a Marantz PMD670 solid state digital recorder. Following trials, I conferred with the other observers to check whether all response behaviors had been recorded, and if the other observers had detected that any additional males beyond the intended subject had responded but were undetected by myself. When multiple individuals had responded, the trial was discarded. This issue occurred regularly because of the very high densities achieved by the focal species. To check distance estimates made during the trial, distances to perches used during approach were measured with a tape measure or, for longer distances, re-estimated from the speaker location. Transcriptions of the recorded observations were compared to audio recordings of the playback trials before analysis as a secondary check on the timing of events during trials. The time period from the start of the song playback through the end of the five minute silent period following the song stimulus, a total of seven minutes, was taken as the response period for Experiment 1. Note that the response period is shorter (four minutes) in Experiment 2.

Experiment 2: Song playback transect

To examine geographic variation in territorial response to heterospecific versus conspecific song, I performed a series of song playbacks across five populations in a transect perpendicular to the geographic boundary between the two focal species' distributions (Figure 1). Localities for *N. fuelleborni* experiments were Mt. Rungwe (9.16° S, 33.61° E; elevation ~1600-1900m), Mufindi (8.6 °S, 35.3 ° E; elevation ~1840-1950), and Nyumbanitu (7.81° S, 36.39 ° E; elevation ~1550-2000m); localities for *N. moreaui* experiments were Ndundulu (7.77° S, 36.49° E; elevation 1800-2250m) and Mafwemiro (6.94° S, 36.59 E; elevation ~1850-2100m).

In this experiment, I employed a repeated-measures design, where each subject was given the opportunity to respond to three different stimulus types: conspecific song, heterospecific song, and a 'control' song. *N. olivacea* was chosen for the control song species because it is a forest-dependent sunbird that occurs syntopically with the two focal species across their distributions, but is distantly related to the focal species and sings extremely dissimilar songs. Aggressive responses to *N. olivacea* should therefore result exclusively from attempts to exclude an ecological competitor, and should represent neither competition for mates nor misdirected aggression with failure to distinguish between conspecific and *N. olivacea* song (Peiman and Robinson 2010). The inclusion of a stimulus representing an actual competitor for the focal subject species in this playback design could be regarded as an improvement in experimental realism versus the use of a negative control like white noise. I expected *a priori* that some individuals would respond aggressively to the control stimulus, and even that such responses could vary by species or population. I argue that there is more to be gained from the use of a control representing an ecologically relevant stimulus from the organismal environment in such a playback design than from a negative control for which there is no biological basis to expect individuals to respond.

Playback tracks consisted of 60 s of silence, and subsequently a 2-minute stimulus period followed by 3 minutes of silence. The three-minute silent period was chosen as a compromise between the likelihood that an individual might leave the playback area during an overly long silent period, and that the response to the previous stimulus might be continuing if the silent period was too short. I examined responses to Experiment 1 as a guide to determine an appropriate response period. For duration measurements calculated afterwards, the responses of individuals who started any stimulus period within 8m were calculated from when they moved towards the speaker. Six playback tracks were produced, representing all possible orders of stimulus types. To produce these tracks, we selected songs as in Experiment 1, but *moreaui* and *fuelleborni* recordings were bandpass filtered at 2.5 to 10kHz instead of 1.5 to 11kHz. *N. olivacea* songs are lower in frequency, and were bandpass filtered at 1.5 to 10kHz. The 6 tracks incorporated a total of 6 *fuelleborni*, 6 *moreaui*, and 4 *olivacea* recordings. Outside of indicated differences, trials were performed as in Experiment 1. In Experiment 2, we sought to perform 18 trials for each population. Responses were recorded for the four minute period beginning with the start of each 2-minute song stimulus period and ending at 2 minutes after, unlike in Experiment 1 where the response period was 7 minutes.

Analyses

For both experiments, I analyzed responses using two-part analyses with generalized/general linear (mixed) models. The first analysis for both experiments tested which factors were responsible for whether an individual approached to within 8m or not, interpreted to indicate whether a song stimulus was initially perceived as a threat (Jankowski et al. 2010). For the subset of individuals who made approaches, I then analyzed the duration of time spent within 8m as an indicator of total aggression. This value was correlated with subjective scores of aggression based on the array of behaviors exhibited during responses, which were too diverse in form among individuals to reduce in dimensionality using multivariate techniques (e.g. principal components analysis). That is, the distribution of individual response variables (e.g. time spent singing, number of calls, fly-bys) is zero-inflated because many individuals exhibited only a subset of these response types. Reduction of dimensions for multivariate techniques (e.g. principal components analysis) assume normality of variable distributions, and zero-inflated distributions cannot be transformed to improve normality.

During Experiment 1, approaches were never instigated by the presence of a mount without song playback. Consequently, mount species was not considered to have any effect on the initial approach to within 8m. Thus neither the mount species nor the mount identity was included as fixed or random effects in the binomial (i.e. logistic regression) model. I used the glm function in R (R Core Team 2012) to fit a binomial-family generalized linear model, equivalent to a logistic regression, with song species (heterospecific versus conspecific) and subject species (*fuelleborni* or *moreaui*) as effects. For individuals that made an initial response, I tested the combined effects of song species and mount species on the duration of response by fitting a generalized linear mixed model with a poisson distribution to the response variable (duration spent within 8m) using the package MCMCglmm (Hadfield 2010). As has been recommended for simulated intrusion experiments with limited exemplar stimuli (Kroodsma et al. 2001)and as implemented in mixed model frameworks by multiple territorial intrusion studies (Grant and Grant 2002; Newman et al. 2006; Uy et al. 2009) I controlled for the variation associated with individual exemplars for mounts (2 exemplars per species) and for songs (7 *moreaui* and 8 *fuelleborni* exemplars) by including the exemplar identities as random effects of recording and mount identity. Some practitioners of GLMM advise a minimum of 5-6 levels for each random effect to achieve adequate precision in estimates for the variance components of the model, and in this case the 'mount identity' random effect has only four levels. However, precise estimates for variation associated with each individual mount identity are not of primary interest in this study. Instead, I use the random effect to control for the limited number of exemplars (i.e. they are treated as 'nuisance' parameters), and the variation explained by the fixed effects is of primary interest. This usage of the mixed modeling framework is consistent with previous use of linear mixed models (LMM) for territorial intrusion experiments as above. To make statistical inferences regarding the fixed effects, I use Bayesian Markov-chain Monte Carlo (MCMC) simulation with random walk Metropolis-Hastings sampling to estimate confidence intervals for the fixed effect parameters. I ran the MCMC chain for 1,300,000 iterations, with a 300,000 iteration burn-in, to produce confidence intervals for the fixed effects. Because sample size was limited in the reduced data set (i.e. subjects who approached to within 8m), especially for *N. moreaui* responses to heterospecific songs, the data for both species were pooled and analyzed without a 'subject species' fixed effect. This left the four stimulus categories of interest with a sample size range of 7-11 (conspecific song/conspecific mount: 9, conspecific song/heterospecific mount: 11, heterospecific song/conspecific mount: 7, heterospecific song/heterospecific mount: 9; total total *n*=36). Because the stimuli were randomized and I was testing the hypothesis of species recognition experimentally, I did not use model selection or stepwise backward regression to reduce the model, and instead use the MCMC parameter estimates to assess the significance of song species, mount species, and their interaction. The model was initiated with conspecific song and conspecific mount as part of the intercept estimate, such that contrasts with heterospecific signals of the fixed effects can be assessed from the parameter estimates.

For Experiment 2, I modeled the binary 'approach or no approach' variable separately for each subject species, using a fully Bayesian implementation of the generalized linear mixed model (package MCMCglmm in R: Hadfield 2010, R Core Team 2012), including controls for repeated measures and playback track identity coded as random effects (although in the Bayesian GLMM framework there is no distinction between fixed and random effects, they are still coded separately in MCMCglmm; Hadfield 2010). As recommended by Hadfield (2010) for logistic regression-like MCMCglmm models, the model did not include an intercept. When subjects were within 8m at the beginning of a stimulus, an approach was indicated by movement towards the speaker from their position at the beginning of the stimulus. The full model was used for hypothesis testing by examining 95% credibility intervals of treatment contrasts (i.e. the null hypotheses were that linearized predictors of the binary response to 'conspecific' (CS), 'heterospecific' (HS), and 'control' songs were equal, H_0 : cs-hs=0; cs-control=0, hs-control=0). The MCMC chain was run with 140,000 iterations with a 40,000 iteration burn-in, sampled every 100 iterations. I checked sampling adequacy of the MCMC chains by visually assessing autocorrelation and the sampled distributions of parameter estimates, and also by checking effective sample size estimates of parameters. Effective sampling estimates for the parameters for both models were >400.

To compare conspecific and heterospecific responses in Experiment 2, I subtracted the value for duration spent within 8m during the heterospecific response period from the same value for the conspecific response period for each subject. This approach has the advantage of completely accounting for among-subject variation in response. I analyzed only those trials where an approach was recorded for at least one of the three stimulus classes (including individuals who did not respond to conspecific stimuli but did respond to non-conspecific stimuli avoids overestimating conspecific aggression – see Peiman and Robinson 2010 p. 138). These differences were determined to be approximately normally distributed (Shapiro-Wilks, p=.4298), and hence were fit with general linear mixed models, one for each species. I estimated per-population means by including population as a fixed effect while controlling for the track identity by coding it as a random effect (as in the binomial response model). These models, as in the binomial modes, were intercept-free. I similarly generated this model in a Bayesian framework using MCMCglmm. I again ran the MCMC chain with 140,000 iterations with a 40,000 iteration burn-in, as in the binomial model.

Results

Territorial response behaviors

Males responded to simulated territorial intrusion in both experiments by approaching the speaker and/or mount, calling, singing, singing soft song, erecting yellow pectoral tufts, and/or facing the mount in the multimodal experiment. No individuals physically attacked the mount during trials used for analysis, although this occurred once in a pilot trial, during which the subject perched on the mount's back and repeatedly pecked the back of the mount's head with a good deal of force. Outside of this instance, the most intense behavioral responses to simulated intrusion appeared to be soft song of similar structure to the long-distance song form used in playback stimuli (which I term "perched song" for these species – see Chapter 1: Figure 2e-f, and Chapter 2: Figure 2) or the "chase song" type reserved for intense intra-specific interactions, which can also be delivered at either low or high amplitude. Chase song appears to have less structural consistency than perched song, and consists of series of rapidly delivered elements with relatively higher frequency bandwidth (JPM unpublished data), though these signals are difficult to analyze because they are almost always uttered simultaneously by multiple males.

During some trials, male-female pairs approached the speaker or speaker/mount together. These approaches seemed to occur when males were in the process of following females as they moved around a territory, when song playback began. In such instances, some males instantaneously responded vocally to the song stimulus but hesitated to leave the female to approach the speaker or speaker/mount until the female also turned its attention towards the playback stimulus. This context-dependence may result in an underestimate of male aggression to playback signals if latency of approach is used as a proxy for aggression, but when such pair behaviors were apparent, males tended to exhibit strong aggressive responses (including when response strength was measured by duration of approach).

Experiment 1 (multimodal) modeling results

In the multimodal experiment, approaches occurred in 36 of 62 trials. *N. fuelleborni* males responded in 10 of 16 trials with conspecific song, and 13 of 15 trials with heterospecific song. Conversely, *N. moreaui* responded in 13 of 15 trials with conspecific song, and 5 of 16 trials with heterospecific song. A likelihood ratio test of nested models indicates that a model containing an interaction of subject species with song stimulus species is an improvement over a model without one ($p<.01$). Neither subject species nor song stimulus species is a significant predictor of approach by itself ($p>.05$). Thus, *N. moreaui* responded less frequently to heterospecific than conspecific songs, whereas the higher frequency response of *N. fuelleborni* to heterospecifics than conspecifics was not statistically significant. Details of the full model can be found in Table 1.

For the subset of individuals that responded, the contributions of the song species and mount species effects were minimal in the model, and the pattern of responses does not correspond to the *a priori* prediction based on the species recognition hypothesis (Figure 2a). The strongest responses (longest duration of approach) in the raw data were made to, in order from highest to lowest: conspecific songs with heterospecific mounts, heterospecific songs with heterospecific mounts, heterospecific song with heterospecific mount, and conspecific song with conspecific mount (Figure 2b). In the Poisson-family model developed with MCMCglmm (Hadfield 2010), the confidence intervals for the fixed effects as estimated by MCMC simulation include zero, and I fail to reject the null hypothesis that approaches within 8m are similar among the four stimulus types (Table 2).

Experiment 2 models within-individual response correlation

Frequencies of response were ordered in both species from highest to lowest as conspecific, sibling taxon, and *N. olivacea* ('control'). In the binomial GLMMs done for each species from this experiment, linearized predictions for *moreaui* ($n=31$ subjects, repeated measures) are significantly greater for conspecifics than for the sibling taxon, and greater for the sibling taxon than for *N. olivacea*. In *fuelleborni* ($n=41$, repeated measures), the contrast between conspecific and sibling taxon responses is not significantly different from 0, and both of these responses were of greater frequency than for *N. olivacea* (Figure 3).

For analyses of response duration to conspecifics versus the sibling taxon, I begin by testing within-individual correlation between responses to *moreaui* and *fuelleborni* songs (test=Kendall's tau, $n=72$, $z=0.904$, $p=.366$, $tau=0.0769$). This test is a rank test, so differences in magnitude are not taken into consideration. Regardless, the use of individual-level response differences for modeling accounts for any within-individual correlation. Details of the general linear mixed models generated to examine within-species population-level variation are shown in Table 3. Population means and standard errors of raw data are presented in Figure 4. I find statistical support for population level variation in *fuelleborni*, and less so in *moreaui*. Examined at a population-by-population level across the two models, only two populations appeared to exhibit discrimination: Mufindi (*fuelleborni*) and Mafwemiro (*moreaui*). Both of these populations are distant from contact (Figure 1).

Discussion

It is evident from the results of these two territorial intrusion experiments that the species recognition hypothesis on its own cannot fully account for the results of either experiment. Evidence for the resource overlap hypothesis is mixed. By examining the results of these experiments in the context of the natural history of the focal species, and the levels of interbreeding found in Chapter 2, I suggest alternate hypotheses for the drivers of territorial responses and for the evolution of response to sibling taxa signals in this species. In the multimodal experiment (Experiment 1), the *a priori* hypothesis was that individuals should mount territorial responses more frequently to songs of their own species, and that the intensity of response (here modeled as the duration of approach to within 8m), should be greatest to the conspecific song/conspecific mount combination, with lesser intensity responses as the signal combination decreases in similarity to the territory holder (Figure 2a). I find support for higher frequency of initial response to conspecific song only in *N. moreaui*, with a trend in the opposite direction for *N. fuelleborni*. The duration of responses for both species in Experiment 1 is not consistent with predictions made from the species recognition hypothesis (and similarly inconsistent with the resource overlap hypothesis if resource overlap is assumed to be highest among conspecific males).

The goals of Experiment 2 were threefold: to check whether the results of Experiment 1 were indicative of species-level responses and heterospecific response differences to song; to test whether high levels of aggressive responses to *N. moreaui* by *N. fuelleborni* are restricted to the vicinity of the contact zone consistent with aggression induced by presence of a threatening heterospecific; and to compare responses to a mostly allopatric sibling species with responses to an ubiquitously sympatric congener that is a competitor for nectar resources. Experiment 2 indicates that despite the great extent of song divergence and the extremely limited sympatry between *N. moreaui* and *N. fuelleborni*, signal similarity due to recent shared ancestry results in greater levels of response to allopatric sibling taxa than to the sympatric, congeneric competitor. I argue that the relevance of signal similarity between *moreaui* and *fuelleborni* is made evident by considering the context of the signals of co-occurring species (Nelson 1989) – the songs of the focal species occupy a signal space that seems to be quite dissimilar from the birds with which both species co-occur (pers. obs). This hypothesis should be further explored. Geographic variation in heterospecific response is evident in *fuelleborni*, and somewhat ambiguous in *moreaui* based on these results. Strong responses to *moreaui* by *fuelleborni* are not limited to the contact zone area, suggesting that response to a sibling species may be stochastic away from contact areas in the absence of selection for response specificity. As previously suggested, associative learning of signals with individuals in sympatry should always be considered as an explanation for territorial responses to heterospecifics in nature. I argue that associative learning as a generalized mechanism for the development of territorial aggressive responses to heterospecifics is likely to be so widespread that instances of heritable variation in territorial aggression limited to particular heterospecifics most likely represent rare circumstances where especially strong selection drives genetic assimilation of an otherwise learned behavior.

Inferences from the multimodal experiment (Experiment 1)

Despite the great differences between species in many measurable song dimensions for individuals available for individuals to use in song discrimination (Chapter 2), *N. fuelleborni* responded more frequently to *N. moreaui* songs than to conspecific songs in Experiment 1 (13/15 versus 10/16), though the difference in response did not achieve statistical significance. The trend, however, is opposite the pattern predicted from the species recognition hypothesis. It is additionally opposite the pattern expected for the resource overlap hypothesis when greatest resource overlap is assumed for conspecifics. The assumption that resource overlap is highest for conspecifics is most comfortably made for species with complete reproductive isolation, in which case the mate resource axis is completely unshared. However, because a small number of individuals with common ancestry are present in the contact zone, it is evident that heterospecific males can compete for mates in these species. If it is assumed that resource overlap is approximately equal in the two species, the resource overlap hypothesis would predict equal frequencies of response to heterospecifics and conspecifics, consistent with *N. fuelleborni* responses if the trend is not considered to be indicative of a difference. With respect to this consideration, it is worth addressing the estimated effect sizes from this experiment – the estimated confidence intervals for the effect size for heterospecific response in Experiment 1 for *N. fuelleborni*, calculated as natural log of the odds ratio and its confidence intervals (Nakagawa and Cuthill 2007), do not include zero (Figure 5). This result is made all the more striking when considering the Experiment 1 responses of *N. moreaui*, whose frequencies of response to song are consistent with the species recognition hypothesis (or the resource overlap hypothesis if greater resource overlap for conspecifics is assumed). Experiment 1's results indicate asymmetry in response frequency to heterospecifics for the two populations examined (Ikokoto and Image) – the possibility that this asymmetry occurs at a species level is further examined in Experiment 2.

For the individuals that responded, the duration of approach did not vary significantly by treatment based on confidence intervals for the parameters of the GLMM. Nor did the pattern of mean responses resemble the pattern expected by the species recognition hypothesis across species (Figure 2a). This evidence suggests that after song initially elicits a territorial response, any combination of acoustic and visual signals of these two species represents a similarly threatening intrusion. This result is consistent with the resource overlap hypothesis if it is assumed that resource overlap is similar between species. This assumption is probably justified to some extent in this instance, as the species share similar autecology, bioclimatic niches (Chapter 2), and interbreed to some extent in sympatry such that the resource axis representing mating activities is shared between species. However, the subtle morphological differences between species and the signal differences (especially song) would suggest that despite that most resource axes should be shared, there are likely to be: 1) subtle differences in foraging ecology (e.g. distribution of flower shapes used) and 2) female preferences based on signal differences that limit the overlap along the mating axis. Thus, it might still be expected that subtle differences should be seen based on the resource overlap hypothesis, and given the low sample size it could be argued that this experiment failed to find such differences because of low power. However, the trend in response is opposite that expected under the resource overlap and species recognition hypotheses for mount species, and the weakest responses by treatment were to the combination of conspecific song and conspecific mount. The combination of results from this experiment: lack of greater frequency of response to conspecific song for one of two species and lack of a pattern consistent with greater intensity of response to conspecifics introduces a number of questions. The possibility that antagonistic interactions in the contact zone drive this pattern is addressed in the next section.

Inferences from the geographic variation experiment (Experiment 2)

A common underlying assumption of studies focused on species recognition, frequently with an interest in simulating sympatry (Grant and Grant 2002) to test whether signals have diverged sufficiently for discrimination to be possible, is that responses to heterospecific signals are geographically invariant. Tests where geographic variation is of central interest tend to examine responses to heterospecifics versus conspecifics in sympatric or allopatric populations with respect to the heterospecific species of interest. The inclusion of an ‘allopatric’ population has been viewed as a control for the ecological context of the experiment (Peiman and Robinson 2010), and typically only a single ‘allopatric’ population is included. In such studies, sympatric populations have the opportunity to interact with heterospecific individuals, and these interactions induce a change in response either through natural selection or by triggering a plastic response through associative learning of signals as indicative of fitness costs to the territory holder. The resource overlap hypothesis accounts for this type of geographic variation in response, except that in cases where ‘allopatric’ populations exhibit a stronger response the persistence of an aggressive response to a signal that is differentiable and that does not represent a territorial threat in sympatry must be explained.

Based on the results of Experiment 1, an investigation of geographic variation in the responses of both species to each other was desirable. Experiment 1 was performed in populations in the vicinity of the contact zone, but where heterospecifics are rare. *Fuelleborni*’s responses to *moreaui* song suggested that even where it is extremely rare, the presence of *moreaui* could drive strong heterospecific responses. By comparison, the low level of response to *fuelleborni* song by *moreaui* in Experiment 1 could have indicated either insufficient frequency of *fuelleborni* individuals (or more specifically individuals exhibiting *fuelleborni* song, as non-territorial *fuelleborni* – females and floaters - may not exhibit song) to drive heterospecific response in *moreaui* or the failure of *moreaui* to respond adaptively to a competitor. A third option was that *moreaui* adaptively exhibits limited response to *fuelleborni* despite contact, if *fuelleborni* does not represent a territorial threat.

The song stimulus-only geographic variation experiment resulted in a complex picture of territorial aggression to heterospecifics versus conspecifics in *fuelleborni* and *moreaui*. I discuss the geographically invariant results first. The clearest and most obvious result is that both *moreaui* and *fuelleborni* respond more frequently to each others’ songs than they do to the songs of *N. olivacea*, an ubiquitous competitor for nectar resources, despite that most subjects in the experiment have never encountered individuals of their sibling taxon (Grether 2011). Thus, despite the obvious differences between species, the retained similarities between *moreaui* and *fuelleborni* songs that result from their recent shared ancestry are sufficient even in allopatric populations to evoke initial investigative responses at the same level as conspecific signals. Indeed, analysis of the binary variable (approach vs. no-approach) indicates that there is no discrimination between conspecifics and heterospecifics at this level for *fuelleborni*, although this discrimination occurs in *moreaui*. When viewed in an adaptationist cost-benefit framework(Bradbury and Vehrencamp 1998; Ord and Stamps 2009), this result in *fuelleborni* suggests that the relative costs of initial response to a novel signal with similarities to conspecific signals are low, or that the costs of ignoring such a signal are high, or some combination of the two (Ord and Stamps 2009). The consistency of this asymmetry in conspecific-heterospecific discrimination between *moreaui* and fuelleborni across experiments begs explanation. The trajectory of song phenotypes in both species suggests an explanation, and indeed a hypothesis motivated by receiver psychology was considered before Experiment 2 was performed. *Fuelleborni* songs can include an exceptionally diverse array of different sounds, including heterospecific mimicry. Thus, it is likely that many sounds produced by *moreaui* within songs bear a strong resemblance to sounds used by *fuelleborni* individuals. The range of sounds used by *moreaui* is comparatively smaller (e.g. most *moreaui* elements within songs are very short – see Chapter 2, Figure 2). This comparison suggests that the specificity of perception of conspecific song could be more narrow in *moreaui* than *fuelleborni*, which may explain some of the asymmetry in initial heterospecific discrimination.

The second analysis performed for Experiment 2, examining geographic variation of within-individual differences in response duration to heterospecific versus conspecific songs, did not fit any of the *a priori* hypotheses for geographic variation based on allopatric/sympatric comparisons. Within *fuelleborni*, strong heterospecific responses were recovered at Nyumbanitu, a contact zone population. This finding confirmed that heterospecific aggression is high in the vicinity of contact, as found in Experiment 1. At Nyumbanitu, response differences to heterospecifics versus conspecifics are indistinguishable from zero, as estimated from credibility intervals of parameters from the Bayesian GLMM analysis. This result again suggests that males near the contact zone treat the heterospecific sibling taxa as similarly threatening to a territory holder as conspecifics, consistent with high resource overlap as predicted by the resource overlap hypothesis. At Mufindi, ~160km from the nearest *moreaui*-dominated population, *fuelleborni* males respond less to *moreaui* songs than to conspecific songs. This pattern is similar to that found in other studies that have recovered an interaction-induced increase in territorial response in sympatry with discrimination in allopatry. However, at Mt. Rungwe, ~350 km from the nearest *moreaui*-dominated population, responses to *moreaui* were again strong relative to conspecific responses. This inconsistency among the allopatric spatial replicates suggests more complexity to geographic variation in territorial response to heterospecifics than has previously been reported. Why would responses vary geographically in this way? I suggest that Nelson's (1989) hypothesis that variation in the sound environment as a function of geographic variation in co-occurring species may result in local differences in the features of song used to recognize conspecifics or territorial threats in *fuelleborni*. However, I know of no extant species that are present in one of the two allopatric populations, and not present in the other, and that have songs that are similar enough to these two species to suggest that they might drive such differences. The song that is most similar to *fuelleborni* in either of these populations is likely to be that of the Yellow-browed Seedeater *Serinus whytii*, which is present in both areas, although it is perhaps more common at Mufindi. How the presence of *Serinus whytii* would affect the perception of conspecific song in a way that would change responses to *moreaui* is also, however, unclear. If it truly is more common at Mufindi, *Serinus whytii*'s presence in the sound environment might simply result in higher specificity in territorial responses of *fuelleborni* there, thereby reducing the response to *moreaui*. Among remaining candidates as influences on the psycho-perceptual sound environment, there are two sunbird species that are found in the Udzungwa and have not been reported at Rungwe (*N. rufipennis* and *N. ludovicensis*), but both species are absent or extremely uncommon at Mufindi and therefore unlikely to drive such a mechanism. Geographical variation in the features used as perceptual cues to recognize conspecifics may also occur independent of variation in the community of co-occurring species. Such variation might occur if selection for the use of particular features to identify conspecifics is weak.

The GLMM analysis of differences in conspecific-minus-heterospecific responses revealed no species-level differences across the data set as a whole, although there is a trend for *moreaui* to respond more strongly to conspecifics versus heterospecifics than does *fuelleborni*. Furthermore, the two populations that exhibited the highest level of heterospecific response relative to conspecific response in Experiment 2 were *fuelleborni* populations. Across both experiments, *fuelleborni* contact zone populations exhibited stronger responses to *moreaui* than the reverse, although the difference in response duration for Experiment 2 was not tested with contrasts statistically, and here I rely on expected mean values from the GLMM as indicators of population-level differences. There are a variety of biological explanations that could be proffered for this apparent difference, including: 1) *fuelleborni* are more easily induced to respond to *moreaui* in sympatry because of perceptual or associative learning differences between the species; and 2) *moreaui* individuals more frequently occur as immigrants in *fuelleborni*-dominated populations in the contact zone than the reverse. This latter statement has support based on molecular sampling and informal surveys – small numbers of pure *moreaui* individuals were found at both Ikokoto (a single *moreaui* male) and Nyumbanitu (two pure *moreaui* males and three individuals of mixed ancestry), whereas no pure *fuelleborni* individuals have yet been found on the *moreaui* side of the transition at either Image or Ndundulu – but further tests of this hypothesis would necessarily include more extensive surveying and sampling at these localities inside and outside the breeding season.

Conclusions

The failure of the species recognition hypothesis to account for variation in territorial response in this experiment, and the difficulty of interpreting the results in the context of the resource overlap hypothesis, highlights an issue with theoretical approaches in this and similar studies. As discussed in the introduction, from an adaptationist perspective, organisms should optimize their territorial responses to improve individual fitness. Because the array of interacting individuals and species is in flux, and the other environmental contexts of territoriality are also changing, territorial response should be sufficiently flexible to be calibrated based on environmental input, and sufficiently plastic that threat levels represented by specific individuals or categories of individuals can be learned. One issue that a comparison of the species recognition hypothesis (a psycho-perceptual hypothesis) and the resource overlap hypothesis (based on competition) bring forth is that there are two aspects of territorial response behavior that must be accounted for separately: 1) perception and categorization/recognition of signals, and 2) calibration of appropriate threshold for initial response, and for response magnitude based on the type and magnitude of threat. An optimality modeling approach seems the best way to move forward. Individuals should maximize the marginal fitness benefits of territorial responses based on their current environment and the threats or opportunities represented by an intruder. What is perhaps critical to consider in the evolution of territorial behavior is that threats from intruders should not be measured for a single resource unless defense of that single resource is completely correlated with fitness; instead, the threat from an intruder is its impact on the reproductive fitness of the territory holder.

With regard to the evolution of response to sibling taxa, the results of these experiments raise issues of theory and experimental design. The geographic variation in *fuelleborni* in relative response to *moreaui* and conspecific song was not predicted by any of the hypotheses considered before the experiment. The strong response to *moreaui* song at Mt. Rungwe, 350 km from the nearest *moreaui* population, may seem maladaptive given the discrimination that occurs at Mufindi, closer to the nearest *moreaui* population. However, because the songs of both sunbirds resemble one another in such a way that both are quite distinct from other sounds encountered in the sound environment at both Mufindi and Rungwe, it would not be entirely surprising for both species to fail to discriminate. The discrimination seen at Mufindi may therefore be more difficult to explain. It suggests a narrowing of the psycho-perceptual concept of conspecific song at Mufindi relative to the variation between *fuelleborni* and *moreaui* song. I suggest in this chapter that the commonness of the very distantly related Yellow-browed Seedeater *Serinus whytii* at Mufindi might result in a refined concept of conspecific song at that locality because of the similarity of *S. whytii* and *N. fuelleborni* song. However, this pattern may also result from stochastic processes (cultural and/or genetic drift). These results highlight the need for spatial replication in studies comparing reciprocal responses in allopatry and sympatry for two taxa.

Table 1: Logistic regression for binary approach to <8m from speaker for Experiment 1 (mount/song presentation), categorized by subject species and song stimulus species.

		95% CI for exp b		
	B (SE)	Lower	exp b	Upper
Constant	0 (0.5)	0.375	1	2.665
subject: moreaui	4 (0.816)	0.807	1.386	19.819
playback song: HS	1.012 (0.769)	0.610	2.75	12.407
subject moreaui*song HS	-3.186**(1.140)	0.004	0.041	0.386

Logistic regression implemented with glm() in R v.2.13.0 (R citation). Model fit initiated with subject species = ‘fuelleborni’ and stimulus song = ‘conspecific’ as part of intercept. HS=heterospecific. Null deviance: 84.33 (61 d.f.); residual deviance: 74.47 (58 d.f.). Model X^2 (3) = 8.7, p<.05. ** *p*<.01.

Table 2: Results of a Poisson-family GLMM for duration of approach within 8m (s) for the multi-modal (mount and song) simulated territorial intrusion experiment. DIC=301.6. The parameter types are in quotes because there is no technical distinction between them in the Bayesian modeling approach used (Hadfield 2010). The effects designated as ‘Random’ are included within the G-structure variance. Effective sample sizes for fixed parameters were ≥1000.

Parameter type	Parameter mean	Lower 95% CI	Upper 95% CI
‘Random’	*Mount ID*		
	$1.2x10^{-16}$	$1.55x10^{-17}$	$3.6x10^{-16}$
	Song ID		
	.11	.00025	.53
(Intercept)			
	4.1	3.1	5.2
‘Fixed’	*Song species (hs)*		
	.068	-1.6	1.7
	Mount species (hs)		
	1.1	-.32	2.4
	*Song (hs)*mount(hs)*		
	-.57	-2.7	1.5

Table 3: Details of general linear mixed models as developed with package MCMCglmm to analyze differences in within-individual response to conspecifics versus sibling taxon song in Experiment 2. Credibility intervals estimated from sampling the posterior distribution. See text for further modeling details. Var G = G-structure variance (estimated variance of random effects). Var R = R-structure variance (estimated residual variance). Asterisks indicate significance of hypothesis that the given level of the fixed effect (population) has non-zero slope (* p<.05, ** p<.01). Population was the single fixed effect, and stimulus identity (track identity) was the random effect.

Species	DIC	Var G	Var R	Pop mean	Pop lower CI	Pop Upper CI
moreaui	365.2	208.6	7137	*Ndundulu*		
				28.7	-17.4	70.4
				Mafwemiro		
				58.2*	13.0	99.6
fuelleborni	491.4	72.85	8791	*Nyumbanitu*		
				0.8	-47.1	48.3
				Mufindi		
				77.0**	25.2	128.2
				Rungwe		
				-3.0	-52.9	45.8

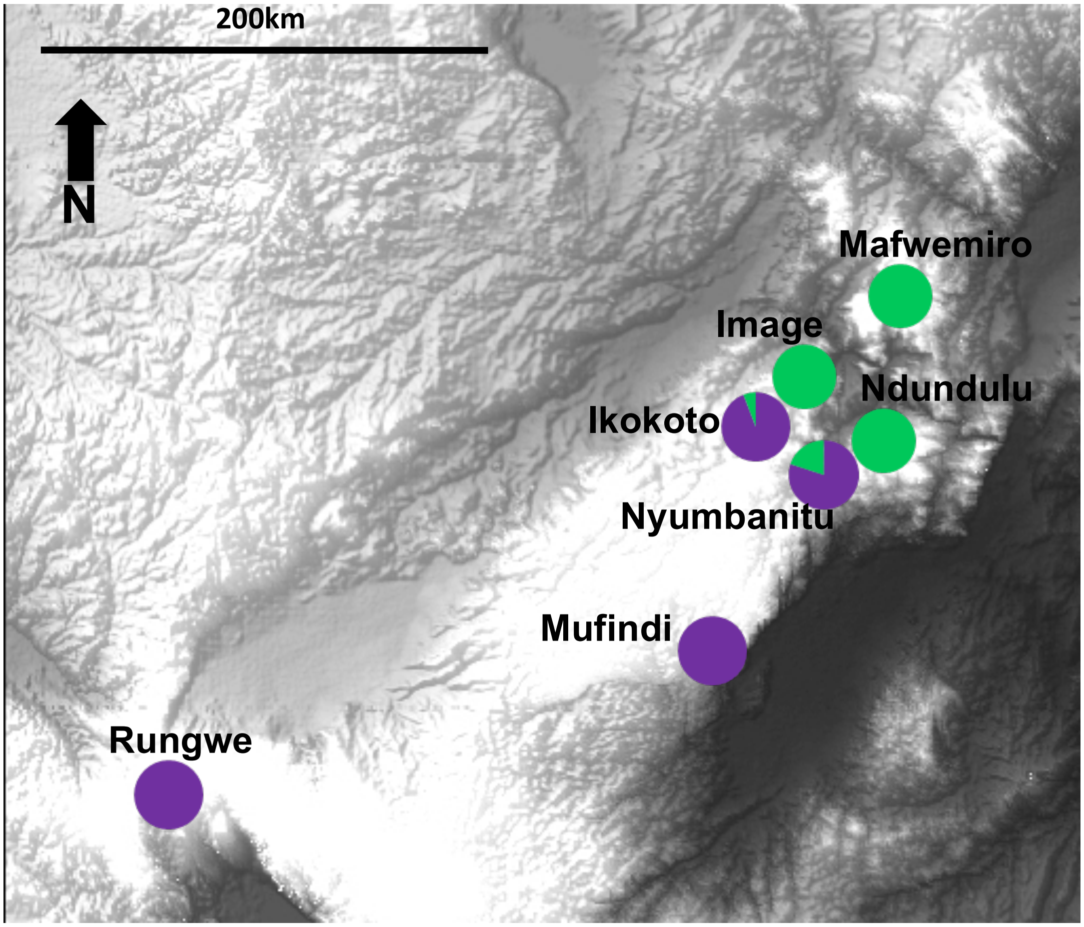


Figure 1a: Map of the study area. Colors in the pie charts indicate frequencies of species as assigned using STRUCTURE (Pritchard et al. 2000) for the named populations (see Chapter 2). Purple represents *fuelleborni* and green represents *moreaui*. Territorial intrusion experiments were conducted in 2009 (Experiment 1) at Ikokoto and Image; and 2010 (Experiment 2) in the remaining five populations. Note that there are two 'arms' of the contact zone area: the Image-Ikokoto contact area and the Ndundulu-Nyumbanitu contact area. See Chapter 2 for additional details. The species of subject individuals was confirmed by plumage or song in the contact zone areas where limited sympatry occurs.

Figure 1b: An elevational profile across the contact zone area showing that all tested populations are at similar elevation (and within generally similar abiotic conditions – see Chapter 2).

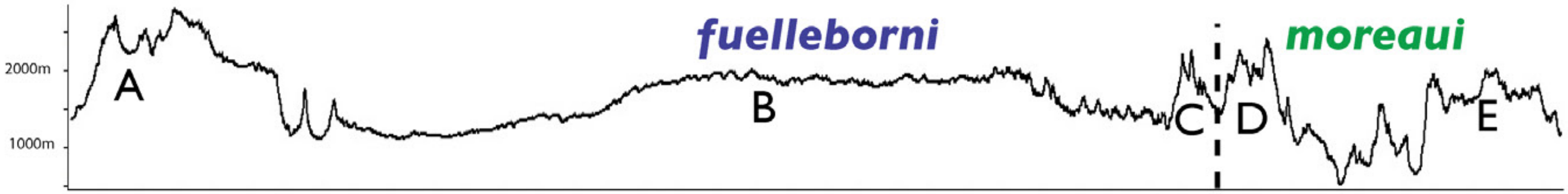
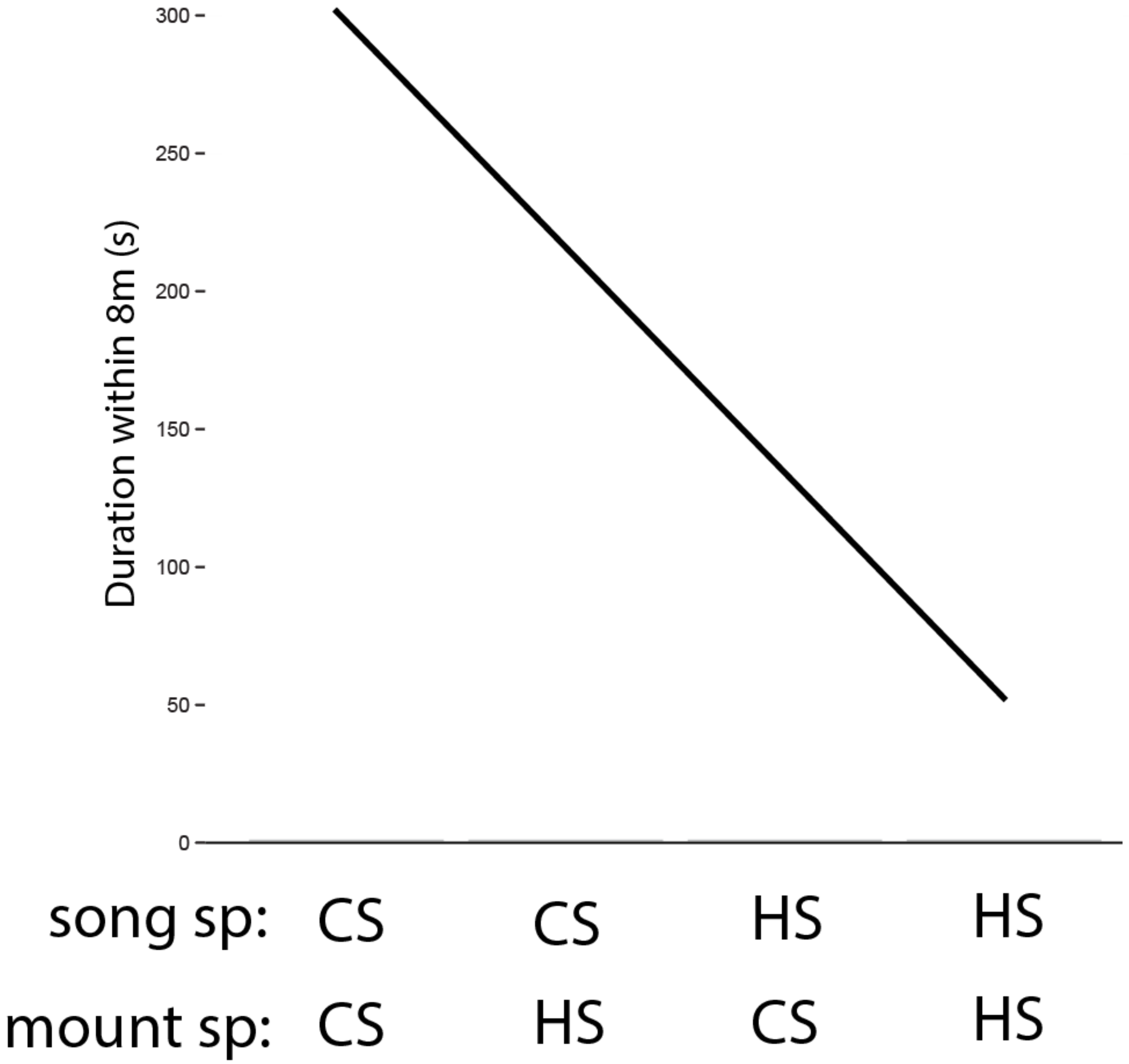


Figure 2a: Expected response pattern by treatment based on the species recognition hypothesis, for the multimodal experiment (Experiment 1). Responses for the conspecific song/heterospecific mount treatment are expected to be stronger than for the heterospecific song/conspecific mount treatment because of the relatively great differences in song and minor differences in plumage and morphology.



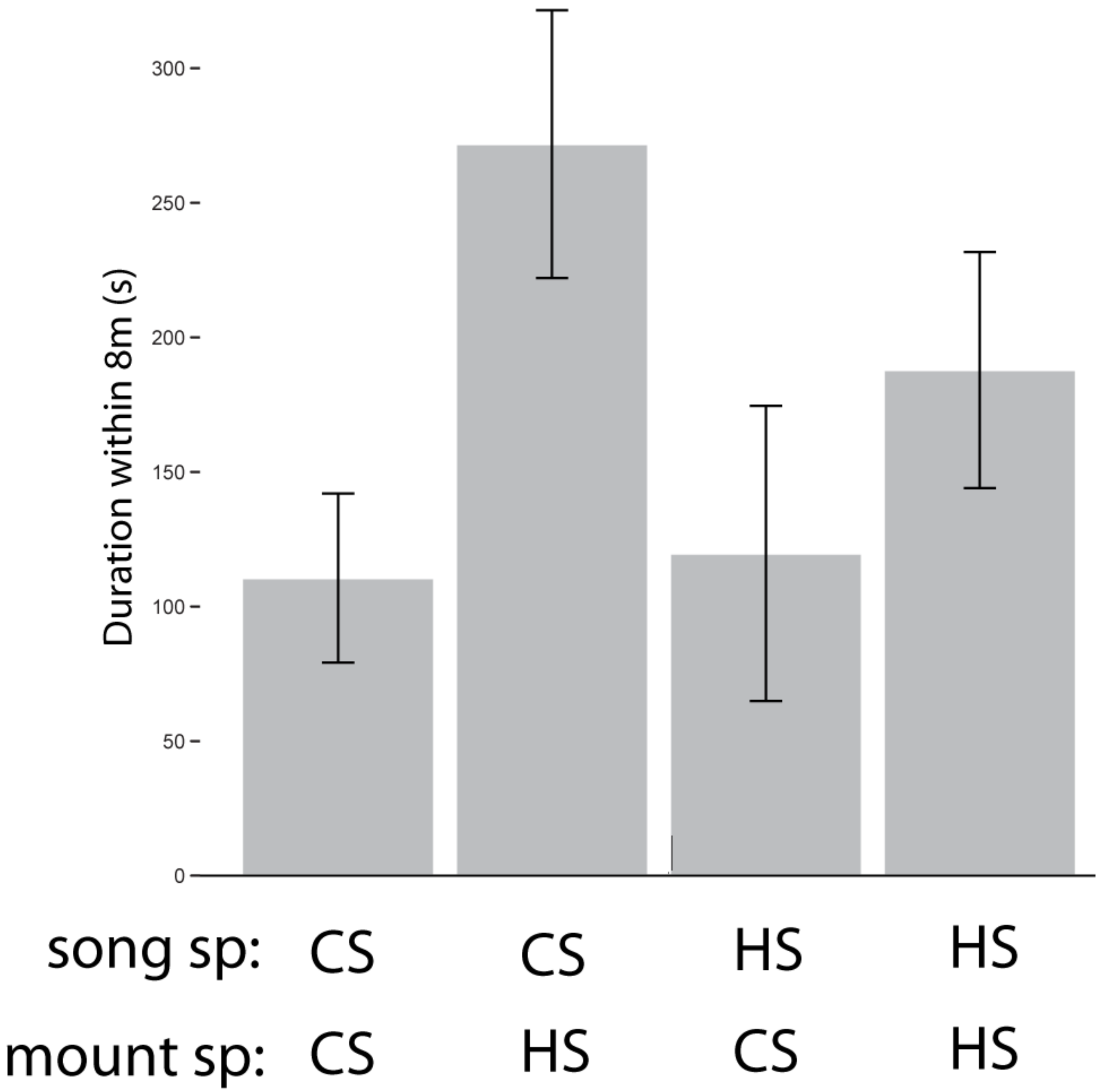
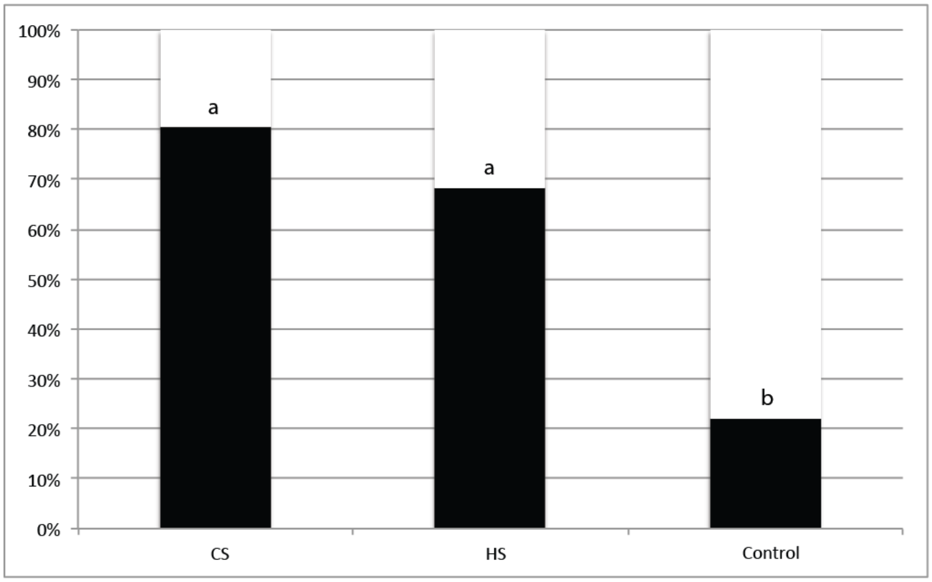


Figure 2b: Means and standard errors for approach duration within 8m, pooled for *moreaui* and *fuelleborni* subjects because of limited sample sizes. Responses were similar within each species.

fuelleborni (n=41)



moreaui (n=31)

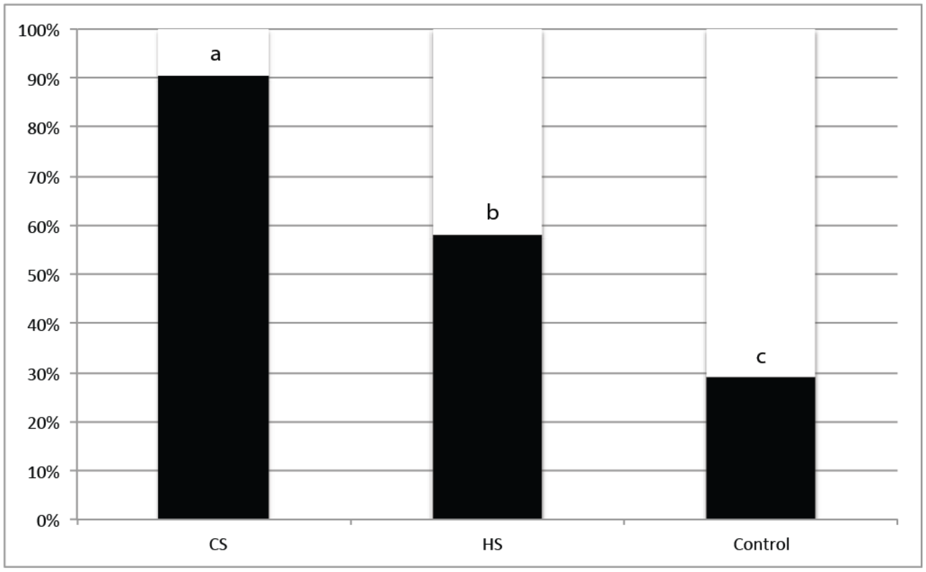


Figure 3: Percentage of trials where subjects responded by approaching within 8m to each of the three stimulus types: CS = conspecific song; HS = heterospecific (either *moreaui* or *fuelleborni*) song; and Control = *N. olivacea* song. The letters above bars indicate statistical significance: each letter represents a statistical grouping based on contrasts performed after Bayesian generalized linear mixed modeling using ‘approach’ or ‘no-approach’ as a binomial response. The experiment is a repeated-measures experiment, and the modeling framework takes into account within-individual variation and variation due to each of the 6 song tracks used (see Methods).

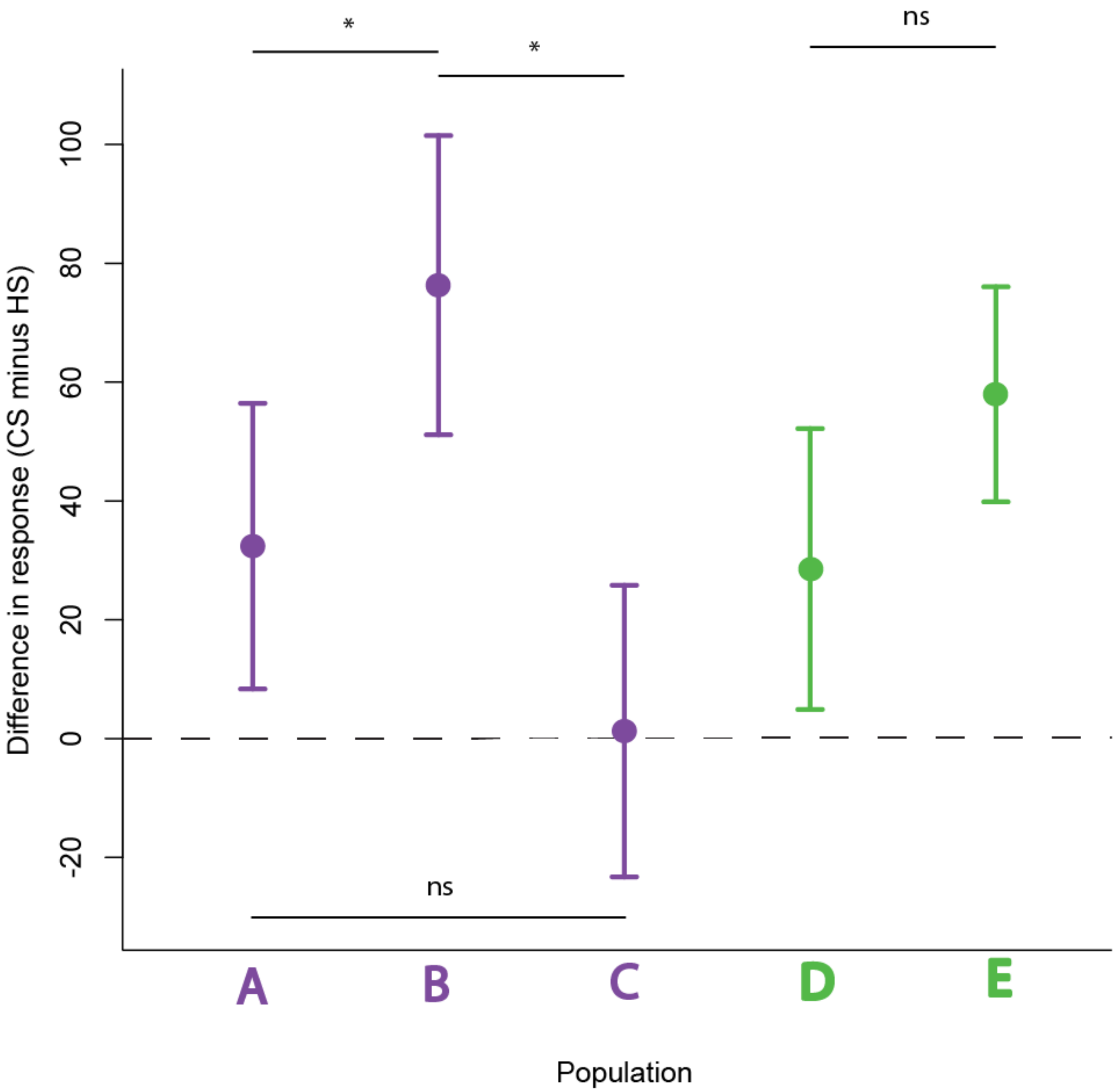


Figure 4: Population means ±SE of individual differences in responses to conspecifics versus heterospecifics (CS – HS for *fuelleborni* and *moreaui* reciprocally). *Fuelleborni* populations are in purple, and *moreaui* in green. Significance comes from contrasts between populations based on Bayesian general linear mixed models independently fit for each species. *=significance at p<.05, ns=not significant. A = Mt. Rungwe, B= Mufindi, C=Nyumbanitu, D=Ndundulu, E=Mafwemiro.

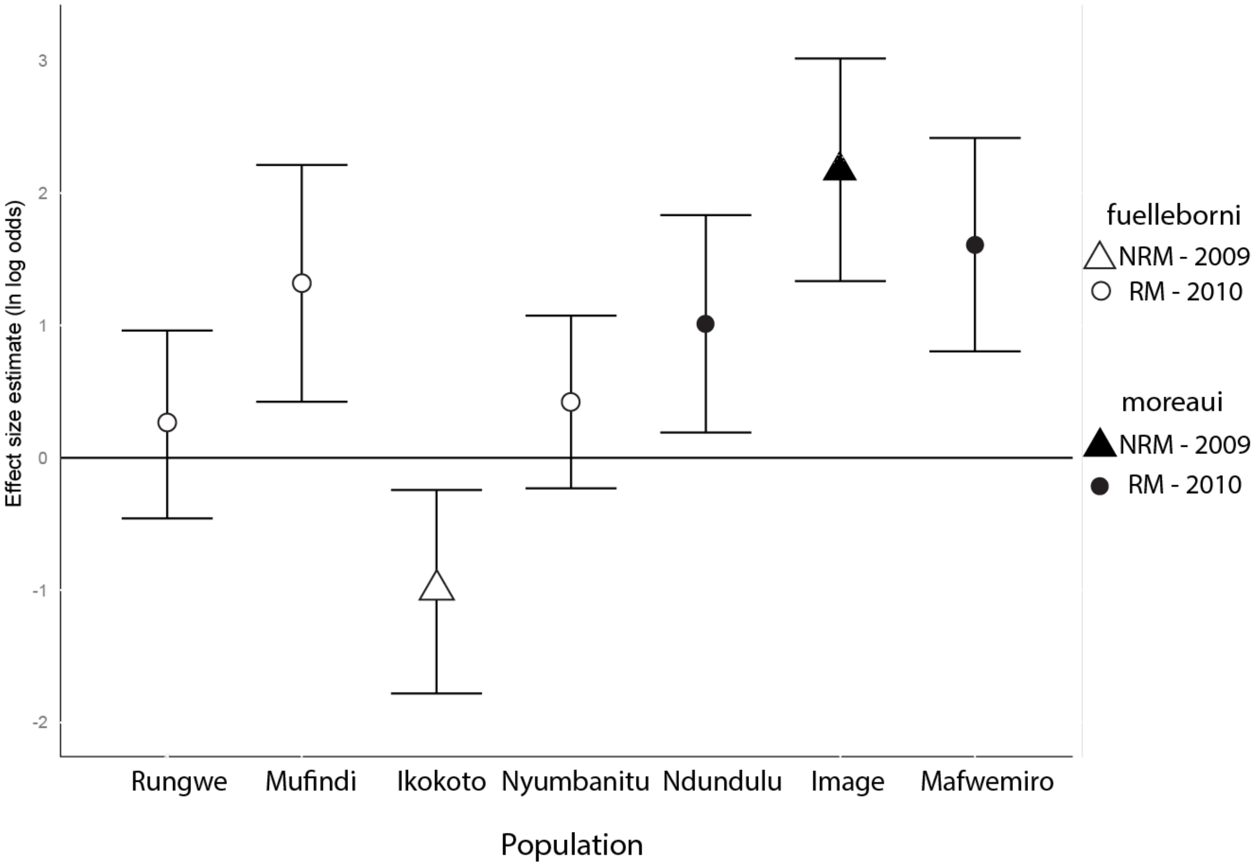


Figure 5: Effect size estimates for the natural log of log odds of approach versus no approach to heterospecific versus conspecific songs. Positive values indicate greater likelihood of responding to conspecific songs. Negative values indicate greater likelihood of responding to heterospecific songs. The estimates from the 2010 experiment have been corrected for repeated measures. The populations are aligned according to spatial orientation.

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Appendix 1: Samples with localities and museum sources for molecular analyses in Chapter 2. Sample identities with a 'JPMxxx/year' form are blood samples.

Sample/Catalog Number	Locality	SOURCE
JH148	Misuku Hills, Malawi	FMNH
JH149	Misuku Hills, Malawi	FMNH
JH150	Misuku Hills, Malawi	FMNH
JH151	Misuku Hills, Malawi	FMNH
JH152	Misuku Hills, Malawi	FMNH
JH153	Misuku Hills, Malawi	FMNH
JH154	Misuku Hills, Malawi	FMNH
JH155	Misuku Hills, Malawi	FMNH
JH157	Misuku Hills, Malawi	FMNH
RB1346	Livingstone Mountains, Tanzania	ZMUC
RB1365	Livingstone Mountains, Tanzania	ZMUC
RB1344	Mt. Rungwe, Tanzania	ZMUC
RB1348	Mt. Rungwe, Tanzania	ZMUC
RB1356	Mt. Rungwe, Tanzania	ZMUC
RB1359	Mt. Rungwe, Tanzania	ZMUC
RB1363	Mt. Rungwe, Tanzania	ZMUC
RB1423	Mt. Rungwe, Tanzania	ZMUC
JPM 060	Mt. Rungwe, Tanzania	MVZ
JPM 061	Mt. Rungwe, Tanzania	MVZ
JPM 062	Mt. Rungwe, Tanzania	MVZ
JPM 063	Mt. Rungwe, Tanzania	MVZ
JPM 064	Mt. Rungwe, Tanzania	MVZ
JPM 065	Mt. Rungwe, Tanzania	MVZ
JPM 066	Mt. Rungwe, Tanzania	MVZ
JPM 067	Mt. Rungwe, Tanzania	MVZ
JPM 068	Mt. Rungwe, Tanzania	MVZ
JPM050/2008	Mt. Rungwe, Tanzania	MVZ
JPM8	Mt. Rungwe, Tanzania	MVZ
JPM9	Mt. Rungwe, Tanzania	MVZ
JPM015	Mt. Rungwe, Tanzania	MVZ
JPM17/2008	Mufindi, Tanzania	MVZ
JPM20/2008	Mufindi, Tanzania	MVZ
JPM21/2008	Mufindi, Tanzania	MVZ
JPM22/2008	Mufindi, Tanzania	MVZ
JPM26/2008	Mufindi, Tanzania	MVZ
JPM32/2008	Mufindi, Tanzania	MVZ
JPM33/2008	Mufindi, Tanzania	MVZ
JPM34/2008	Mufindi, Tanzania	MVZ
JPM36/2008	Mufindi, Tanzania	MVZ

JPM37/2008	Mufindi, Tanzania	MVZ
JPM38/2008	Mufindi, Tanzania	MVZ
JPM39/2008	Mufindi, Tanzania	MVZ
JPM40/2008	Mufindi, Tanzania	MVZ
JPM42/2008	Mufindi, Tanzania	MVZ
JPM43/2008	Mufindi, Tanzania	MVZ
JPM44/2008	Mufindi, Tanzania	MVZ
JPM021	Ikokoto, Tanzania	MVZ
JPM022	Ikokoto, Tanzania	MVZ
JPM023	Ikokoto, Tanzania	MVZ
JPM024	Ikokoto, Tanzania	MVZ
JPM025	Ikokoto, Tanzania	MVZ
JPM026	Ikokoto, Tanzania	MVZ
JPM027	Ikokoto, Tanzania	MVZ
JPM028	Ikokoto, Tanzania	MVZ
JPM029	Ikokoto, Tanzania	MVZ
JPM030	Ikokoto, Tanzania	MVZ
JPM031	Ikokoto, Tanzania	MVZ
JPMB013/2009	Ikokoto, Tanzania	MVZ
JPMB017/2009	Ikokoto, Tanzania	MVZ
JPMB018/2009	Ikokoto, Tanzania	MVZ
JPMB019/2009	Ikokoto, Tanzania	MVZ
JPM034	Kihulula, Tanzania	MVZ
JPM035	Kihulula, Tanzania	MVZ
RCKB1549	Nyumbanitu, Tanzania	MVZ
RCKB1554	Nyumbanitu, Tanzania	MVZ
RCKB1572	Nyumbanitu, Tanzania	MVZ
RCKB1583	Nyumbanitu, Tanzania	MVZ
RCKB1587	Nyumbanitu, Tanzania	MVZ
JPM052	Nyumbanitu, Tanzania	MVZ
JPM053	Nyumbanitu, Tanzania	MVZ
JPM055	Nyumbanitu, Tanzania	MVZ
JPM056	Nyumbanitu, Tanzania	MVZ
JPM057	Nyumbanitu, Tanzania	MVZ
JPM058	Nyumbanitu, Tanzania	MVZ
JPM059	Nyumbanitu, Tanzania	MVZ
JPM003/2010	Nyumbanitu, Tanzania	MVZ
JPM054	Nyumbanitu, Tanzania	MVZ
RCKB1580	Nyumbanitu, Tanzania	MVZ
JPM037	Selebu Mountain, Tanzania	MVZ
JPM039	Selebu Mountain, Tanzania	MVZ
JPM040	Selebu Mountain, Tanzania	MVZ
JPM041	Selebu Mountain, Tanzania	MVZ

JPM042	Selebu Mountain, Tanzania	MVZ
JPM001/2008	Image, Tanzania	MVZ
JPM002	Image, Tanzania	MVZ
JPM002/2008	Image, Tanzania	MVZ
JPM003	Image, Tanzania	MVZ
JPM003/2008	Image, Tanzania	MVZ
JPM004	Image, Tanzania	MVZ
JPM006/2008	Image, Tanzania	MVZ
JPM007/2008	Image, Tanzania	MVZ
JPM009/2008	Image, Tanzania	MVZ
JPM010/2008	Image, Tanzania	MVZ
JPM011/2008	Image, Tanzania	MVZ
JPM012/2008	Image, Tanzania	MVZ
JPM013/2008	Image, Tanzania	MVZ
JPM014/2008	Image, Tanzania	MVZ
JPM016/2008	Image, Tanzania	MVZ
RB153	Uvidunda Mountains, Tanzania	ZMUC
RB606	Mang'alisa, Tanzania	ZMUC
RB609	Mafwemiro, Tanzania	ZMUC
JH108	Mafwemiro, Tanzania	ZMUC
JH109	Mafwemiro, Tanzania	ZMUC
JH110	Mafwemiro, Tanzania	ZMUC
JH112	Mafwemiro, Tanzania	ZMUC
JH113	Mafwemiro, Tanzania	ZMUC
JH114	Mafwemiro, Tanzania	ZMUC
RB1342	Wota, Tanzania	ZMUC
RB1361	Wota, Tanzania	ZMUC
RB1422	Wota, Tanzania	ZMUC
138653	Ndundulu, Tanzania	ZMUC
138660	Ndundulu, Tanzania	ZMUC
138665	Ndundulu, Tanzania	ZMUC
138666	Ndundulu, Tanzania	ZMUC
138669	Ndundulu, Tanzania	ZMUC
138713	Ndundulu, Tanzania	ZMUC
138720	Ndundulu, Tanzania	ZMUC
138721	Ndundulu, Tanzania	ZMUC
138722	Ndundulu, Tanzania	ZMUC
138725	Ndundulu, Tanzania	ZMUC
138753	Ndundulu, Tanzania	ZMUC
138788	Ndundulu, Tanzania	ZMUC
139006	Ndundulu, Tanzania	ZMUC
139068	Ndundulu, Tanzania	ZMUC
139079	Ndundulu, Tanzania	ZMUC

139080	Ndundulu, Tanzania	ZMUC
139089	Ndundulu, Tanzania	ZMUC
139161	Ndundulu, Tanzania	ZMUC
139169	Ndundulu, Tanzania	ZMUC
139179	Ndundulu, Tanzania	ZMUC
139229	Ndundulu, Tanzania	ZMUC
140436	Ndundulu, Tanzania	ZMUC