

THE ROLE OF CULTURAL DIVERGENCE IN REPRODUCTIVE
ISOLATION IN A TROPICAL BIRD,
THE RUFOUS-COLLARED SPARROW (*ZONOTRICHIA CAPENSIS*)

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Dissertation submitted to the faculty of Virginia Polytechnic Institute and State
University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

In

Biological Sciences

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7 May 2012

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Keywords: tropics, reproductive isolation, female choice, song dialects, population
divergence, *Zonotrichia capensis*

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ABSTRACT

In birds, song can evolve quickly through cultural transmission and due to errors in the learning process may result in regional dialects. A lack of dialectal recognition may be a critical component of reproductive isolation through female mate preference. I investigated the role of cultural divergence in reproductive isolation in a widespread Neotropical passerine the rufous-collared sparrow (*Zonotrichia capensis*). In Chapter II, I investigated, the role of female preference for and male territorial response towards, local and non-local dialects in two allopatric populations. Females in both populations preferred their local dialect to the dialect of an allopatric population only 25 km away. In contrast, males showed similar territorial response to all conspecific dialects. Premating reproductive isolation based on culture may exist among the study populations. In chapter III, I investigated if cultural divergence can drive population divergence by examining dialects and variable microsatellite loci among eight populations of *Z. capensis*. I investigated the presence of population divergence and then identified the mechanism that may be driving the pattern. Apart from culture, a geographic barrier (the Andean ridge), elevation, and geographic distance were potential

mechanisms of population divergence that I considered. All sites exhibited localized dialects, except for two closest neighboring populations. Populations exhibited genetic differentiation with support for the presence of five genetic clusters. Culture does not appear to be driving population divergence because song dialects and genetic population structure were not correlated. Populations separated by an Andean ridge did not display higher genetic or song differences than distance would predict, suggesting that the ridge is not driving population divergence. Elevation was not correlated to song or genetic differentiation. Both song divergence and microsatellite allele frequency differentiation were correlated with geographic distance suggesting a pattern of isolation by distance. Overall, geographic distance is the best predictor of population divergence in this system. Cumulatively, I found that culture might promote assortative mating via female mate choice, perhaps generating partial reproductive isolation; however, song dialect differences among contiguous populations is not currently driving population divergence in this species.

DEDICATION

To all the little non-descript brown birds out there. You are much more beautiful than most people know.

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CHAPTER I. INTRODUCTION

Tropical biodiversity

The spectacular increase of species richness from polar regions to equatorial regions is evident across most groups of organisms (Hillebrand 2004). For example, in North America there are 620 species of trees (Currie and Paquin 1987), whereas, in the New World Tropics there are an estimated 22,500 tree species (Fine and Ree 2006). Similarly, $\frac{3}{4}$ of the world's bird species are found in tropical latitudes (Stutchbury and Morton 2001). Despite this striking pattern, the ultimate cause of the latitudinal biodiversity gradient is largely unknown (Schemske 2009).

A current hypothesis to explain the observed high species richness in the tropics is a higher rate of speciation at lower latitudes (Martin and McKay 2004; Mittelbach et al. 2007). Population divergence is the first step in speciation. Many tropical species exhibit among-population divergence over short geographic distances (Chek et al. 2003; Martin and McKay 2004); for example, variation in morphological and phenotypic traits over short distances can be observed in tropical populations of frogs (Ryan et al. 2003; Funk et al. 2009; Boul et al. 2007) and birds (Nyari 2007; Seddon and Tobias 2007). The observed divergence over short geographic distances suggests a great potential for the evolution of reproductive isolation and subsequent speciation in the tropics.

Mechanisms driving or maintaining diversity

In the classic allopatric speciation model, reproductive isolation is typically attributed to the presence of geographic barriers and increasing physical distance between populations (Mayr 1947; Coyne and Orr 2004); populations diverge genetically because of the lack of gene flow

across these physical barriers, genetic drift and possibly selection caused by differing environments. Geographic barriers, such as mountains and rivers, might facilitate speciation in the tropics by impeding gene flow among allopatric populations (Brumfield and Capparella 1996; Bates et al. 1999). However, a geographic barrier is not a prerequisite for reproductive isolation. Ecological isolation, when species occupy different habitats or breed at different times of the year, may play a large role in the divergence of populations (Rundle and Nosil 2005). Further, divergent mating signals may lead to a lack of cross attraction among individuals resulting in behavioral isolation thus facilitating speciation (Price 2008).

Sexual selection can reinforce and potentially mediate population divergence through mate choice (Boul et al. 2006; Andersson 1994; Price 2008; Coyne and Orr 2004). Among isolated populations, variation in sexually selected traits, such as mating signals, may increase and then act as a barrier to breeding (Price 1998), which might be a common phenomenon in the tropics. Upon secondary contact if females do not recognize the non-local signal, they may not mate, thus eliminating gene flow among populations. However, for sexual selection via female choice to take place, the signaler and the receiver must co-evolve assuring that the receiver recognizes the signal. Examining divergent sexually selected mating signals among tropical populations may aid in understanding the process and spatial scale of reproductive isolation.

Bird song and assortative mating

Bird song may provide a useful tool for estimating premating reproductive isolation among populations. Male birds sing to deter potential male intruders from invading the space that they are occupying, and to attract females and stimulate them (Nowicki and Searcy 2005). Females typically do not sing, and therefore, use male songs to locate conspecific males and to

assess their quality for mating (Nowicki and Searcy 2005). A male's song may provide females with information about his health, social status, and place of birth (Searcy and Andersson 1986). Since females use song to discriminate among males and to choose a mate, female mate preference for certain songs may drive sexual selection (Price 2008).

Among oscine birds, song is learned (Marler and Tamura 1964). Young males learn their song from their fathers and neighboring males (Grant and Grant 1996; Bell et al. 1998). Errors in reproducing songs may create regional differences (Slater 1989), and when bird populations become geographically isolated, their respective songs may diverge into substantially different dialects (Nelson et al. 2001; Nottebohm 1969). When birds of different populations, with distinct dialects, come into contact with each other, the implications may be substantial. Song, which is often the first point of contact, may serve as the primary isolating mechanism between birds (Martens 1996). In the extreme case, individuals from opposing populations do not mate with one another because they do not recognize each other as conspecifics, consequently inhibiting gene flow. Therefore, song dialects may represent a behavioral pre-zygotic mechanism whereby a culturally transmitted trait may affect the genetic structure of populations via assortative mating (Baker and Cunningham 1985; Rothstein and Fleischer 1987). However, the degree to which geographic variation in bird song acts as a reproductive isolating mechanism, driving speciation, is a long-standing controversy in evolutionary biology (Nottebohm 1969; Baker and Cunningham 1985; Rothstein and Fleischer 1987; Chilton and Lein 1996; Slabbekoorn and Smith 2002; Soha et al. 2004; Grant and Grant 2002; MacDougall-Shackleton and MacDougall-Shackleton 2001; Podos 2007).

Research questions and study system

This dissertation explores the role of culture as a behavioral isolating mechanism among populations of a widespread emberizid, the rufous-collared sparrow (*Zonotrichia capensis*). *Zonotrichia capensis* is a Neotropical passerine that is distributed from Chiapas, southern Mexico to Tierra del Fuego, Argentina and ranges from sea level up to 4000m in some areas and occupies a variety of typically open habitats. Starting in the late 1960's, *Z. capensis* were recognized to have distinct dialects between populations in South America, most notably in northwestern Argentina, and since then have been the focus of numerous song studies (Nottebohm 1969, 1975; King 1972, Handford and Nottebohm 1976; Loughheed and Handford 1992; Loughheed et al. 1989). Songs of *Z. capensis* are comprised of two parts: the theme which includes 1-5 short notes and one long note descending in frequency, followed by a trill which is composed of several fast repetitive notes. The song in *Z. capensis* is stereotypic thus presenting a good opportunity to examine the role of cultural evolution. The basis of my dissertation has been to examine geographic variation in the song of *Z. capensis* and how it relates to female mate choice and population genetic divergence.

Chapter summaries

Chapter II: Danner, J. E., R. M. Danner, F. Bonier, P. R. Martin, T. W. Small, and I. T. Moore. 2011. Female, but not male, tropical sparrows respond more strongly to the local song dialect: Implications for population divergence. *The American Naturalist* 178: 53–63. (<http://www.jstor.org/action/showPublication?journalCode=amernatu>)

I investigated the role of song in reproductive isolation between two allopatric populations of *Z. capensis*. With playback experiments, I examined female preference for, and male territorial response to, local and non-local dialects in the Andes of Ecuador. I found that, in

both populations, females strongly prefer the local dialect over a dialect from only 25km away, yet separated by unsuitable higher elevation habitat (Andean pass at 4200m). Conversely, males responded similarly aggressively to all conspecific dialects regardless of origin. Based on the observed female mate choice, the opportunity for assortative mating and potentially reproductive isolation exists between these populations. Further, these results suggest that female response to songs, not male territorial response, may be important for estimating reproductive isolation among populations.

Chapter III: Cultural evolution and genetic structure in a tropical bird, the rufous-collared sparrow (Zonotrichia capensis)

In *Z. capensis*, I investigated the role of culture in driving population genetic divergence. Specifically, I examined dialects and genetic population structure among eight populations on a transect bisected by the Andean ridge. I investigated the presence of population divergence and then identified the mechanism that may be driving the observed pattern. Besides culture, alternative mechanisms of genetic divergence were a geographic barrier (an Andean ridge), elevation, and geographic distance. All populations exhibited high site-specific songs, except for two neighboring populations that were the closest geographically. Analysis of variable microsatellites indicated genetic differentiation between populations and there was high support for the presence of five population genetic clusters. There was no relationship of either the Andean ridge or elevation with song or genetic differences. Both song divergence and microsatellite allele frequency differentiation were correlated with geographic distance, suggesting a pattern of isolation by distance. There was no relationship between song dialects and genetic population structure, indicating that culture is not driving population divergence. In

this system, geographic distance, not the Andean barrier, elevation, or song differentiation, is the best predictor of population divergence.

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Female, but Not Male, Tropical Sparrows Respond More Strongly to the Local Song Dialect: Implications for Population Divergence

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Submitted August 11, 2010; Accepted March 15, 2011; Electronically published May 25, 2011

Dryad data: <http://dx.doi.org/10.5061/dryad.8837>.

ABSTRACT: In addition to the observed high diversity of species in the tropics, divergence among populations of the same species exists over short geographic distances in both phenotypic traits and neutral genetic markers. Divergence among populations suggests great potential for the evolution of reproductive isolation and eventual speciation. In birds, song can evolve quickly through cultural transmission and result in regional dialects, which can be a critical component of reproductive isolation through variation in female preference. We examined female and male behavioral responses to local and nonlocal dialects in two allopatric populations of rufous-collared sparrows (*Zonotrichia capensis*) in the Andes Mountains of Ecuador. Here we show that female sparrows prefer their natal song dialect to the dialect of an allopatric population that is just 25 km away and separated by an unsuitable higher-elevation habitat (pass of 4,200 m), thus providing evidence of prezygotic reproductive isolation among populations. Males showed similar territorial responses to all conspecific dialects with no consistent difference with respect to distance, making male territoriality uninformative for estimating reproductive isolation. This study provides novel evidence for culturally based prezygotic isolation over very short distances in a tropical bird.

Keywords: reproductive isolation, tropics, female choice, song dialects, *Zonotrichia capensis*.

Introduction

Many tropical species exhibit among-population divergence over short geographic distances (Fouquet et al. 2007; Kosciński et al. 2008). Phenotypic traits, including coloration and vocalizations, vary over short distances in tropical frogs (Boul et al. 2006) and birds (Nottebohm 1969; Nyari 2007; Podos 2007; Seddon and Tobias 2007; Tobias and Seddon 2009), and bird subspecies, defined by

morphology, occur at higher densities at lower latitudes (Martin and McKay 2004; Martin and Tewksbury 2008). Similarly, greater neutral genetic divergence is found among bird (Hackett and Rosenberg 1990; Chek et al. 2003) and plant (Eo et al. 2008) populations in the tropics, especially when separated by topographic barriers, such as large rivers and mountains (Brumfield and Capparella 1996; Bates et al. 1999; Aleixo 2004).

Given the importance of population divergence for allopatric speciation, the morphological and genetic differences observed among tropical populations over short distances suggest a great potential for the evolution of reproductive isolation and eventual speciation. When populations are separated, variation among sexually selected traits, such as male mating signals, may increase and then act as a premating barrier to interbreeding (Coyne and Orr 2004). For instance, divergent sexually selected vocal signals in tropical frogs (Ryan et al. 2003; Hoskin et al. 2005; Boul et al. 2007) have been shown to lead to behavioral isolation among populations. However, direct measures of components of reproductive isolation among populations, such as measures of female preference for local versus foreign mates or hybrid fitness, are rare (Mittelbach et al. 2007; Schemske 2009; but see Boul et al. 2007; Kay and Schemske 2008). Estimating reproductive isolation is critical for understanding the process and spatial scale of speciation among populations.

Song plays an important role in female mate choice and reproductive isolation in birds (Nowicki and Searcy 2002). In oscines, song is learned (Marler and Tamura 1964) and dialects can vary among populations (Nottebohm 1969), arising through both genetic and cultural evolution (Lynch 1996; Podos and Warren 2007). These dialects can have important implications for reproductive isolation among populations (Irwin et al. 2001). A lack of female recog-

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nition of or preference for dialects from other populations may serve as a primary prezygotic isolating mechanism among bird populations (Martens 1996; Price and Bouvier 2002; Price 2008). Thus, female response to song could be an informative measure of reproductive isolation among allopatric populations. However, estimates of reproductive isolation among allopatric populations of birds typically focus on phenotypic or neutral genetic differences (Mayr and Ashlock 1991; Helbig et al. 2002; Price 2008) or on territorial response to songs from other populations (Ratcliffe and Grant 1985; Grant and Grant 2002; Irwin et al. 2005; Seddon and Tobias 2007; Uy et al. 2009). Studies of tropical species rarely consider female mate choice, despite its significance in prezygotic isolation and speciation of birds (Price and Bouvier 2002; Price 2008).

We examined both female preference for and male aggressive response toward male song over differing geographic distances in a nonmigratory tropical bird, the rufous-collared sparrow (*Zonotrichia capensis*). Despite the large geographic range of *Z. capensis*, variation in song dialects can be observed over short geographic distances (20 km; Loughheed et al. 1989) and in the presence or absence of geographic barriers (Nottebohm 1969, 1975; Handford and Nottebohm 1976; Moore et al. 2005). We specifically asked the following two questions: How does female preference for male song dialect vary with geographic distance between populations? And given that male response is often used to estimate biological species limits in birds, how do patterns of female preference for male song compare with male territorial response to the same songs? We predicted that females would prefer the local dialect. Furthermore, we predicted that females would display a higher level of discrimination among dialects than males because of asymmetric fitness costs and benefits of responding to distant song dialects (Searcy and Brenowitz 1988; Andersson 1994; Baker and Boylan 1999).

Material and Methods

Zonotrichia capensis is a widespread Neotropical emberizid sparrow that breeds from southern Mexico to Tierra del Fuego and occupies a wide range of elevation (from sea level to 4,000 m). We conducted song-playback experiments on females and males from each of two allopatric equatorial populations of *Z. capensis* in the Andes Mountains in Ecuador (Papallacta: 0°21'S, 78°9'W, elevation of 3,300 m; Pintag: 0°22'S, 78°22'W, elevation of 2,900 m). The two populations are roughly 25 km apart, yet they are separated by unsuitable higher-elevation habitat (an Andean pass with an elevation of 4,200 m) and exhibit limited gene flow and significant genetic differentiation at microsatellite loci (Moore et al. 2005). Furthermore, the populations experience different seasonal weather patterns

and asynchronous reproductive phenologies (Moore et al. 2005). Last, the males in the two study populations sing different song dialects (Moore et al. 2005; fig. 1).

We broadcast songs from conspecific populations that occur at different distances from each focal population, as well as a song from a distantly related control species, and recorded the behavioral responses of both females and males. Female preference was estimated by the number of copulation solicitations given in response to songs. Male response was measured by behaviors that characterize territoriality, including their approach toward the broadcast speaker and vocalizations.

Female Trials

We used mist nets to capture 13 female and 4 male *Z. capensis* from each of the two Ecuadorian populations before the start of their respective breeding season (Papallacta: late July–August 2007; Pintag: May–June 2008). The experiments were conducted at different times of the year to correspond with the respective breeding seasons at each site. We implanted females with two subcutaneous silastic implants filled with 17 β -estradiol (length = 12 mm, inner diameter = 1.47 mm, outer diameter = 1.96 mm; Dow Corning; O'Loughlen and Beecher 1997) to heighten the females' reproductive receptiveness and to control for individual hormonal variation (Moore 1982). It is worth noting that smaller doses of 17 β -estradiol might have sufficed, but this dose was chosen on the basis of previous studies. We allowed each bird 7 days to recover from surgery and to allow hormone levels to stabilize before performing the song-preference trials. To avoid interference by local male birds during the experiments, we moved the Papallacta birds to Quito (0°8'S, 78°27'W, elevation of 2,800 m; 65 km west of Papallacta) and the Pintag birds to Nanegalito (0°06'S, 78°42'W, elevation of 1,553 m; 75 km northwest of Pintag). At these neutral sites, the local birds were not breeding and thus did not interfere with the behavioral trials. While the trials were not taking place, females were housed in individual cages (33 cm \times 50 cm \times 50 cm) indoors and provided natural light. The females were not visually isolated from each other. Males were kept in individual cages in an outdoor aviary. All birds had free access to food (seed and egg mixture) and water.

All song trials were completed outdoors between 0700 and 1000 hours and between 1500 and 1800 hours, to avoid the hottest parts of the day and to mimic the birds' natural activity patterns. Each female bird had two trials a day, one in the morning and one in the late afternoon (weather permitting), for two consecutive days (four trials in total per bird, one of each song type). We presented each female with one of four replicate recordings of dif-

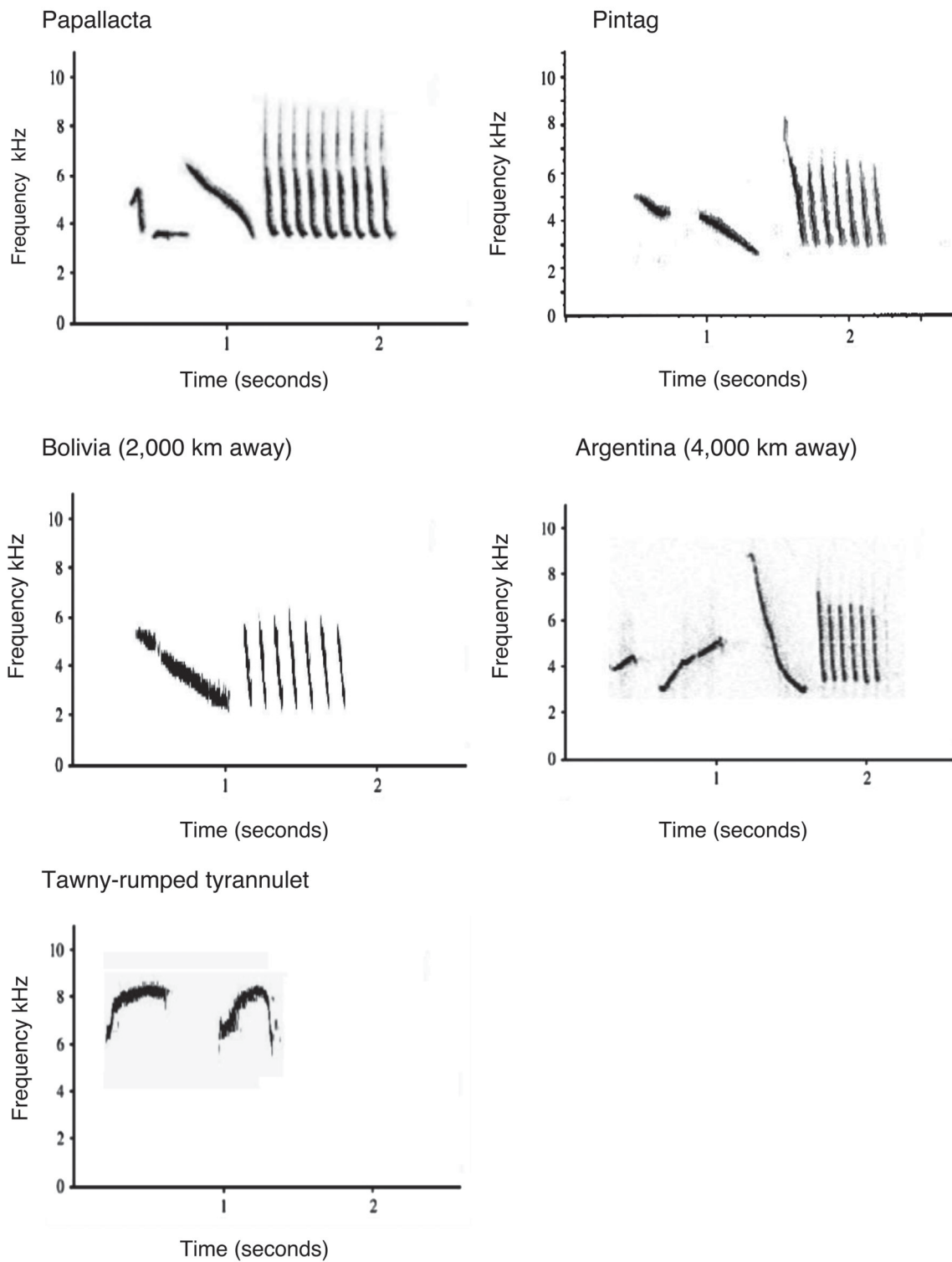


Figure 1: Example sonograms of *Zonotrichia capensis* song dialect treatments used in male (all songs) and female (all songs except Bolivia) playback experiments. Papallacta and Pintag sonograms are from Moore et al. (2005).

ferent males singing one of each of the following song types in random order: (1) local dialect, (2) nearby non-local dialect (25 km away), (3) distant nonlocal dialect (4,000 km away; Buenos Aires, Argentina), and (4) control song from another local bird species, the tawny-rumped tyrannulet (Tyrannidae: *Phyllomyias uropygialis*; fig. 1). We used songs from the distantly related tyrannulet because its range overlaps with *Z. capensis*, but it is ecologically distinct (and therefore not a competitor) and does not resemble *Z. capensis*. To diminish the pseudoreplication that can occur in playback studies and to account for within-dialect variation, for each of the four song treatments we used songs from four different individuals (Kroodsma 1989). To reduce background noise, all songs were band-pass filtered between 2 and 10 kHz using RAVEN software (Charif et al. 2004), which did not remove any part of the song, and were then standardized to an amplitude of 0.65–0.70 relative to the dynamic range of the sound file. Additionally, all songs were broadcast at an amplitude of 80 dB measured 1 m from the speaker using a sound meter (Radio Shack model 33-4050).

For each song trial we placed the focal caged female 10 cm from a caged nonbreeding adult male decoy with the playback speaker on the opposite side of the male's cage. We randomly assigned one of four adult males to serve as the decoy in each trial. Each female was exposed to a different male decoy for each trial. The male decoys were captured from the same population as the females and were in nonbreeding condition (based on a lack of an enlarged cloacal protuberance). Male plumage does not differ between breeding and nonbreeding seasons. The caged male decoys did not sing or show obvious courtship behavior during any of the trials. The male decoys were used in randomized order to isolate the female's response to song cues from their response to any visual cues.

Each trial consisted of a 10-min acclimation period (no stimulus: male decoy covered, no song), followed by a 3-min trial during which we uncovered the male decoy and broadcast one of the four prerecorded songs at a rate of 10 songs per minute, which represented high yet natural song rates on our study plots (Moore et al. 2005). Before bringing the female to the testing arena, we covered the male decoy's cage with a cloth that had a 15-m-long string tied to the end. At the start of each 3-min trial, the observer slowly pulled the string to uncover the male decoy and simultaneously began broadcasting the song treatment. Trials were observed from indoors 15 m away, through a window and from behind a blind. All trials were videotaped (Sony Handycam model DCR-HC48) from 2 m away. We familiarized the females with the video camera by placing it in the indoor aviary 1 week before the trials started and left it in the room while trials were not taking place. During the trials we videotaped the female's be-

havioral responses and quantified female responsiveness to male song using a copulation solicitation display assay (e.g., Searcy 1992; Searcy et al. 2002; Derryberry 2007). We counted only full solicitation displays, defined as postures consisting of an elevated tail, arched back, and raised head in addition to wing flutter and a solicitation call. We considered only full solicitations in our analysis because in the wild only full copulation solicitation displays precede copulation (J. E. Danner, personal observation). All trials were scored by watching the videos. One reviewer (J. E. Danner) scored all of the female behavioral trials to avoid individual variation. To avoid scoring bias, the volume of the video player was muted, and the identification of the bird and the treatment was not revealed until after the trial.

At the end of all song trials, we removed all hormone implants and collected blood for hormone analysis to confirm the efficacy of the estrogen implants. Blood samples were returned to Virginia Tech, where we performed radioimmunoassays (Moore et al. 2002) to determine plasma 17β -estradiol levels.

We used preliminary generalized linear models in R software (R Development Core Team 2010) to determine whether birds became habituated with respect to the trial order, whether they responded more strongly to a certain playback tape, and whether females had a stronger response to certain male decoys. Using the same methods, we tested for effects of song type on the number of copulation solicitation displays. To account for differences in individual female behavior, we also included female identification as a predictor. Because no females from *Papallacta* displayed copulation solicitation displays toward the control song, we removed the control song from the *Papallacta* data set when constructing generalized linear models. Data were fitted with Poisson regressions (function `glm`; R Development Core Team 2010) and tested for overdispersion by examining ϕ (residual deviance/residual degrees of freedom). Data were overdispersed with respect to models from both populations that included trial order, playback tape, or male decoy and to the model from *Papallacta* with the additive effects of song and female (all $\phi > 1.5$). Therefore, we refitted the data for those tests with negative binomial models (function `glm.nb`; Venables and Ripley 2002). We then tested the data for zero inflation by comparison to zero-inflated models (function `zeroinfl`; Zeileis et al. 2008) with Vuong tests (function `vuong`; Vuong 1989; Jackman 2010). None of the models were zero inflated (all Vuong test statistics were between -0.89 and 0.29 ; all $P > .05$). Effects of variables were tested by comparison to null models with likelihood ratio tests (function `lrtest`; Zeileis and Hothorn 2002). When likelihood ratio tests revealed that variables were significant, we compared the effects of variable levels (e.g., song types) on the num-

ber of copulation solicitation displays with Tukey's multiple-comparison tests (function `glht`; Hothorn et al. 2008). To account for potential behavioral differences between populations, we performed separate analyses for each population.

Male Trials

We conducted song-playback experiments on territories of 15 breeding males in Papallacta (October 2008) and 16 breeding males in Pintag (February 2009). The experiments were held at different times of the year to correspond with the respective breeding seasons at each site. The day before the start of the trial, J. E. Danner observed and identified territorial males. J. E. Danner marked the center of each male's territory and placed flagging at 5-m intervals on either side to aid with distance estimation. All playback experiments were completed between 0700 and 0930 hours. We broadcast the same song types as were used in the female experiments, plus an additional song type from Bolivia (2,000 km away; fig. 1) to achieve a higher level of discrimination among the treatments, in a randomized order to the territorial male. For each trial, we placed a speaker in the center of the territory and played each song stimulus at a rate of 10 songs per minute for 2 min followed by 2 min of poststimulus silence before presenting the next song until all five stimuli had been presented. To reduce any carryover effects, we waited until the male was moving away and was at least 8 m from the playback speaker before starting the next song treatment (the average amount of time between poststimulus silence and next song treatment was 2 min). For each 2-min period of playback and 2 min of poststimulus silence, we quantified (1) the minimum distance from speaker (m) and (2) the number of songs produced by the focal male. The observer (J. E. Danner) recorded the behaviors from at least 15 m away. For each population, we combined the above-described behavioral data into a single variable using principal component analysis (JMP software, ver. 7.0; SAS Institute). Principal component 1 loadings were -0.71 for closest approach and 0.71 for number of songs. The first principal component explained 57.39% of the variance in the Papallacta male data and 68.65% of the variance in the Pintag male data. We then performed mixed-model analysis of the first component (PROC MIXED; SAS software, ver. 9.1; SAS Institute) with random effects (trial order and male bird) and fixed effects (song treatment) to determine whether male response differed among songs. We used post hoc least square means tests for pairwise comparisons of behavioral responses to each song type.

The females and the males were exposed to different experimental protocols and therefore were not directly or

statistically compared. The experimental tests performed on females cannot be performed on males because there is no behavioral measure that we could use for male mate preference in a caged experiment. Similarly, we could not perform a female mate-choice trial in the field because of interfering variables, including variable female reproductive condition and territorial males that would respond to the playback speaker, which would likely affect the results (e.g., Nelson and Soha 2004). Last, the number of song treatments used for the male and female experiments were different because females may become habituated in a caged experiment and may not respond as strongly to many treatments.

Results

In both populations, song treatment had a significant effect on the number of copulation solicitations given by females (likelihood ratio tests; Papallacta: $df = 2$, $\chi^2 = 11.29$, $P = .003$; Pintag: $df = 3$, $\chi^2 = 69.29$, $P < .0001$). Female *Zonotrichia capensis* from both Papallacta and Pintag preferred their local song dialect, giving significantly more copulation solicitation displays in response to their local dialect than to any other treatments (Tukey's post hoc tests; Papallacta: $z = -4.44$, $P < .0001$ for local vs. Pintag, $z = -4.39$, $P < .0001$ for local vs. Argentina; Pintag: $z = -4.87$, $P < .001$ for local vs. Papallacta, $z = -4.35$, $P < .001$ for local vs. Argentina, $z = -4.91$, $P < .001$ for local vs. control; fig. 2). The responses of Papallacta and Pintag females to both of the nonlocal dialects did not differ from each other (Papallacta: $z = 0.30$, $P = .95$ for Pintag vs. Argentina; Pintag: $z = 0.96$, $P = .760$ for Papallacta vs. Argentina). In Pintag, females responded more strongly to the Argentina song than to the control song of a distantly related species (*Phyllomyias uropygialis*; $z = -2.66$, $P = .035$) but similarly to the Papallacta and control songs ($z = -1.99$, $P = .175$). Female behavioral response in both populations was not significantly affected by treatment order (likelihood ratio tests; Papallacta: $df = 1$, $\chi^2 = 0.88$, $P = .347$; Pintag: $df = 1$, $\chi^2 = 2.07$, $P = .150$), playback tape (likelihood ratio tests; Papallacta: $df = 9$, $\chi^2 = 14.21$, $P = .115$; Pintag: $df = 12$, $\chi^2 = 17.73$, $P = .124$), or the presence of a particular male decoy (likelihood ratio tests; Papallacta: $df = 3$, $\chi^2 = 3.88$, $P = .275$; Pintag: $df = 3$, $\chi^2 = 0.45$, $P = .931$). The average (\pm SD) 17β -estradiol content was 3.46 ± 1.84 ng/mL in the females from Papallacta and 1.57 ± 0.79 ng/mL in the females from Pintag.

In both populations, the song treatments had a significant effect on the male's territorial response (mixed-model analysis; Papallacta: $F_{4,69} = 4.59$, $P = .0024$, $n = 15$; Pintag: $F_{4,74} = 5.57$, $P = .0006$, $n = 16$). Male *Z. capensis* from Papallacta showed a similar territorial re-

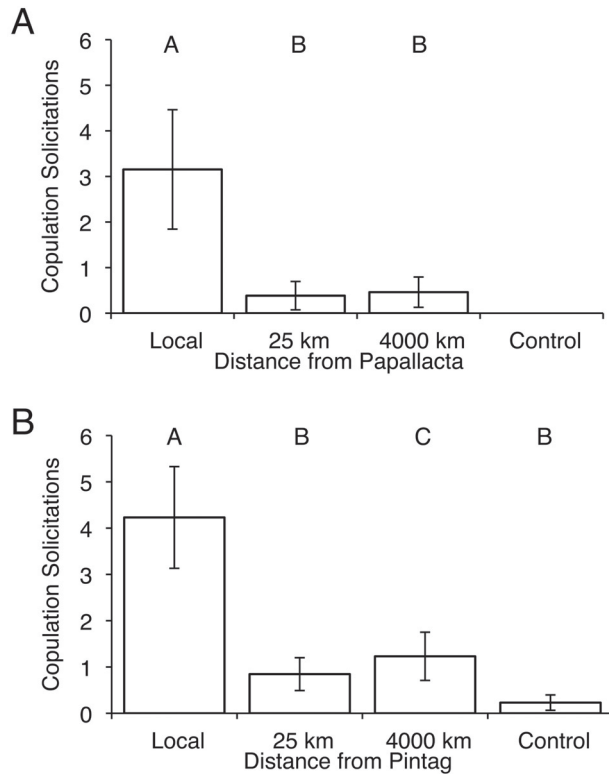


Figure 2: Number of female copulation solicitations in response to each song treatment from each population (mean \pm SE). *A*, Papallacta population. *B*, Pintag population. In both populations, female *Zonotrichia capensis* displayed significantly more copulation solicitations in response to their local dialect. Different letters indicate significantly different responses between song treatments ($P < .05$; $n = 13$ for each population).

sponse to all conspecific song treatments, with no difference in behavior between the local dialect and dialects from populations 25 km away ($t = 0.97$, $df = 69$, $P = .335$), 2,000 km away ($t = -1.14$, $df = 69$, $P = .259$), and 4,000 km away ($t = -1.94$, $df = 69$, $P = .06$; fig. 3*A*). Males from Pintag showed an aggressive response to all conspecific song treatments, with no difference between response to the local dialect and the dialect from 2,000 km away ($t = -1.79$, $df = 74$, $P = .078$) and no difference between the dialects from 25, 2,000, and 4,000 km away ($t = 0.42$, $df = 74$, $P = .674$ for Papallacta vs. Bolivia, $t = -0.3$, $df = 74$, $P = .766$ for Papallacta vs. Argentina, $t = -0.72$, $df = 74$, $P = .472$ for Bolivia vs. Argentina; fig. 3*B*). The Pintag males did show a significant difference in their territorial response between the local and Papallacta dialect ($t = -2.21$, $df = 74$, $P = .03$). In both populations, there was a significant difference between the response to the conspecific dialects and the heterospecific control (Papallacta: $t = -4.03$, $df = 69$,

$P < .001$ for control vs. local, $t = -3.06$, $df = 69$, $P = .003$ for control vs. Pintag, $t = -2.89$, $df = 69$, $P = .005$ for control vs. Bolivia, $t = 2.09$, $df = 69$, $P = .040$ for control vs. Argentina; Pintag: $t = -4.66$, $df = 74$, $P < .001$ for control vs. local, $t = -2.45$, $df = 74$, $P = .017$ for control vs. Papallacta, $t = -2.87$, $df = 74$, $P = .005$ for control vs. Bolivia, $t = 2.15$, $df = 74$, $P = .034$ for control vs. Argentina), with males from both populations displaying significantly reduced aggression to the control song. Male response was not significantly affected by treatment order (ANOVA; Papallacta: $F = 0.47$, $df = 4$, $P = .76$; Pintag: $F = 0.72$, $df = 4$, $P = .58$).

Discussion

Here we show that females from two allopatric equatorial bird populations prefer their local song dialect to all other dialects tested, including those originating from populations only 25 km away. In both populations, female *Zonotrichia capensis* gave significantly more copulation solicitation displays in response to their local dialect than to the song dialects from a population on the other side of an Andean pass (25 km away; fig. 2). There was no difference in the Papallacta female's behavioral response to either of the nonlocal dialects. In Pintag, the female response to conspecific song from the population only 25 km away could not be distinguished from their response to the negative control song of a distantly related sympatric flycatcher. A previous study documented declines in female territorial responses to song dialects over short geographic distances (<30 km) in a tropical suboscine antbird (Seddon and Tobias 2007). Our study is the first to show differences in female mating preference for song dialects over a short geographic distance in a tropical bird.

Males from both populations displayed aggressive territorial responses to all conspecific song dialects regardless of dialect origin. Papallacta males showed a similar territorial response to all conspecific song treatments, including songs from populations 4,000 km away (fig. 3*A*). Pintag males showed aggressive responses to all song treatments, with no consistent difference with respect to distance (fig. 3*B*). Unlike the Papallacta males, the Pintag males displayed a significant difference in territorial response between the local song and the songs from the populations 25 and 4,000 km away but no difference between the local song and the dialect from 2,000 km away. In both the Papallacta and Pintag populations, males responded with significantly less aggression to the heterospecific control treatment than to conspecific songs.

In our study, females from both populations gave few copulation solicitations in response to songs from only 25 km away, despite the presence of a live local male sparrow

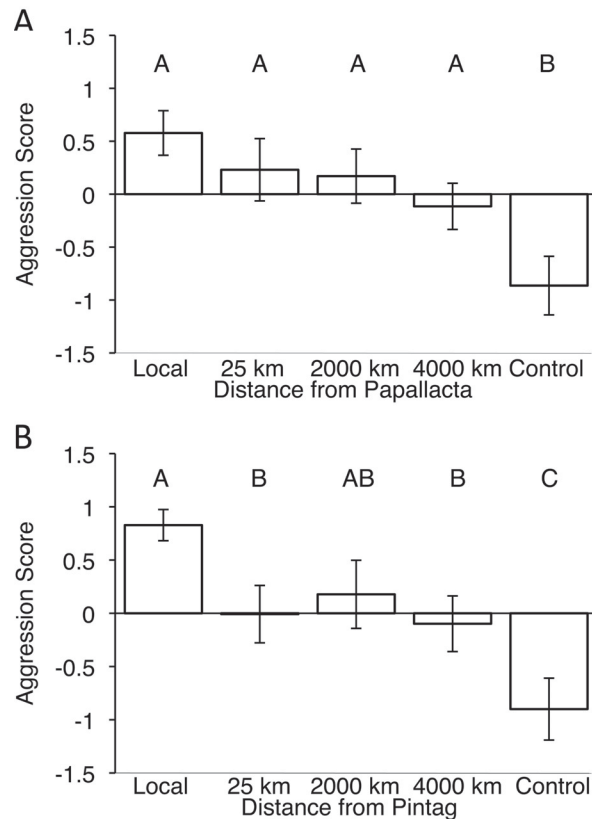


Figure 3: Aggression scores for male *Zonotrichia capensis* (mean \pm SE). A, Papallacta population ($n = 15$). B, Pintag population ($n = 16$). In both populations, male *Z. capensis* showed a similar aggressive response to all conspecific song treatments. The aggression scores are derived from a principal component analysis of territorial behaviors displayed in response to each song treatment. A negative score indicates a lower aggression score in comparison to other treatments. Different letters indicate significantly different responses between song treatments ($P < .05$).

and estrogen implants. While this result suggests that pre-mating reproductive isolation based on mate choice may already exist between the two populations, we cannot conclude that complete reproductive isolation has formed between the populations. Males in the genus *Zonotrichia* learn their songs from tutors within the first 100 days (Marler and Tamura 1964). However, some species have demonstrated the capability to learn new songs after the sensitive phase, indicating that song learning may be more plastic than originally thought. In *Zonotrichia leucophrys*, differences among subspecies in the plasticity of learning, the capacity to learn many songs, and the capability to select which song from its repertoire to sing has largely depended on whether the species is migratory or sedentary (Nelson et al. 1996, 2001). An extended sensitive phase would allow late-dispersing males to imitate their neigh-

bor's song (Bell et al. 1998; Nelson et al. 2001; Slabbekoorn and Smith 2002), thus disrupting reproductive isolation among populations. However, an acoustic signature of the natal population may remain in a dispersing male's repertoire, which would reduce a male's ability to replicate the new population's dialect (Slabbekoorn and Smith 2002). Regardless of song, a female may still choose to mate with the foreign male on the basis of other courtship behavior, such as visual displays. Dispersing females, on the other hand, may have no choice but to mate with a male singing a foreign dialect, which would also disrupt reproductive isolation. Nevertheless, the strong female preference for local songs demonstrated here suggests that reproductive isolation may be evolving among these populations and might continue to evolve with sustained geographic isolation.

Geographic variation in male song dialects and patterns of female preference could result from genetic and/or cultural evolution. Bird song development may be influenced by environmental factors (Loughheed et al. 1989; Slabbekoorn and Smith 2002), by a genetic predisposition to a song template (Marler and Peters 1977; Kroodsma 1996), and, in oscines, by learning (Marler and Tamura 1964). Therefore, differences in song between populations may have evolved by genetic and/or cultural evolution in response to drift (Fitzsimmons et al. 2008) and/or selection (Podos 2001; Kirschel et al. 2009). Similarly, female preference for male song may be influenced by sex-linked genes (Sæther et al. 2007) or sexual imprinting (Clayton 1990; Weisman et al. 1994; Price 2008), and thus differences among populations may have evolved genetically or culturally via drift or selection. Male song and female preference for male song may evolve independently or may coevolve through interactions mediated by sexual selection (Nowicki and Searcy 2002).

If the divergence in female preference for songs that we demonstrate here reflects reproductive isolation among other populations of tropical birds, then current estimates of species limits may be overly conservative and underestimate species diversity. In birds, allopatric populations are typically considered distinct species if they show neutral genetic or phenotypic differentiation at the level found in closely related sympatric species (Mayr and Ashlock 1991; Helbig et al. 2002; Price 2008) or if territorial birds do not respond aggressively to songs or mounted specimens in a simulated territorial challenge (Grant and Grant 2002; Uy et al. 2009). However, neutral genetic markers evolve relatively slowly by genetic drift, whereas phenotypic traits important for reproductive isolation can evolve quickly because of directional selection. Thus, divergence in neutral genes may underestimate species limits among allopatric populations (Hudson and Coyne 2002). Using

levels of phenotypic differentiation of sympatric species as a guide to species limits among allopatric populations may also underestimate species limits if traits important for reproductive isolation evolve before secondary contact.

Similarly, male territorial response might be an inaccurate measure of reproductive isolation because the selective pressures acting on female mate choice versus male territorial response are different (Andersson 1994; Ratcliffe and Otter 1996). Here we show that males and females have differing responses to song dialects, demonstrating that male and female behavioral responses should not be assumed to be the same. The observed lack of male discrimination among conspecific dialects in this study would traditionally be interpreted as evidence for a lack of reproductive isolation among populations. However, female responses from those same populations may indicate the presence of prezygotic reproductive isolation. In a recent study, Seddon and Tobias (2010) demonstrated in two sympatric species of antbirds with convergent songs—the Peruvian warbling antbird (*Hypocnemis peruviana*) and the yellow-breasted warbling antbird (*Hypocnemis subflava*)—that females are capable of discriminating between species and individuals on the basis of their willingness to duet with the playback, although males of the same population failed to discriminate between conspecific and heterospecific song dialects in simulated territorial intrusions. Therefore, male response might not provide predictive value for female response, making male territoriality to song dialects uninformative for reproductive isolation. Female responses do appear to be useful for estimating reproductive isolation among allopatric populations.

Female song preference has rarely been implicated in prezygotic isolation among populations. Previous studies of temperate-zone *Zonotrichia* species have demonstrated that females prefer local dialects to distant nonlocal dialects (local vs. 275 km: Baker et al. 1981, 1982; Sonoran Pass vs. Rocky Mountains: MacDougall-Shackleton et al. 2001; Tioga Pass vs. other regions in the Sierra Nevada: MacDougall-Shackleton et al. 2002) but concluded that song was only a partial barrier to breeding on the basis of a lack of population structure with respect to neutral genes (Baker 1982; Loughheed and Handford 1992; Loughheed et al. 1993; MacDougall-Shackleton and MacDougall-Shackleton 2001). The lack of population structure potentially resulted from their migratory and dispersal behaviors. In a mixed-dialect population of white-crowned sparrows (*Z. leucophrys*) in Alberta, Canada, females displayed no preference among dialects (Chilton and Lein 1996). In a subspecies of song sparrows (*Melospiza melodia*), Searcy et al. (2002) found that females can discriminate among song types at 34 km. Furthermore, in a hybrid zone of song sparrows, where a geographic barrier and at least 50 km separate subspecies,

females responded more strongly to their local song type (Patten et al. 2004).

In a tropical experiment examining female aggression in response to nonlocal dialects, female sharp-beaked ground finches (*Geospiza difficilis*) rarely responded to any dialect and showed no clear sign of discrimination, whereas males of the same population showed differential territorial responses between local and conspecific dialects (Grant and Grant 2002). In another tropical study examining female territoriality of nonlocal dialects in a suboscine—a subspecies of the chestnut-tailed antbird (*Myrmeciza hemimelaena pallens*)—females were more aggressive in response to their local dialect than to that of a neighboring population from at least 30 km away (Seddon and Tobias 2007). This study may suggest the existence of reproductive isolation between two populations with differing dialects over a very short distance. Thus, in some cases female territoriality to songs may be a useful tool in simulating secondary contact and determining response to distinct dialects; however, female territoriality might not give an indication of female mate preference.

Correlation between premating isolating barriers and reduction in gene flow is essential to show that reproductive isolation is occurring among populations (Coyne and Orr 2004; Funk et al. 2009). Our two tropical populations do exhibit genetic differentiation of microsatellites and low gene flow (Moore et al. 2005) that parallel the female preference in our study. However, the differentiation among our two study population dialects might result from a combination of female song preference, differential reproductive phenology (Moore et al. 2005), geographic barriers to dispersal, and/or other sexual or natural selective pressures driving these populations apart. Furthermore, female choice may be an important secondary reproductive barrier for allopatric populations with incomplete barriers to dispersal. Nevertheless, the differentiated populations may persist without further divergence.

Sedentary, isolated populations are abundant at low latitudes; therefore, assortative mating between allopatric bird populations might be a common phenomenon, particularly upon secondary contact in tropical mountainous regions. In regions such as the Andes Mountains, where the habitat is composed of isolated patches, populations may be separated by unsuitable habitat, and thus premating isolation may be established among many populations. Our results provide an example of differences in female preference for song among populations only 25 km apart in the equatorial Andes of South America. If our results are typical of other tropical species, then divergence in the tropics may take place over very short distances, creating great potential for diversification. Our results suggest that the high levels of population differentiation ob-

served at lower latitudes across many taxa (Martin and McKay 2004) may coincide with differences in traits important for reproductive isolation, such as female mate choice. Furthermore, if dispersing males do not change their songs to match new populations and if female preference for song accurately reflects reproductive isolation, then we may be underestimating species richness in tropical birds.

Acknowledgments

We thank M. Cooper, Fundación Terra, the Muñoz family, and Termas de Papallacta for field accommodations and D. A. Lijtmaer and S. C. Loughheed for the Argentina songs. We thank N. Johnson of the Virginia Tech Laboratory for Interdisciplinary Statistical Analysis and J. Hostetler for advice on statistical analyses. All procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee. We acknowledge funding from Sigma Xi (J.E.D.), a Virginia Tech Advance Postdoctoral Fellowship (F.B.), National Science Foundation (NSF) International Research Fellowship OISE-0700651 (F.B.), NSF International Research Fellowship OISE-0602084 (T.W.S.), the Natural Sciences and Engineering Research Council of Canada (P.R.M.), and a Baillie Family Endowment (P.R.M.). I.T.M. acknowledges support from NSF grant IOS-0545735.

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Associate Editor: Ellen D. Ketterson
Editor: Ruth G. Shaw



Male rufous-collared sparrow singing in Papallacta, Ecuador. Photograph by Julie E. Danner.

Chapter III: Cultural evolution and genetic structure in a tropical bird, the rufous-collared sparrow (*Zonotrichia capensis*)

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Abstract

Culture may affect the genetic structure of populations resulting in population divergence and potentially leading to speciation. In oscine birds, song is learned and can evolve quickly through cultural transmissions, resulting in dialects. Localized song dialects could facilitate assortative mating, due to a lack of recognition of conspecifics, restrict gene flow, and result in a genetic subdivision of populations. We investigated the role of cultural divergence in population divergence in a widespread Neotropical passerine, the rufous-collared sparrow (*Zonotrichia capensis*). We examined dialectal differences and population genetic structure in eight populations along a transect that is bisected by the Andean ridge, which exceeds *Z. capensis*' elevational range. We investigated the presence of population divergence and identified the mechanism that may be driving the observed pattern. Apart from culture, a geographic barrier (an Andean ridge), elevation, and geographic distance were alternative mechanisms of genetic divergence that we considered. With the examination of the full song and only the trill, all sites exhibited site-specific song dialects, except for two neighboring populations. There was genetic differentiation among sites and high support for the presence of five population genetic clusters. Populations separated by the Andean ridge did not display higher genetic differentiation or song differentiation than distance would predict, suggesting that the Andean ridge is not driving population divergence. Elevation was not correlated to either song or genetic differences, indicating that gene flow crosses differential elevational gradients. Both song divergence and microsatellite allele frequency differentiation were correlated with geographic distance

suggesting a pattern of isolation by distance. Patterns of cultural divergence were not related to patterns of genetic differentiation, indicating that culture is not driving population divergence. Overall, our results suggest that geographic distance, not geographic barriers, elevation, or culture, is the best predictor of population genetic divergence in this system.

Introduction

Like genes, cultural traits can mutate and evolve through drift (Derryberry 2007; Goodale and Podos 2010) and selection (Podos and Warren 2007; Derryberry 2009; Luther and Derryberry 2011). Genes and culture may each act upon the other to varying degrees. The evolution of culture may in part depend on genes via a genetic template (Soha and Marler 2000; Podos and Warren 2007), and some evidence suggests that culture may affect the genetic structure of populations, resulting in population divergence, potentially leading to speciation (Slabbekoorn and Smith 2002; Price 2008). A pattern of cultural divergence that parallels the pattern of genetic divergence provides evidence of a relationship between culture and genes, though the directionality may remain unknown.

The role of culture in population divergence may in part depend on the mode of speciation separating populations. Among allopatric populations, the pattern of cultural divergence would parallel the pattern of genetic divergence because of a lack of gene flow across physical barriers, genetic drift and possibly selection caused by differing environments (Mayr 1946; reviewed by Coyne and Orr 2004). If the physical barrier is an incomplete barrier, allowing gene flow, differences in culture may reinforce genetic divergence. In the parapatric speciation model, populations may exhibit clinal variation in culture and genetics increasing with distance (Irwin 2000; Irwin et al. 2001, 2005). Differences in culture may be a barrier to breeding among populations that are geographically far apart, yet panmixia may be present among neighboring populations. Under both allopatric and parapatric distributions cultural divergence may facilitate population divergence perhaps leading to speciation.

Under either speciation model, sexual selection via female choice for a cultural trait may reinforce differentiation among populations (Coyne and Orr 2002). Song in birds may be a

culturally transmitted trait and females may exhibit a preference for certain songs (Nowicki et al. 2002). In oscine birds, song is learned (Marler and Tamura 1964) and errors during the learning process may create regional dialects. Females have been shown to exhibit a preference for local dialect (Baker et al. 1981, 1982; MacDougall-Shackleton et al. 2001, 2002; Searcy et al. 2002; Patten et al. 2004; Danner et al. 2011), and localized song-dialects could facilitate assortative mating, due to a lack of recognition, restricting gene flow, thus causing a genetic subdivision among populations (Nottebohm 1969; Baker et al. 1981; Baker and Cunningham 1985; Rothstein and Fleischer 1987; Payne 1973; Balaban 1988; Soha et al. 2004; MacDougall-Shackleton and MacDougall-Shackleton 2001; Irwin et al. 2001). Geographic variation in song dialects may present a good opportunity to study the role of culture in driving population divergence.

A number of previous studies have investigated the role of song variation in relation to population structure. Baker (1975, 1982) found that in a subspecies of white crowned sparrows (*Zonotrichia leucophrys nuttali*) song dialects correspond to genetic structure (Baker and Cunningham 1985). Similarly, in swamp sparrows (*Melospiza georgiana* Balaban 1988), greenish warblers (*Phylloscopus trochiloides* Irwin 2000), white-bellied shortwings (*Brachypteryx major* Robin et al. 2011) and blue tits (*Parus caeruleus* Doutrelant et al. 2000), song divergence has been correlated to population structure. However, for several species, including brown-headed cowbirds (*Molothrus ater* Fleischer and Rothstein 1988), indigo buntings (*Passerina cyanea* Payne and Westneat 1988), two subspecies of white crowned sparrows *Z. l. oriantha* (MacDougall-Shackleton and MacDougall-Shackleton 2001) and *Z. l. pugetensis* (Soha et al. 2004), and Darwin's finches (*Geospiza fortis* and *G. scandens* Grant and Grant 1996, 2002), genetic structure was not found to correspond to dialectal boundaries. Thus relationships between song dialects and population genetic divergence are inconsistent.

We investigated the role of cultural divergence in population divergence in a widespread Neotropical bird species, the rufous-collared sparrow (*Zonotrichia capensis*), that exhibits geographic variation in song dialects. Previous studies on *Z. capensis* in northwestern Argentina found a weak relationship between trill rate dialects and genetic variation (Handford and Nottebohm 1976; Loughheed and Handford 1992). However, larger sample sizes, using microsatellites, fine structural measurements on the trill, and sampling over a small geographic scale may provide a more precise small-scale examination of the relationship between song and genetics. Our study uses variable microsatellites, which are a powerful analytical tool for examining genetic variation among populations on a small geographic scale (Petren 1998) due to their putative selective neutrality, relatively rapid mutation rate, and high variability (Hancock 1999). Further, our previous work has shown female preference for local male song dialects in two allopatric populations of *Z. capensis* (Danner et al. 2011), suggesting that culture may play a role in our study system. We investigated the role of culture in population genetic divergence by examining dialects and genetic differentiation in eight populations of *Z. capensis* along a transect that is bisected by the Andean ridge. Specifically we examined: 1) dialectal differences among sites, and features of the song that are most variable in order to identify dialectal signatures and, 2) population genetic structure in relation to song dialects and geography using variable microsatellites. We also considered three non-cultural mechanisms that may drive patterns of population genetic divergence: a geographic barrier (the Andean ridge), elevation, and geographic distance. Because the Andean ridge (4200 m) is above our study species' elevational range (3500 m), the ridge could increase differentiation in song and genetics by inhibiting gene flow between separated populations, supporting the allopatric speciation model.

Dialects correlated with genetic structure, independent of the geographic barrier and geographic distance would provide evidence that *culture* is driving population divergence. Increased differentiation in song and genetics among populations separated by the Andean ridge would provide evidence that this *geographic barrier* is the mechanism driving population divergence. Correlations between elevation and both dialects and genetics would provide support that *elevation* plays a role in cultural and population divergence. Finally, geographic distance correlated to both dialect and genetic variation via a pattern of isolation by distance would provide evidence that *geographic distance* is the mechanism driving population divergence, supporting the parapatric speciation model.

Study Species

We examined song in *Z. capensis*, which is a widespread non-migratory tropical bird that ranges from Chiapas, Mexico to Tierra del Fuego. Due to its broad geographic range, *Z. capensis* has the potential to adapt to a wide range of environments; however they typically reside in human disturbed habitats. Song dialects have been extensively studied in *Z. capensis* especially in northwestern Argentina (Nottebohm 1969, 1975; King 1972, Handford 1988; Handford and Lougheed 1991; Lougheed and Handford 1992; Lougheed et al. 1989; Lougheed et al. 1993), as well as in its phylogenetically basal sister species the white-crowned sparrow (*Zonotrichia leucophrys*; Baker et al. 1981; Baptista et al. 1977; MacDougall-Shackleton and MacDougall-Shackleton 2001; Nelson and Soha 2004; Nelson and Poesel 2009). Songs of *Z. capensis* are comprised of two parts: the theme which includes 1-5 short notes and one long note descending in frequency, followed by a trill which is composed of several fast repetitive notes (Figure 1). In two Ecuadorian populations males can sing more than one song type (Moore et al. 2005):

Nottebohm (1969) and King (1972) found that in Argentina a small percentage (< 10%) of birds sing 2-3 song types. The difference between an individual's song types is restricted to the theme whereas the trill remains uniform (Nottebohm 1969; King 1972; Moore 2005). In some *Zonotrichia*, the theme portion of the song provides information on individual identification and the trill functions for dialectal identification (Baker 1982; Nottebohm 1975).

METHODS:

Study Sites

We examined song dialects and population structure in eight study sites spanning the east-west and elevational gradient of *Z. capensis* in Ecuador (Figure 1). The sites are on an approximate northwest-southeast transect that transverses an Andean ridge (4200 m) which is above the local subspecies elevational range (3500m). The populations we sampled from west to east are: Bellavista, San Jorge, Pululahua, Tumbaco, Pintag, Papallacta, Baeza, and Yanayacu (Table 1). Bellavista is located on the wetter, western slope of the inter-Andean ridge and the Pichincha volcano separates Bellavista from the inter-Andean populations: San Jorge, Pululahua, Tumbaco, and Pintag. There are pockets of suitable habitat for *Z. capensis* that may connect Bellavista to the inter-Andean sites. The inter-Andean sites are mostly contiguous and located in and around the sprawl of Quito. Pululahua is located at the bottom of a volcanic crater lined with steep walls. An unsuitable higher elevation Andean ridge separates the inter-Andean valley sites from the east slope sites: Papallacta, Baeza, and Yanayacu. Papallacta and Pintag experience seasonal rainfall and their populations exhibit asynchronous breeding cycles (Moore et al. 2005). A deep, narrow valley lined with intact forest connects Papallacta and Baeza and there appears to be no obvious barrier that separates Baeza and Yanayacu. East of Yanayacu is a ridge that

quickly drops off into the Amazon basin. The mean distance separating closest neighboring populations is 24km (max: 32km, min: 16km), whereas the mean distance separating all populations is 55km (max: 124km, min: 16km; Table 2). The populations range in elevation from 1890m to 3300m (average elevation: 2560m).

Field Methods

From each of the eight populations, we captured between 14-42 birds using nylon mist nets and/or potter traps baited with seed or rice. From each bird we collected ~75 μ l of blood from the brachial wing vein and stored the blood in 200 μ l of lysis buffer. We captured and bled for genetic analysis 19 birds in Bellavista, 14 birds in San Jorge, 20 birds in Pululahua, 19 birds in Tumbaco, 28 birds in Pintag, 42 birds in Papallacta, 21 birds in Baeza, and 26 birds in Yanayacu (n = 189: 89 of which were females that were evenly distributed across populations, sex confirmed genetically, see methods below; Table 1).

We recorded songs from 22 males in Bellavista, 11 males in San Jorge, 23 males in Pululahua, 17 males in Tumbaco, 32 males in Pintag, 39 males in Papallacta, 23 males in Baeza, and 22 males in Yanayacu. At each population, JED recorded songs using a directional shotgun microphone (Sennheiser ME66) and a solid-state recorder (Marantz PMD 660). All recordings took place during the pre-breeding or breeding season verified by either breeding condition while birds were in hand (enlarged cloacal protuberance in males or brood patch in females) and/or observations of breeding behavior (ie: nest building, copulation, etc.). Further, male birds only sing during the breeding season (Moore et al. 2004). To minimize any potential effects of habitat on song (reviewed by Slabbekoorn and Smith 2002; *Z. capensis*: Nottebohm 1969, 1975; Handford 1988; Handford and Loughheed 1991; Loughheed and Handford 1992), all recordings

were obtained in open habitats either on cattle pastures or on dirt roads directly adjacent to cattle pastures. All recordings took place between 6:00–10:00 and 16:00–17:00 during the birds' natural activity periods. All males were recorded for at least 3 minutes or until 8 full songs were recorded. To prevent duplicate recordings of unmarked birds, we carefully noted the location and only took recordings from a single bird from each location.

Song analysis

In *Zonotrichia* the trill tends to be temporally stable (Handford 1988) and highly stereotypic (Nottebohm 1969; King 1972; Nottebohm 1975); therefore, most studies use only the trill to assign geographic song dialects (Handford 1988; Handford and Loughheed 1991; Loughheed and Handford 1993; Baptista 1977; Nelson and Soha 2004; Nelson and Poesel 2009). For our study, we performed separate analyses on the theme, trill and then the entire song. Analyses on the full song and the trill will be most useful for assigning dialects due to variability among sites and for comparisons with other studies. For our analyses, we used the song that was of the highest quality (songs with low background noise and no overlap with other songs) to convert into wav files. We isolated the theme from the trill, which was easily identifiable because the trill is composed of repetitive syllables at the end of the recording, using syrinx-PC (syrinxpc.com). To standardize recordings, in Audition 3 (Adobe, San Jose, California) we resampled the songs to 22050Hz, 16-bit, mono, bandpass filtered noises outside of *Z. capensis*' range to 2–10 kHz, and normalized the songs to -1dB. For both the theme and trill, we used Avisoft SASLab Pro's v. 4.52 (Avisoft Bioacoustics, Berlin, Germany), automatic feature to collect the following 5 measurements from the linear amplitude spectra (determined at -24dB, smoothed to an average of 15 points; Figure 2a) a) peak maximum (V), b) peak frequency (Hz;

frequency in the song with the greatest amplitude), c) minimum frequency (kHz) and d) maximum frequency (kHz), and e) bandwidth (maximum frequency–minimum frequency relative to peak frequency). Additionally, we measured the following 12 fine structural characters in Avisoft: f, g) duration (theme and trill), h,i) number of notes (theme and trill), j) bandwidth of longest note in the theme, k) theme rate (number of notes/ duration), l) maximum frequency at the start of the first note in the trill, m) minimum frequency at the end of the last note in the trill, n) maximum and o) minimum frequency averaged across all notes in the trill, p) maximum bandwidth for all the notes in the trill and q) trill rate (number of notes/ duration; Figure 2b). To reduce inconsistencies JED measured all structural characters.

Songs Data Analysis

To examine dialectal differences among our eight study sites we employed six analyses using 1) all song measurements, 2) only theme measurements or 3) only trill measurements. First, we used a non-parametric analysis of variance test (Kruskal–Wallis) because of violations of normality, to determine if each fine structural measurement varied by site. All nine fine structural measurements on the theme (all song measurements uncorrected p value < 0.05) and all 13 fine structural measurements on the trill (all trill measurements uncorrected p value < 0.05) were significant, indicating variation among sites; therefore, all measurements were used in the subsequent analyses. To confirm trill stereotypy among individuals, we examined three banded birds that were recorded over 2-3 days with 11-12 recordings per individual (35 recordings total). We conducted a discriminant function analysis using all fine structural characters. Discriminant function analysis correctly assigned 98% of the songs to the correct individual supporting stereotypy and the use of one song per individual in our analyses. To determine if all structural

characters differed by site, we ran a MANOVA. We conducted a discriminant function analysis (DFA) in JMP v. 9.0 to test if the structural measurements could correctly assign the dialects by site. Following DFA we ran a proportions test to see if incorrectly classified songs were more likely to come from the two closest neighboring populations.

We used coefficient of variation (CV) to determine which structural characters were more stable and variable among populations. We calculated coefficients of variation for within each population (CV_{site}) and among all populations ($CV_{all\ sites}$) for each song measure (Robisson 1993; Mennil 2008; Seddon and Tobias 2010; Vignal et al. 2004). We determined a potential for population coding (PPC) score by calculating $CV_{all\ sites}/CV_{site}$ (adapted from Vignal et al. 2004). A PPC score > 1 indicates that the variability of a song feature among sites is greater than within a single site. A high PPC score suggests that the structural character may be highly site specific and is perhaps a signature for that dialect (Vignal 2004).

Differences in the overall song features among sites were analyzed with a multi response permutation procedure (MRPP; Euclidean distance, 1000 permutations) in the *R* ‘vegan’ package. For MRPP pairwise comparisons we used the Bonferroni method (Sokal and Rohlf 2012). To compare song differences we used function ‘meandist’ to calculate a matrix of mean differences within and among sites. Previous work showed that females in both Pintag and Papallacta preferred their local dialect (Danner et al. 2011). Therefore we used the difference in song between Pintag and Papallacta from the ‘meandist’ calculations as a reference point, assuming that in populations exhibiting higher differences in song than this reference point, females from the respective populations likely show a dialect preference. Next, we tested for differences in the variance among sites using a multivariate homogeneity of group dispersions (‘betadisper’) test that measured individual Euclidean distance to the group’s centroid. We used

Tukey's Honest Significant Difference to test for pairwise differences in the variance among sites.

Lastly, we ran partial Mantel tests (R package 'vegan'), which measures the partial correlation between two dissimilarity matrices, conditioned on a third matrix, to determine if the Andean ridge, elevation, or geographic distance had an effect on song dialects (Table 3). For each of the following partial Mantel tests we examined differences in song based on the 1) full song, 2) theme, or 3) trill measurements. Specifically, we examined if song differences were affected by the Andean barrier while controlling for geographic distance. Further, populations separated by the Andean ridge displaying higher song differentiation than populations not separated by the ridge would provide evidence that the Andean ridge is playing a role in cultural divergence. Then we tested to see if song dialects were correlated with elevation while controlling for either genetics differences or geographic distance. Last we examined if song dialects were correlated to geographic distance while controlling for any genetic differences.

Genetic Techniques

We extracted the DNA from blood samples following the Qiagen Biosprint 96 DNA blood kit's manufacturer's instructions and we electrophoresed a subsample on 1.5% agarose gel to check for proper extraction. We tested 26 microsatellite loci for amplification, which had previously been described in other species (Double et al. 1997; Griffith et al. 1999; Petren 1998; Poesel et al. 2009; Table 4; Appendix Table 1). We PCR amplified thirteen microsatellite loci that consistently amplified and were able to be genotyped to assess variability among individuals. We ran 10 μ l reactions containing 20ng template DNA, 10x polymerase chain reaction (PCR) buffer, 0.2mM each dNTP, between 1 and 3.5 mM MgCl₂, 0.05 μ M Promega

Taq polymerase and 0.5 μ M of each primer. All loci were amplified under the following thermal conditions: 94° for 3 minutes followed by 32 cycles of 94° for 30 seconds, the annealing temperature for 40 seconds, and 72° for 40 seconds then a final extension step of 72° for 10 minutes. Annealing temperatures are provided in Table 4. One primer of each pair was fluorescently labeled, and PCR products were run on an ABI 3130xl genetic analyzer. We used diamond Rox as a size standard and Genemapper v.4.0 (Applied Biosystems) to estimate fragment sizes and infer genotypes. Since males and females are monomorphic, and not all captured individuals were breeding, we identified the sex of each bird using the methods of Griffiths et al. (1998).

Genetic Analysis

We used Genepop (Raymond and Rousset 1995) to statistically compare expected and observed heterozygosity at each locus and sampling locality, and to estimate F_{st} values among sites. We used the Bayesian clustering program Structure v.2.3.3 (Pritchard et al. 2000) to infer population structure among the sites using genotype data. We set K to 1–8 possible population groupings and used a burn in of 50,000 iterations followed by 500,000 iterations for each K, and replicated each K four times to assess stability. We then reevaluated K for 1–8 possible population groupings using location as a prior with the same specifications listed above. We used the program StructureHarvester (Earl and vonHoldt 2011) to calculate ΔK , a measure of the second order rate of change in the likelihood of K (Evanno et al. 2005), to evaluate the optimal number of population groupings. When location was not incorporated into the model, we examined the sex of the individuals that exhibit greater than 30% of their genetic make-up belonging to another cluster.

We used a partial Mantel test (R package ‘vegan’) to determine if the Andean ridge affected population structure while controlling for geographic distance (Table 3). Populations separated by the Andean ridge displaying higher genetic differentiation than populations not separated by the ridge would provide evidence that the Andean ridge was disrupting gene flow among populations. We predicted that populations on the same side of the barrier would be more similar genetically than populations on opposite sides of the geographic barrier. Then we tested to see if genetic differences were correlated with elevation while controlling for either song differences (full/theme/trill) or distance. Last we used partial Mantel tests to determine if genetic population structure was correlated with geographic distance while controlling for differences in song (full/theme/trill).

Relationship between Culture and Genetic Isolation

To test the hypothesis that cultural differences produce genetic isolation, we examined differences in song dialect within and among genetic population clusters. We predicted sites displaying a greater difference in song will have greater genetic differentiation and those differences will be correlated. We ran partial Mantel tests (R package ‘vegan’) to determine if song dialects were correlated to genetic population structure while controlling for geographic distance or elevation (Table 3).

RESULTS

Dialects

Structural characters on the full song were significantly different by site (Manova Wilks’ Lambda: $F = 6.51$, $df = 154, 1083$, $p < 0.0001$). Discriminant function analysis of all raw song

measures assigned 85% of the males (161 individuals) to the correct population from which the song was sampled (Figure 3a). Specifically, 82% of the birds from Bellavista were correctly assigned, 91% in San Jorge, 83% in Pululahua, 88% in Tumbaco, 88% in Pintag, 87% in Papallacta, 78% in Baeza, and 86% in Yanayacu. There was a strong trend for incorrectly assigned songs to be more likely assigned to the two closest neighboring populations on the same side of the ridge ($\chi^2 = 14.90$, $df = 8$, $p = 0.061$).

All theme measurements were significantly different by site (Manova Wilks' Lambda: $F = 5.33$, $df = 63$, 980 , $p < 0.0001$). The discriminant function analysis of the theme measurements was less accurate and only correctly assigned 52% of the males (98 individuals) to the population from which they were sampled (Figure 3b). Specifically, 59% of the birds from Bellavista were correctly assigned, 48% in San Jorge, 51% in Pululahua, 38% in Tumbaco, 48% in Pintag, 64% in Papallacta, 29% in Baeza, and 50% in Yanayacu. Incorrectly assigned songs were not assigned to the two closest neighboring populations on the same side of the ridge ($\chi^2 = 7.4$, $df = 8$, $p = 0.49$).

All trill measurements were significantly different by site (Manova Wilks' Lambda: $F = 8.096$, $df = 91$, 1062 , $p < 0.0001$). Discriminant function analysis of the trill measurements correctly assigned 75% of the males (141 individuals) to the population from which they were sampled (Figure 3c). Specifically, 55% of the birds from Bellavista were correctly assigned, 73% in San Jorge, 87% in Pululahua, 82% in Tumbaco, 91% in Pintag, 72% in Papallacta, 57% in Baeza, and 77% in Yanayacu. Incorrectly assigned songs were significantly more likely to be assigned to the two closest neighboring populations on the same side of the ridge ($\chi^2 = 23.71$, $df = 8$, $p = 0.003$).

All coefficients of variation for PPC scores from the fine structural measurements were greater than 1 (full mean = 11.69; theme mean = 9.81; trill mean = 12.98), suggesting all structural characters may be used for site-specific delineation because the variables have a greater inter- than intra- population variability. The song features that had the highest average PPC score and therefore may be the most useful in determining unique site signatures were trill rate, theme peak maximum, minimum of all trill notes, and minimum of last trill note trill rate, theme peak maximum, trill all minimum notes, and trill last minimum note. Theme maximum frequency, theme bandwidth, bandwidth of longest note in the theme, and trill duration had the lowest PPC score suggesting these features were more stable across sites.

MRPP on all song measurements verified that song features differed significantly among sites (MRPP; $A = 0.129$; $p < 0.0001$). Post hoc tests revealed that all songs significantly differed among sites ($A < 0.029$; all corrected $p = 0.028$), except between Bellavista and San Jorge (corrected $p = 0.11$) and Baeza and Yanayacu (corrected $p = 0.8$). Using ‘meandist’ calculations, the difference in songs between all inter-Andean valley populations and Pintag was greater than the song difference between Pintag and Papallacta. Bellavista – Tumbaco and Tumbaco – Papallacta also had a greater difference in songs than Pintag – Papallacta. For the full song, average individuals’ distance to their respective site’s centroid did not differ among sites, indicating that each site had a similar variance in songs ($df = 7$, F value = 1.825, $p = 0.085$). Post-hoc analyses indicated that Bellavista and Yanayacu significantly differed in their variance, with Bellavista exhibiting more song variability (Tukey’s HSD: $p = 0.033$), whereas all other sites were similar (Tukey’s HSD: all $p > 0.163$).

MRPP on the theme measurements verified that features in the theme differed significantly by site (MRPP; $A = 0.1$; $p = 0.001$). Post hoc tests revealed that the theme differed

significantly among 60% of the sites ($A < 0.046$; all corrected $p = 0.028$). However, there was no difference in the theme among the following sites: Bellavista– San Jorge (corrected $p = 1.0$), Bellavista– Tumbaco (corrected $p = 0.083$), San Jorge– Tumbaco (corrected $p = 0.39$), Pululahua– San Jorge (corrected $p = 1.0$), Yanayacu– Bellavista (corrected $p = 0.08$), Baeza– San Jorge (corrected $p = 0.12$), Papallacta– San Jorge (corrected $p = 0.139$), Pululahua– Yanayacu (corrected $p = 0.167$), Bellavista– Papallacta (corrected $p = 1.0$), Baeza– Pintag (corrected $p = 1.0$), San Jorge– Yanayacu (corrected $p = 1.0$), and Yanayacu– Baeza (corrected $p = 0.08$). Using ‘meandist’ calculations, the following sites exhibited a greater difference in the theme than Pintag and Papallacta: Pintag and all inter-Andean valley sites, San Jorge– Papallacta, and Tumbaco and all sites except for San Jorge and Yanayacu. Average individuals’ distance to their respective centroid did not significantly differ among sites ($df = 7$, F value = 1.815, $p = 0.09$) and post-hoc analysis indicated that all site pairwise comparisons exhibited similar variance (Tukey’s HSD: all $p > 0.18$), indicating similar within site variability in the theme portion of the song.

MRPP on trill measurements verified that song features differed significantly among sites (MRPP; $A = 0.168$; $p < 0.0001$). Post hoc tests revealed that the trill differed significantly among sites ($A < 0.029$; all corrected $p = 0.028$), except for Papallacta and Tumbaco ($p = 0.056$), Bellavista and San Jorge (corrected $p = 0.11$), and Baeza and Yanayacu (corrected $p = 0.36$). Using ‘meandist’ calculations, the difference in songs between Bellavista and all other sites was greater than the song difference between Pintag and Papallacta. Pintag – San Jorge, Pintag – Pululahua, and Pintag – Tumbaco, Pululahua– Tumbaco, and Tumbaco– Yanayacu also displayed a greater difference in songs than Pintag – Papallacta. For measurements on the trill, average individuals’ distance to their respective site’s centroid significantly differed among sites ($df = 7$,

F value = 4.68, $p < 0.0001$), indicating dissimilar within site variability of the trill. All site pairwise comparisons with Bellavista were significantly different, with Bellavista exhibiting more trill variability than the other sites, except those with Tumbaco and Papallacta (Tukey's HSD: Bellavista–San Jorge $p = 0.03$, Bellavista–Pululahua $p < 0.0001$, Bellavista–Pintag $p = 0.0008$, Bellavista–Baeza $p = 0.006$, Bellavista–Yanayacu $p = 0.003$). All other sites exhibited a similar variance in the trill (Tukey's HSD: all $p > 0.163$).

There was no relationship between the Andean ridge and differences in song while controlling for geographic distance (for full song, theme, and trill partial Mantel: all $p > 0.3$; Figure 4; Table 3). Further, there was no relationship between differences in song and elevation while controlling for genetics (for full song, theme, and trill partial Mantel: all $p > 0.5$; Figure 5) or distance (for full song, theme, and trill partial Mantel: all $p > 0.5$). However, full song and trill differences were correlated with geographic distance while controlling for genetic differences (full song partial Mantel: Mantel $r = 0.42$, $p = 0.008$; theme partial Mantel: Mantel $r = 0.04$, $p = 0.385$; trill partial Mantel: Mantel $r = 0.42$, $p = 0.005$; Figure 4).

Genetic Results

Only one locus (C07) routinely showed significant deviations from Hardy-Weinberg expectations across multiple populations. Five loci completely conformed to Hardy-Weinberg equilibrium (HWE) after sequential Bonferroni correction. One locus had a single population that did not meet HWE, five loci had two populations that were out of HWE, and 1 locus had 3 populations that were out of HWE. Locus C07 had 5 populations that significantly deviated from HWE (all by heterozygote deficiency). We were unable to determine the cause of this deviation, and thus we removed locus C07 from further analysis.

The Andean ridge did not affect the genetic differentiation among populations while controlling for geographic distance (partial Mantel $r = -0.048$, $p = 0.562$; Figure 6; Table 3). Further, there was no relationship between genetic differentiation and elevation while controlling for songs (for full song, theme, and trill partial Mantel: all $p > 0.8$; Figure 7) or distance (partial Mantel: Mantel $r = -0.09$, $p = 0.64$). However, genetic divergence (F_{st}) was significantly correlated with geographic distance while controlling for song differences (full song partial Mantel: Mantel $r = 0.83$, $p = 0.0003$; theme partial Mantel: Mantel $r = 0.76$, $p = 0.0004$; trill partial Mantel: Mantel $r = 0.827$, $p = 0.0003$; Figure 6).

The most conservative approach in Structure, using no prior information on sample location, resulted in $K = 2$ population clusters; assigning Baeza and Yanayacu as a single population and all other sites as a single population (Figure 8a). Individuals that exhibit 30-49% of their proportional genetic make-up belonging to another cluster were male biased (one female: seven males). In comparison, there was an even sex ratio (4 females: 4 males) for individuals that exhibit 50-90% of their proportional genetic make-up assigned to another cluster. When we used location as a prior, $K = 3, 4$, and 5 populations appear to cluster (Figure 8b-d). Structure Harvester (Earl and vonHoldt 2011), ΔK calculations (Evanno et al. 2005) gave the highest weight to the occurrence of 5 population clusters (appendix, Figure 1), therefore we used $K = 5$ for our cluster groupings. In all cases Baeza and Yanayacu were lumped as a single cluster. When $K = 3$, all inter-Andean valley sites were lumped as a single cluster, and Papallacta was a separate cluster from Baeza and Yanayacu. When $K = 4$ Bellavista was separated from other inter-Andean valley sites, and the east slope was the same as when $K = 3$. $K = 5$ was similar to $K = 4$, except Puluahua was a separate cluster from San Jorge and Tumbaco, and the genetic composition of the Pintag population appeared to be mixture of Puluahua and San

Jorge/Tumbaco. In all cases when location was used as a prior, there was evidence of some mixed ancestry, perhaps due to introgression, in San Jorge, Puluahua, Tumbaco and Pintag, all inter-Andean valley sites.

Relationship between Culture and Genetic Isolation

In all three analyses of the song, dialects were more similar within genetic clusters than among genetic clusters on the east slope. Overall, differences in song were greater among the inter-Andean and the west slope sites than among the east slope sites, yet song differences were similar within and among genetic clusters. Genetic differentiation was not correlated to song dialect differentiation while controlling for geographic distance (for full song, theme, and trill partial Mantel: all $p > 0.94$; Figure 9; Table 3) or elevation (for full song, theme, and trill partial Mantel: all $p > 0.85$)

DISCUSSION

Here we show site-specific song types indicative of dialect structure over a short geographic distance, with only two populations displaying considerable song similarity (Figure 3). Sites also exhibited genetic population structure, with support for the presence of five genetic clusters (Figure 8). Neither the Andean ridge nor elevation played a role in differences in song or genetics (Figures 4-7). Among sites, differences in song dialect (Figure 4) and genetic differentiation (Figure 6) increased with geographic distance; however, cultural divergence was not correlated to genetic isolation (Figure 9). Our results provide evidence that geographic distance, not the Andean Ridge, elevation, or culture, is the mechanism driving population divergence in this system.

Cultural Divergence

Along a 124 kilometer transect, *Z. capensis*' full song and trill element exhibited significant song differentiation among eight populations (Figures 1, 3). Trill rate, theme peak maximum, minimum of all trill notes, and minimum of last trill note were the most variable song features among sites and thus may be used to identify dialectal signatures. In contrast, many features in the theme were similar among sites, leading to low assignment accuracy in a DFA (Figure 3b), thus indicating that the theme measurements are not useful for assigning dialects. Further, analyses on the full song and on the trill had similar results; therefore our discussion will focus on the full song and trill.

For the full song, average distance to each site's centroid did not differ among sites, indicating similar within population song variability. In comparison, for the trill, all sites exhibited similar within population trill variability, except for Bellavista, which had higher variation than Tumbaco and Papallacta. Overall, song variation was high among sites, leading to high assignment accuracy in a DFA, thus suggesting that a bird would be able to distinguish among dialectal groups. Discriminant function analysis showed few similarities in song features between sites, except for our two easternmost populations, Baeza and Yanayacu, which displayed considerable overlap in song measurements (Figures 3a,c). Incorrectly classified songs were more likely to be assigned to a neighboring population than a non-neighboring population, indicating greater similarity of songs among adjacent populations. Many pairs of sites exhibited greater divergence in song than Papallacta and Pintag (full song: 6 population pairs; trill: 12 population pairs).

Potential Mechanisms Driving Pattern of Culture

We examined the potential mechanisms of the Andean ridge, elevation and geographic distance to explain the observed pattern of cultural divergence. Populations separated by the Andean ridge do not exhibit higher differences in song than populations on the same side of the ridge, indicating that there is no relationship between the ridge and song differentiation (Figure 4). Although geographic barriers such as mountains, rivers, and fragmented habitats have been shown to play roles in suboscine song divergence (Seddon and Tobias 2007; Tobias et al. 2009), examples of geographic barriers driving cultural divergence in tropical oscines are rare. Similarly, songs may diverge along elevational gradients, due to performance limits at high altitudes, as seen in cardueline finches (Snell-Rood and Badyaev 2008); however, we did not find a relationship between elevation and song dialects (Figure 5). Although not examined in this study, variation in bill morphology due to natural selection and adaptation to local food resources may also be a mechanism of cultural divergence (Handford and Lougheed 1991, 1992; Podos 2001; Podos and Nowicki 2004). However, we sampled songs from birds in similar habitats that presumably contained similar food resources, thus reducing the potential for divergent foraging ecology to affect culture.

We found that songs were increasingly different with distance between populations (Figure 4), suggesting that distance rather than the geographic barrier or elevation better predicts song divergence. Pintag and Bellavista displayed the most distinctive dialects compared to other sites. Interestingly, birds from Pintag sang a unique dialect even though genetic divergence with other sites was low, whereas birds in Bellavista displayed higher genetic divergence from other sites. In contrast, Baeza and Yanayacu sang the most similar songs, displayed low F_{st} values, and

were lumped as a single genetic cluster, thus providing evidence for a strong connection between these populations.

While geographic distance has been demonstrated to play a role in cultural evolution in temperate zone species (Irwin 2000; Searcy et al. 2002), to the best of our knowledge, examples in tropical oscines do not exist. Among tropical suboscine populations, clinal variation in songs can be observed in warbling antbirds (*Hypocnemis cantator* Isler et al. 2005; *H. peruviana*, *H. subflava* Tobias and Seddon 2009), but not in chestnut tailed antbirds (*Myrmeciza hemimelaena* Seddon and Tobias 2007). In other classes of tropical organisms, acoustic differences increased with geographic distance among populations of a singing mouse (*Scotinomys teguina* Campbell et al. 2010) and populations of tungara frogs (*Physalaemus pustulosus* Ryan et al. 1996; Pröhl et al. 2006).

Genetic Divergence

Our results provide evidence of genetic structure among the eight study sites. The most conservative Structure test, when location was not incorporated into the model, resulted in two genetic population clusters (Figure 8a). The two easternmost sites, Baeza and Yanayacu, were lumped as a single population, whereas the other east slope population, Papallacta, was assigned to a population cluster with the inter-Andean and west slope sites, suggesting that the Andean ridge is not playing a role in population divergence. When sampling location was incorporated into the model, ΔK gave high support to the presence of five genetic clusters, which appears to reflect the spatial distribution of the study sites. In this case, similar to the previous model, the two easternmost sites Baeza and Yanayacu were lumped as a single population. In contrast, Papallacta was in its own cluster, and the inter-Andean sites displayed two population clusters;

one cluster was comprised of Tumbaco and San Jorge, sites that are close geographically (16 km), and the other cluster was composed of the site at the bottom of a volcanic crater, Pulumahua. Pintag appeared to be a mixture of the two inter-Andean valley clusters. Lastly, the site on the west slope, Bellavista, was assigned as a single population (Figure 8d).

Potential Mechanisms Driving Pattern of Genetic Structure

Genetic differences among populations can typically be attributed to geographic barriers such as rivers and mountain ranges (Brumfield and Capparella 1996; Cheviron et al. 2005; Alexio 2004; Naka et al. 2012), or fragmented habitats (Seddon and Tobias 2007; Bates 2002). The Andean ridge separating our study sites allows us to examine the role a potentially major geographic barrier plays in genetic differentiation. Interestingly, there was no relationship between the Andean ridge and genetic differentiation based on F_{st} values (Figure 6). Further, populations separated by the Andean ridge did not display higher genetic differentiation than distance would predict, indicating that gene flow is not limited by the Andean ridge in *Z. capensis* and that the ridge is a leaky barrier. Some populations not separated by the ridge exhibited high genetic differentiation, indicating that some other mechanism, such as distance or ecological variables, may be more important than this barrier. For example, Papallacta was more genetically similar to populations on the inter-Andean side of the ridge than to the closer east slope populations (Figure 8a-d). These patterns indicate that the Andean ridge is not playing a role in population divergence in this system.

In contrast, many bird populations separated by the Andes Mountains are recognized as distinct taxa (Brumfield and Capparella 1996; Burney and Brumfield 2009). Further, the radiations of numerous bird families (furnariidae Derryberry et al. 2011), genera (*Muscisaxicola*

Chesser 2000; *Tangara* Burns and Naoki 2004; *Thamnophilus* Brumfield and Edwards 2007), and species (*Thamnophilus caerulescens* Brumfield 2005) have been attributed to the Andes. The Andes may be a particularly disruptive barrier for sedentary lowland species (Burney and Brumfield 2009; Naka et al. 2012). In other taxa, the Andes have been shown to play a role in the divergence of frog (Katten et al. 2004) and mouse populations (Salazar-Bravo et al. 2001). Further, other geographic barriers, such as rivers, have been shown to play a role in genetic population structure among tropical bird populations (Alexio 2004; Naka et al. 2012); however not in Amazonian frogs (Boul et al. 2006; Funk et al. 2009).

Genetic population structure could be driven by elevation if populations exhibit differences in physiological parameters due to demanding cold and hypoxic environments that parallel elevation (Cheviron et al. 2011). Among our study sites, we did not find a relationship between elevation and genetic populations structure. Our sites varied little in elevation (differences in elevation among populations range: 0– 1410m, average 580m), which may explain a lack of a relationship between these variables. Similarly, in the Peruvian Andes, Cheviron and Brumfield (2009) found that elevation had no effect on gene flow based on nuclear DNA of *Z. capensis* when examined over a larger range (maximum elevational gain 3900m). In contrast, the same study found a relationship between mitochondrial F_{st} and elevation (Cheviron and Brumfield 2009).

In the parapatric speciation model, increased geographic distance results in more genetically distinct populations without the presence of a geographic barrier (Irwin et al. 2001, 2005). Consistent with this model, our results show that F_{st} values increased with geographic distance separating populations (Figure 6). These results provide evidence for a pattern of isolation by distance driving population genetic divergence. Similarly, among populations of *Z.*

capensis in the Peruvian Andes, nuclear F_{st} (but not mitochondrial DNA) was associated with geographic distance (Cheviron and Brumfield 2009). In tropical suboscines, clinal variation in genetic differentiation without the presence of a geographic barrier has been reported (*Thamnophilus caerulescens* Brumfield 2005). In contrast, in a review of 40 Neotropical bird species, geographic distance did not appear to predict genetic differentiation (*Z. capensis* not sampled; Burney and Brumfield 2009). In tropical frogs, genetic divergence was correlated to geographic distance in some species (*P. pustulosus* Ryan 1996; Pröhl et al. 2006), but not others (*Physalaemus petersi* and *P. freibergi* Funk et al. 2009; *Dendrobates pumilio* Wang and Summers 2010).

Song has been suggested to be a potential premating barrier to breeding, causing a reduction of gene flow among populations, and resulting in genetically separated populations (Nottebohm 1969; Baker and Cunningham 1985; Rothstein and Fleischer 1987; Balaban 1988; Martens 1996; Price 2008). Our results indicate that song is likely not a mechanism driving population structure in *Z. capensis* because there was no consistent relationship between song differences and genetic differentiation (Figure 9). Populations on the inter-Andean slope demonstrate high variability in differences in song within and among genetic clusters. On the east slope, namely between Papallacta and Baeza–Yanayacu, the high genetic isolation over a short distance (32 km) without the presence of a geographic barrier, and the moderate song differentiation, suggests that song is not driving population divergence among these populations. Rather, both song differentiation and genetic differentiation increase with geographic distance, with a stronger relationship between genetics and distance, indicating that distance is the best predictor of population divergence in this study system,

In tropical oscines, there are limited examples of culture as a driver of population divergence. Interestingly, Tobias et al. (2010) have shown in suboscines that song is not correlated to genetics despite its presumed genetic basis. In contrast, tropical frogs (Ryan 1996; Funk et al. 2009) and singing mice (Campbell et al. 2010) display relationships between vocalizations and genetic population structure. Thus, the role of song in driving genetic population structure is inconsistent among tropical populations.

Initiation and Maintenance of Cultural Divergence

Our results show geographic variation in culture over a short geographic distance. Geographic patterns of culture may arise due to drift (Fitzsimmons et al. 2008) and/or selection pressures (Podos 2001). Microsatellites have a high mutation rate; however, it is suggested that cultural traits such as song in oscines can mutate at an even faster rate (Lynch 1996). Culture evolves because mutations that arise from errors or innovations during the song-learning phase are incorporated into an individual's song (Marler and Tamura 1964; Slater 1989). Cultural mutations are then passed through populations via young birds that learn songs from parents (Grant and Grant 1996) and neighbors (Bell et al. 1998). Cultural mutations are transmitted at a proportionately faster rate through small populations (Price 2008). Differential selection of these mutations driven by female choice (Derryberry 2007) and male-male aggression (Luther and Derryberry 2012; Goodale and Podos 2010) among sites may lead to geographic variation in culture.

For geographic differences in song to be maintained, individuals must disperse within their dialectal group (Nelson et al. 2001; Podos and Warren 2007). If individuals disperse outside of their dialectal group new songs may be introduced into the recipient populations, perhaps

leading to the homogenization of dialects. Typically birds disperse after the song crystallization period (Grant and Grant 1996); however, some species, such as the brown-headed cowbird, (*Molothrus ater*) learn their songs post-dispersal (Rothstein and Fleischer 1987). In other species, bird song may have longer crystallization periods (Baptista and Petrinovich 1984; Petrinovich and Baptista 1987) or be more plastic post-crystallization (Nelson et al. 1996), such that dispersers can match the local dialect post-dispersal thus leading to genetic mixing (Bell et al. 1998; Nelson et al. 2001; Slabbekoorn and Smith 2002). If males match the song of the population into which they disperse, geographic variation of dialects would be maintained despite gene flow, thus disrupting the relationship between song dialects and genetic divergence. In *Z. leucophrys* the plasticity in song learning and the capacity to learn new songs largely depends on if the population is migratory or sedentary, with migratory sparrows exhibiting more plasticity in song learning (Nelson et al. 1996, 2001). Migratory species typically disperse greater distances than sedentary species; therefore, we predict that *Z. capensis* disperses short distances and may not have the capability to learn new songs post-dispersal. A dispersing male's inability to sing the local song dialect may result in reduced fitness if females prefer local dialects (Baker et al. 1981, 1982; MacDougall-Shackleton et al. 2001, 2002; Searcy et al. 2002; Patten et al. 2004; Danner et al. 2011).

In birds, females typically disperse greater distances than males (Greenwood and Harvey 1982). Here we show an even sex ratio of individuals that exhibit more than 50% of their genetic make-up belonging to another cluster. However, most individuals with 30-49% of their genetic make-up assigned to another cluster were males. This suggests that male *Z. capensis* may disperse greater distances than females, as seen in *Z. leucophrys* (Morton 1997). Further, we show localized song dialects with some areas of high genetic isolation between populations and

other areas of low genetic isolation, suggesting that individuals may disperse within and among dialectal groups. However, the greater similarity of songs among neighboring sites supports the prediction of short dispersal distances in *Z. capensis*. Among our study sites, distance and habitat features may be the largest factors that limit dispersal (Beier and Noss 1998; Ibarra-Macias et al. 2011).

The equatorial region has a unique set of abiotic factors including relative aseasonality, constant photoperiod, geographic barriers, and varying climatic conditions, which could give rise to biotic mechanisms such as breeding asynchrony, mate recognition and mate choice, facilitating speciation. Genetic divergence among populations is a critical early step in the process of speciation and is typically attributed to geographic barriers. Our study provides evidence for isolation by distance rather than isolation by geographic barrier, elevation, or song dialect. Song dialects may promote assortative mating through female mate choice, perhaps generating partial reproductive isolation, however, song dialect differences among contiguous populations do not appear to have driven population divergence in this study system.

Acknowledgements:

We thank Bellavista Cloud Forest Reserve research station, San Jorge Ecological, Pululahua Hostel, the Muñoz family, Murray Cooper, Termas de Papallacta, and Yanayacu Biological Station for their accommodations. We thank Nancy Rotzel and T. Brant Ryder for their guidance in the lab, Daniel Mennill and Jeffrey Podos for their help with the song analyses, and Jeffrey Walters for comments on this paper. All procedures were approved by the Virginia Tech IACUC. We acknowledge funding from a Smithsonian Institution pre-doctoral fellowship (JED), Virginia Tech Graduate school (JED), the Center for Conservation and Evolutionary

Genetics for all laboratory supply costs, and The American Ornithologists' Union (JED). I.T.

Moore acknowledges support from NSF IOS-0545735.

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TABLES:

Table 1: Our eight study sites with corresponding coordinates, elevation, and sample size of songs analyzed and individuals used for genetic analyses. W= west, I= inter-Andean valley, E= east.

site	northerly	westerly	elevation	slope	N songs	N genetics
Bellavista	S 00°00	W 078°41	2300	W	22	19
San Jorge	S 00°07	W 078°31	3200	I	11	14
Pululahua	N 00°02	W 078°29	2300	I	23	20
Tumbaco	S 00°13	W 078°24	2500	I	17	19
Pintag	S 00°22	W 078°22	2900	I	32	28
Papallacta	S 00°21	W 078° 9	3300	E	39	42
Baeza	S 00°27	W 077°51	1890	E	23	21
Yanayacu	S 00°36	W 077°53	2100	E	22	26

Table 2: Geographic distances (km) separating the eight study sites.

	Bellavista	Baeza	Papallacta	Pintag	Pululahua	San Jorge	Tumbaco	Yanayacu
Bellavista	0							
Baeza	109	0						
Papallacta	77	32	0					
Pintag	58	57	25	0				
Pululahua	30	92	60	50	0			
San Jorge	27	83	51	33	18	0		
Tumbaco	42	67	35	20	30	16	0	
Yanayacu	124	16	47	72	107	98	82	0

Table 3: Partial Mantel tests used to evaluate potential mechanisms of cultural and genetic divergence grouped by part of song. First two letters are being compared while controlled for the third letter. S= song, G= genetics, R= Andean ridge, E= elevation, D= distance. Bold face indicates significance.

Partial Mantel Comparison	Theme		Trill		Full Song	
	Mantel r	<i>p</i> value	Mantel r	<i>p</i> value	Mantel r	<i>p</i> value
SR, D	0.084	0.37	-0.4	0.972	-0.358	0.948
SE, D	-0.022	0.522	-0.367	0.965	-0.36	0.96
SE, G	-0.052	0.583	-0.42	0.982	-0.434	0.984
SD, G	0.04	0.385	0.417	0.005	0.415	0.007
GR, D	-0.048	0.568	-0.048	0.568	-0.048	0.568
GE, D	-0.088	0.637	-0.088	0.637	-0.088	0.637
GE, S	-0.17	0.798	-0.257	0.933	-0.31	0.966
GD, S	0.76	0.0004	0.83	0.0003	0.829	0.0003
SG, E	-0.422	0.95	-0.273	0.85	-0.403	0.945
SG, D	-0.3	0.939	-0.44	0.998	-0.5	0.999

Table 4: Characteristics of 13 microsatellite loci used on equatorial populations of *Zonotrichia capensis*. Forward and reverse primer sequence, MgCl₂ concentrations, annealing temperature (Ta), sample size (N), number of alleles (Na), size range (bp), and original reference are listed.

Locus	Primer sequence forward	Primer sequence reverse	MgCl mm	Ta	N	Alleles per locus	size range (bp)	Original Reference
Zole_A08	ACCCAAAGTGCAAATCCCATC	ACAAAGTCCCGTTTTCTTGC	1.6	59	187	10	223-264	Poesel et al. 2009
Zole_C06	CCAGCCTGATTTCCCATGC	TGTTGAGCATCTCTGGAGG	1	59	188	15	182-240	Poesel et al. 2009
Zole_C07	TGCCAGCAACTCTGCCTC	TGAGCTTCCAGCCCTCAG	1	59	188	25	163-256	Poesel et al. 2009
Zole_C11	TCCATGCTTCTGAACTGCC	ACACCTGCTTTTCTGACTG	1.6	59	184	15	134-194	Poesel et al. 2009
Zole_C12	TAGGCAGGACAGCAAGAC	ACTACCAGAACCACTAGGGG	3.5	59	188	13	193-233	Poesel et al. 2009
Zole_E02	GAGAACAGTCAAAGGGCCC	GGCATCCTTCCCCTCAGTC	2.5	59	187	7	227-241	Poesel et al. 2009
Zole_F11	AACCAAGCCACCACAATGC	GACAGGCACTAGGATGGGAG	2.75	59	189	11	212-252	Poesel et al. 2009
Zole_G03	GCCAGGTACACATCCTGC	CTGGCAITCCAAGCTGGG	2.5	59	189	15	191-251	Poesel et al. 2009
Gf01	TAGCAITTCATGTAGTGTATTAA	TTATTATGTTCATATAAACTGCATG	2.5	50	188	22	142-184	Petren 1998
Gf06	GCTATTGAGCTAACTAAATAAACAAC	CACAAATAGTAATTAAGGAAGTACC	2.5	50	188	6	183-195	Petren 1998
Zole_H02	ACTGTTCTTTTCTCCACCCAC	GGTTGAATCCAGGTGGAAAC	2	59	189	16	160-220	Poesel et al. 2009
Meyu4	CCTGGCAGGTTTTATTGTGG	AGTGCAGCAAAAAGAGGAACC	1	55	187	17	162-202	Double et al. 1997
Pd0u5	GATGTTGCAGTGACCTCTCTG	GCTGTGTTAATGCTATGAGG	2	54	183	12	192-216	Griffith et al. 1999

Figure Legends:

Figure 1: Map of Ecuador (© Google 2012) with example sonogram for each sampling location.

Dashed line represents approximate location of Andean ridge.

Figure 2a: Example amplitude spectra denoting measurement locations on the theme and trill portion of a song: a) peak maximum (V), b) peak frequency, c) minimum frequency (kHz) and d) maximum frequency (kHz), and e) bandwidth.

Figure 2b). Waveform and spectrograph depicting measurement locations of fine structural characters on a song from Baeza. f, h) duration and number of notes in theme g, i) duration and number of notes in trill, j) bandwidth of longest note in the theme, l) maximum frequency at the start of the first note in the trill, m) minimum frequency at the end of the last note in the trill, n) maximum and o) minimum frequency averaged across all notes in the trill, p) maximum bandwidth for all the notes in the trill.

Figure 3: Canonical plot of discriminant function analysis using a) all 22 structural measures on the full song, b) 9 structural measures on the theme, or c) 13 structural measures on the trill.

Each circle represents the 95% confidence limit on the multivariate mean for each study site.

Non-overlapping circles represents sites that are significantly different in song dialects. Baeza and Yanayacu have overlapping circles indicating that these sites have very similar song structures. Letters represent individual males and their sampling location: Bellavista (B), San Jorge (S), Pululahua (U), Tumbaco (T), Pintag (I), Papallacta (A), Baeza (Z), and Yanayacu (Y).

Figure 4: Population pairwise comparisons of differences in a) full song, b) theme, or c) trill measurements and geographic distance. To compare song differences among sites, we first used MRPP (R ‘vegan’ package) to calculate overall differences in song features by site and then we used function ‘meandist’ to calculate mean distance to each site’s centroid. Population pairs separated by the ridge, marked as black triangles, do not show higher differences in song than distance would predict. Populations in the same genetic cluster are represented by open circles (east slope) and open squares (inter-Andean slope), and populations that are in separate genetic clusters are notated by filled circles (east slope) and filled squares (inter-Andean). Structure outcome $K = 5$ is used for population genetic clustering. Song differences among sites were correlated with geographic distance.

Figure 5: Population pairwise comparisons of differences in a) full song, b) theme, or c) trill measurements and elevation. Populations separated by the Andean ridge are marked as black triangles, populations in the same genetic cluster are represented by open circles (east slope) and open squares (inter-Andean slope), and populations that are in separate genetic clusters are notated by filled circles (east slope) and filled squares (inter-Andean). Structure outcome $K = 5$ is used for population genetic clustering. Song differences among sites were correlated with geographic distance.

Figure 6: Population pairwise comparisons of differences in F_{st} value and geographic distance. Populations that are furthest apart geographically display the highest F_{st} values, supporting a pattern of genetic isolation by geographic distance. Populations separated by the Andean ridge, marked as black triangles, do not show higher genetic isolation than distance would predict,

indicating that the ridge is not driving population divergence. Populations in the same genetic cluster are represented by open circles (east slope) and open squares (inter-Andean slope), and populations that are in separate genetic clusters are notated by filled circles (east slope) and filled squares (inter-Andean). Structure outcome $K = 5$ is used for population genetic clustering.

Figure 7: Population pairwise comparisons of differences in F_{st} value and differences in elevation. Populations separated by the Andean ridge are marked as black triangles, populations in the same genetic cluster are represented by open circles (east slope) and open squares (inter-Andean slope), and populations that are in separate genetic clusters are notated by filled circles (east slope) and filled squares (inter-Andean). Structure outcome $K = 5$ is used for population genetic clustering.

Figure 8: Program STRUCTURE diagrams produced between 2 and 5 population clusters. Populations are shown from west to east. The Andean ridge marked as a dashed line is between populations Pintag and Papallacta. a) $K = 2$: Baeza and Yanayacu were assigned as a single population with all other sites as a second population, when no prior information on sampling location was used. b-d) $K = 3, 4,$ and 5 sampling location was used as a prior. Structure and Structure harvester (Earl and vonHoldt 2011) gave heavy weight to 5 population clusters.

Figure 9: Population pairwise comparisons of differences in a) full song, b) theme, or c) trill measurements and F_{st} value. There was no consistent pattern between dialectal structure and genetic structure, indicating that culture is not driving population divergence. Populations separated by the Andean ridge are marked as black triangles, populations in the same genetic

cluster are represented by open circles (east slope) and open squares (inter-Andean slope), and populations that are in separate genetic clusters are notated by filled circles (east slope) and filled squares (inter-Andean). Structure outcome $K = 5$ is used for population genetic clustering.

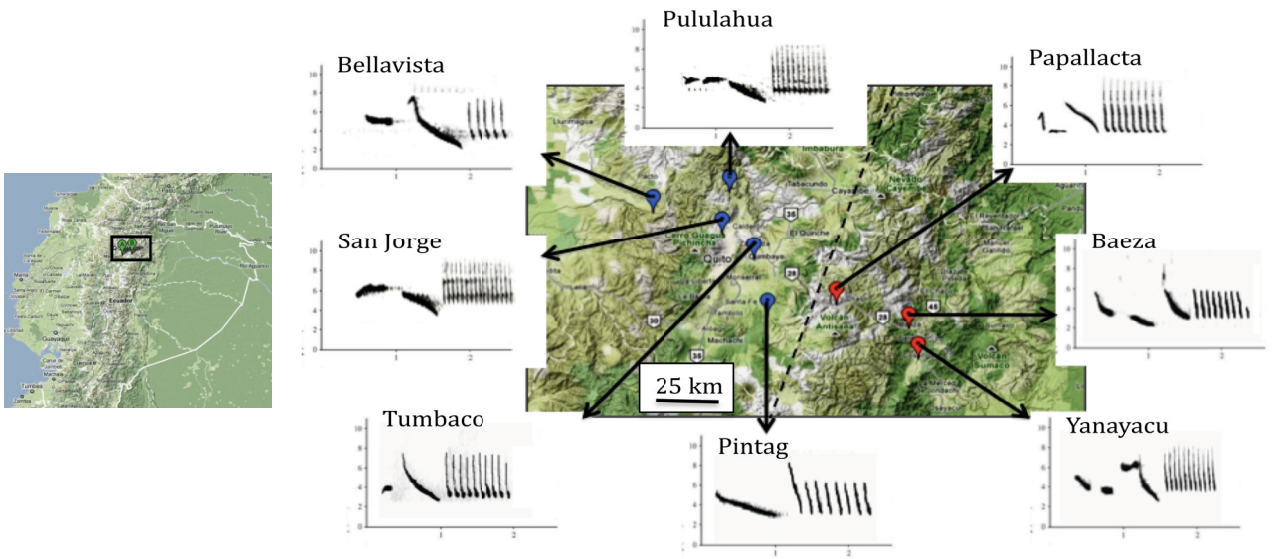


Figure 1

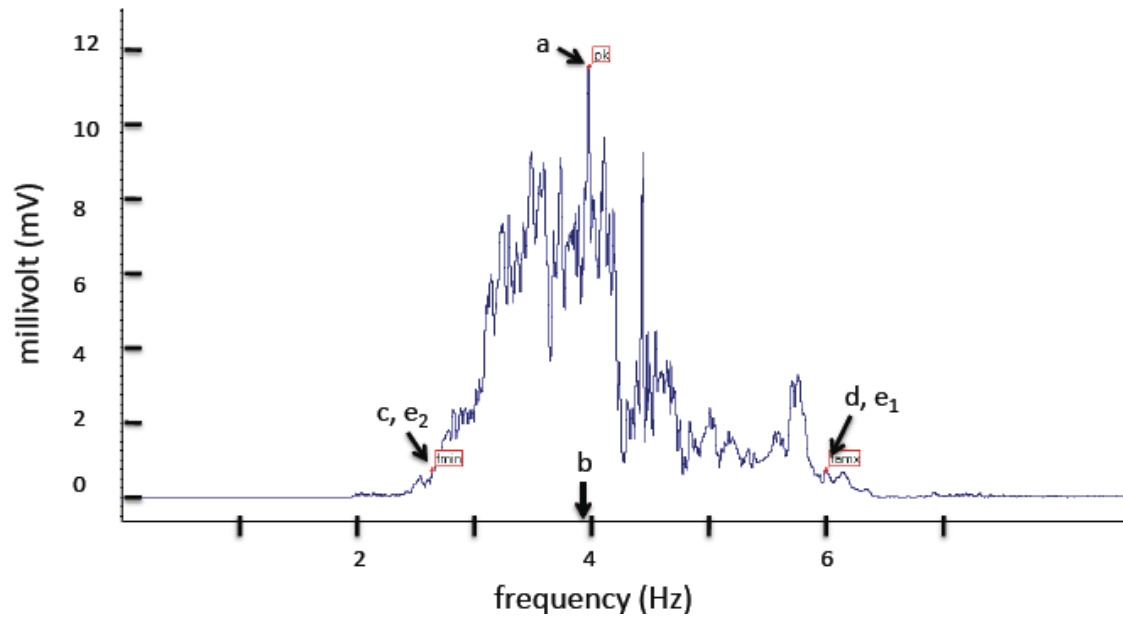


Figure 2a

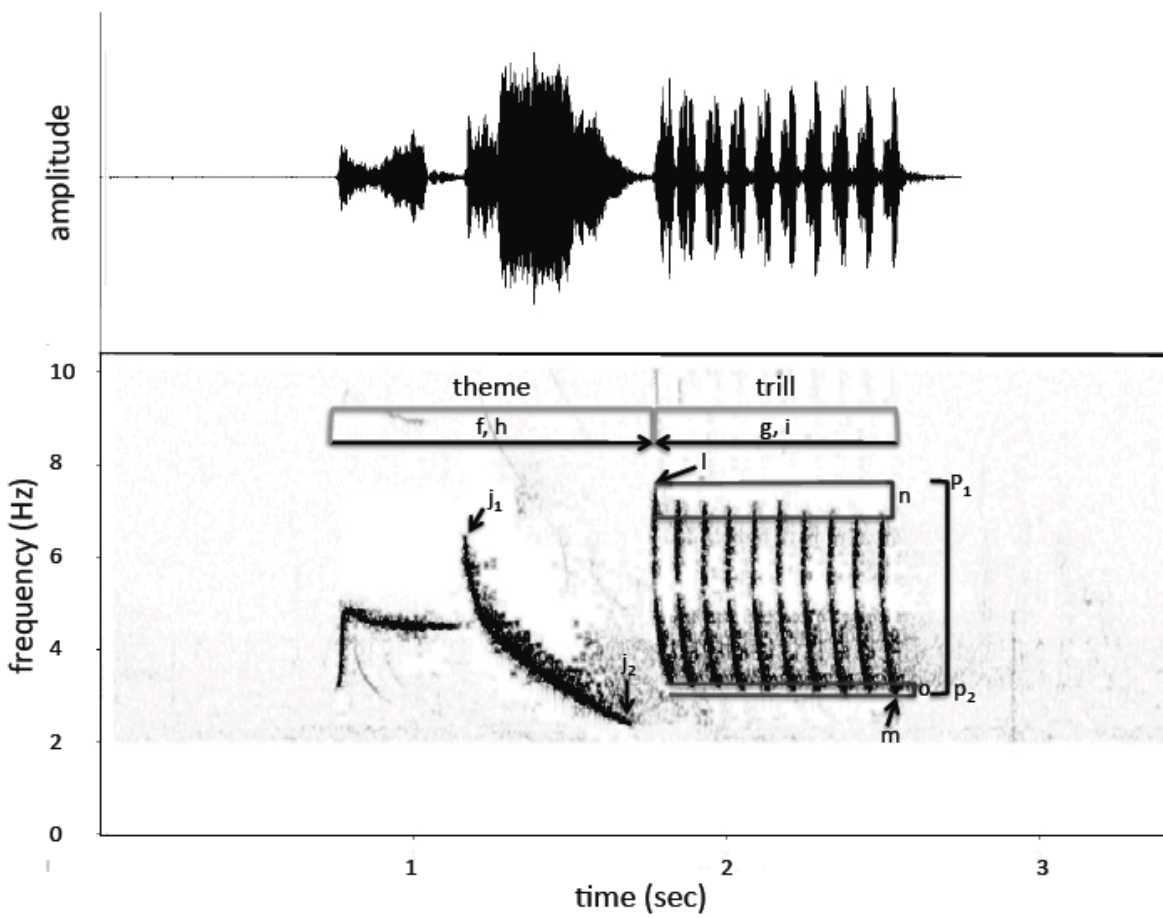


Figure 2b

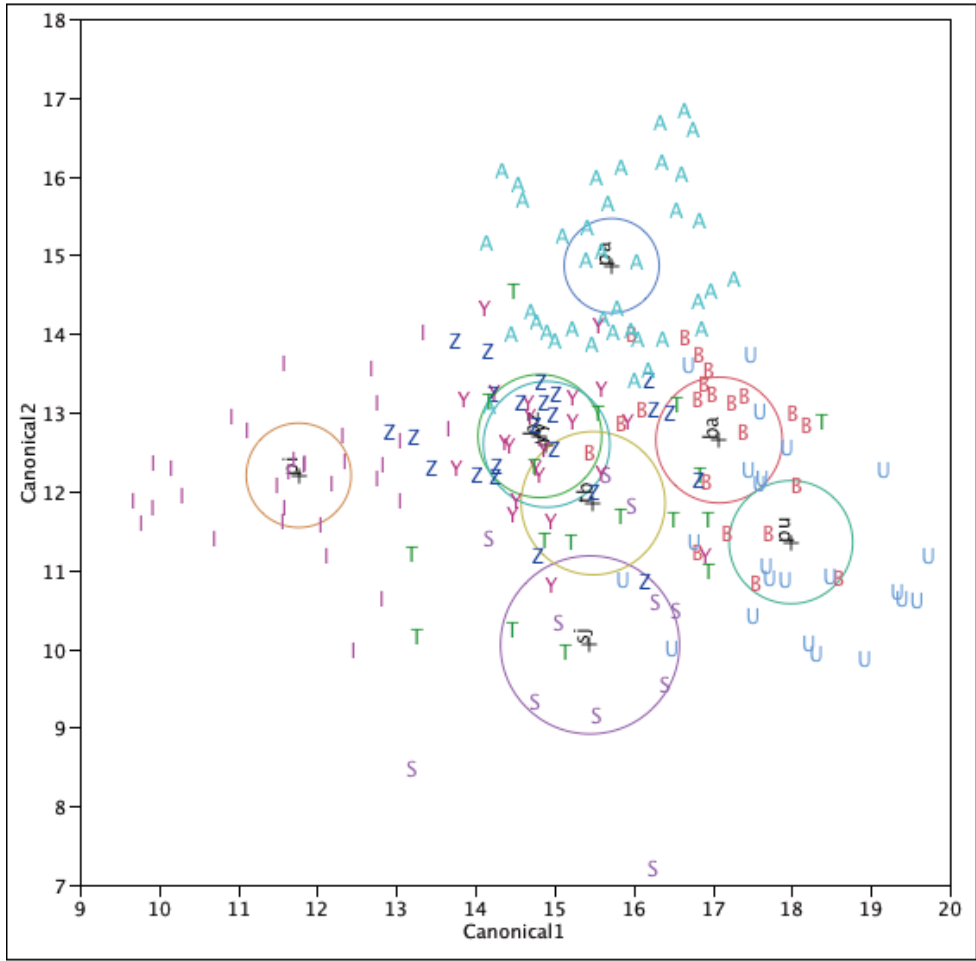


Figure 3a

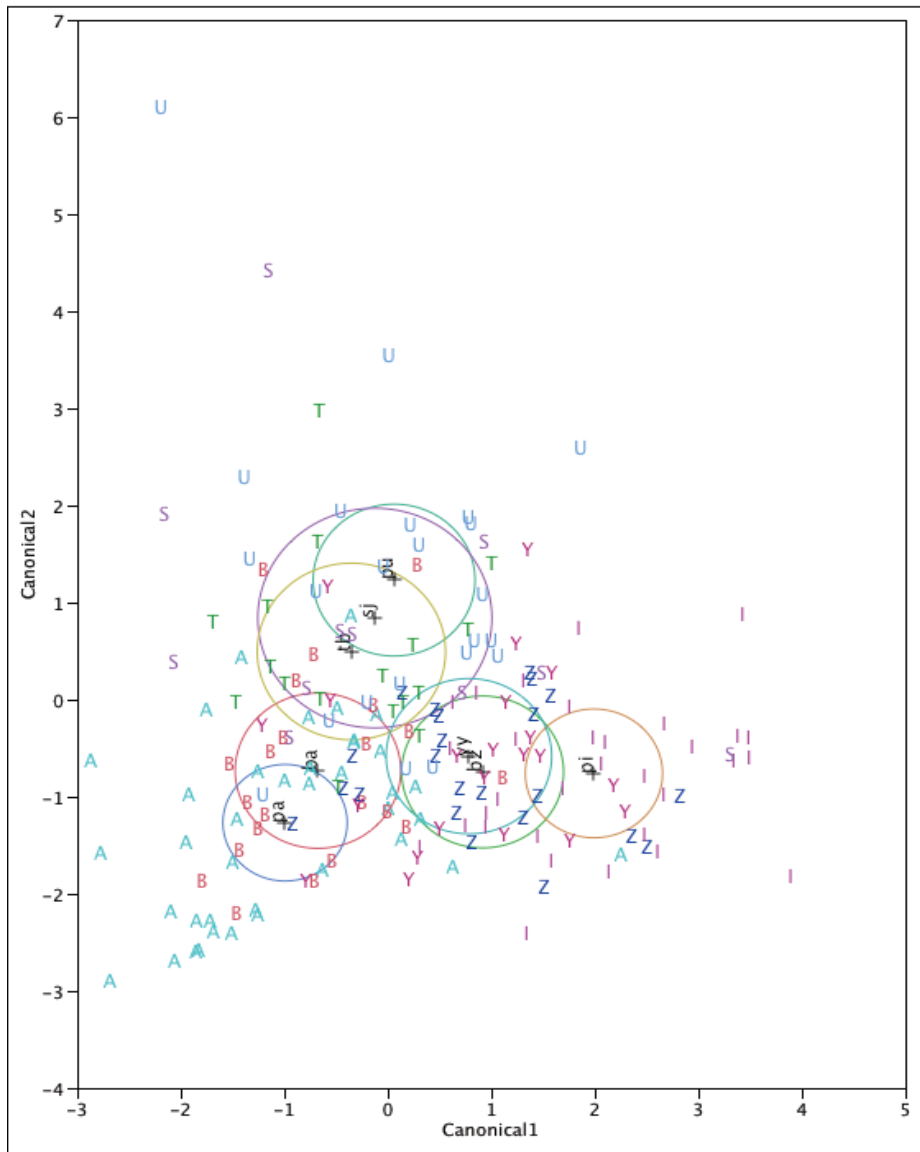


Figure 3b

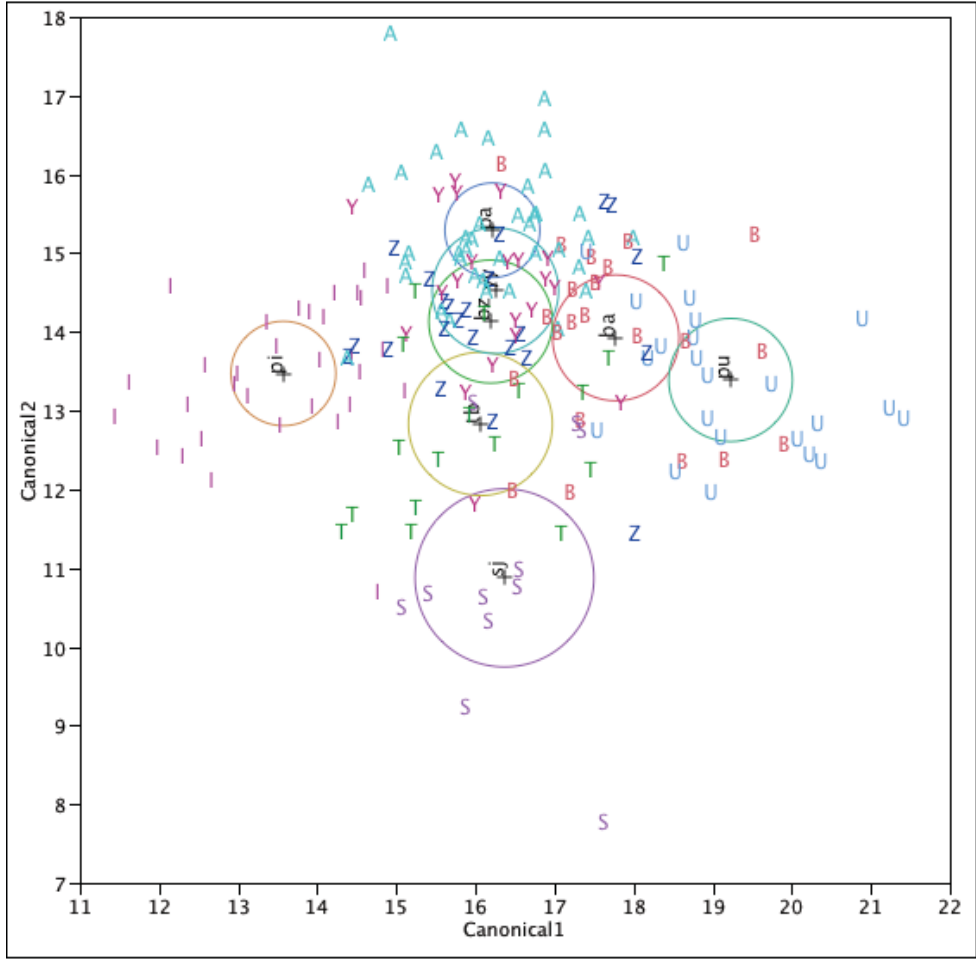


Figure 3c

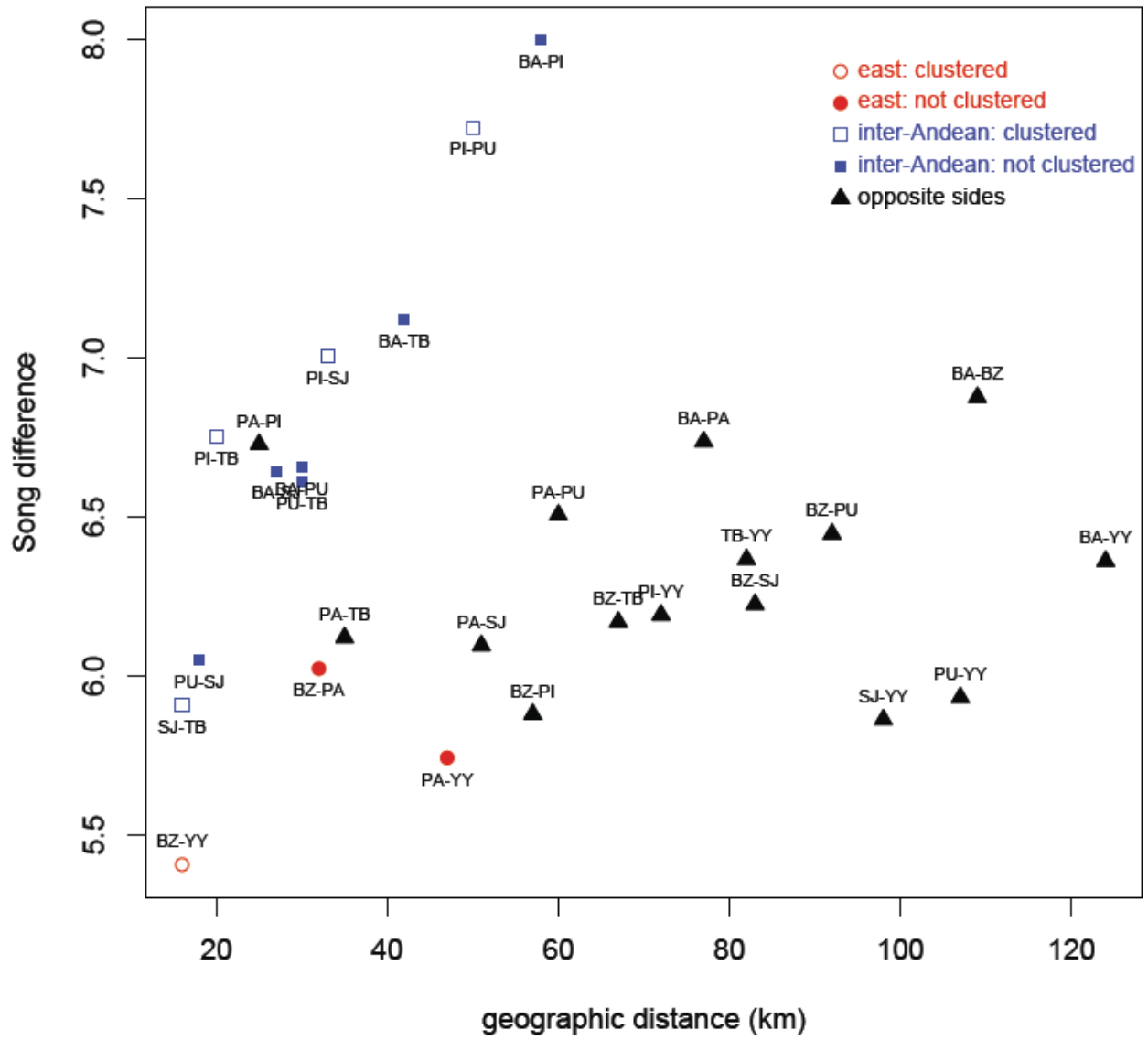


Figure 4a

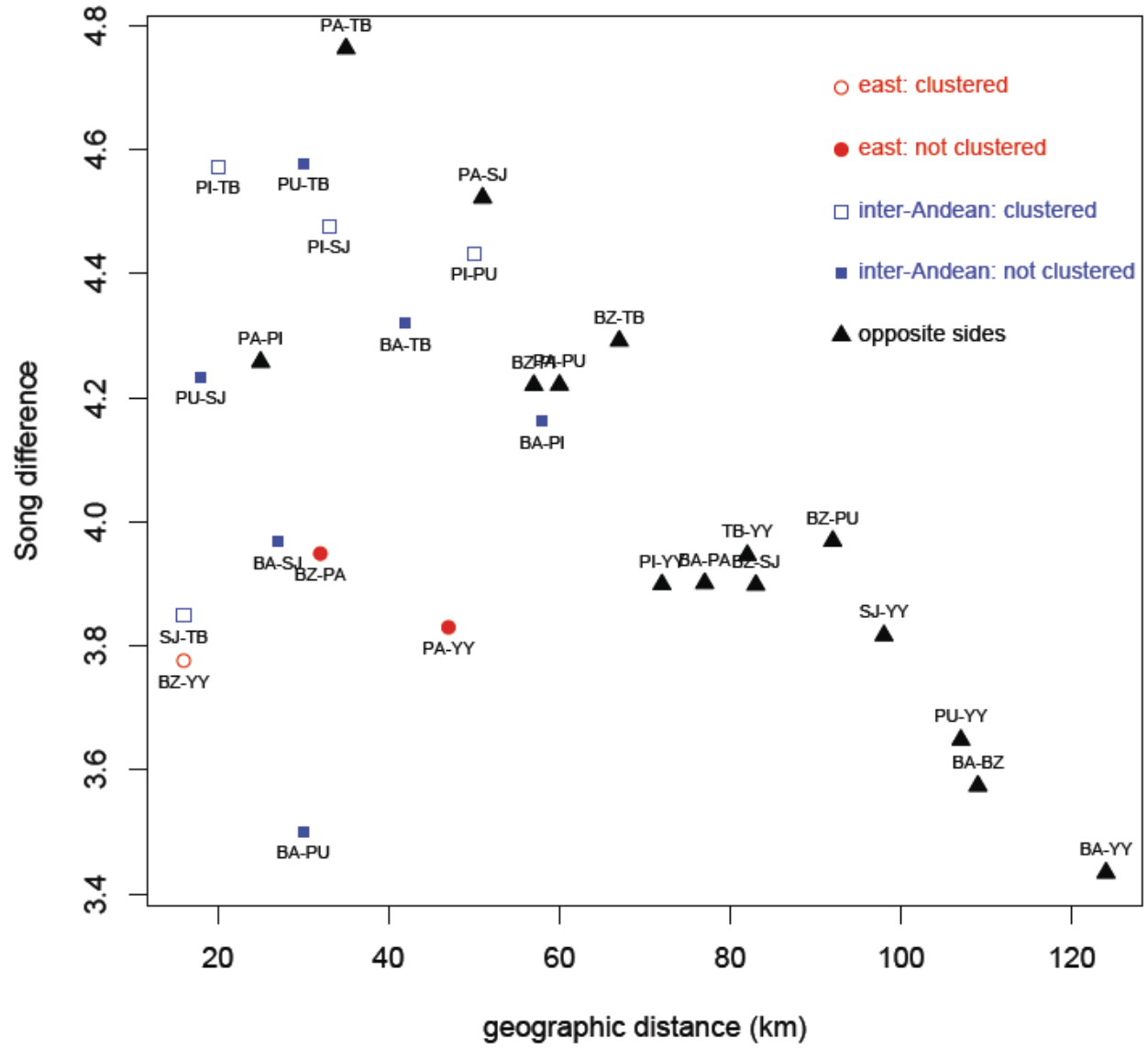


Figure 4b

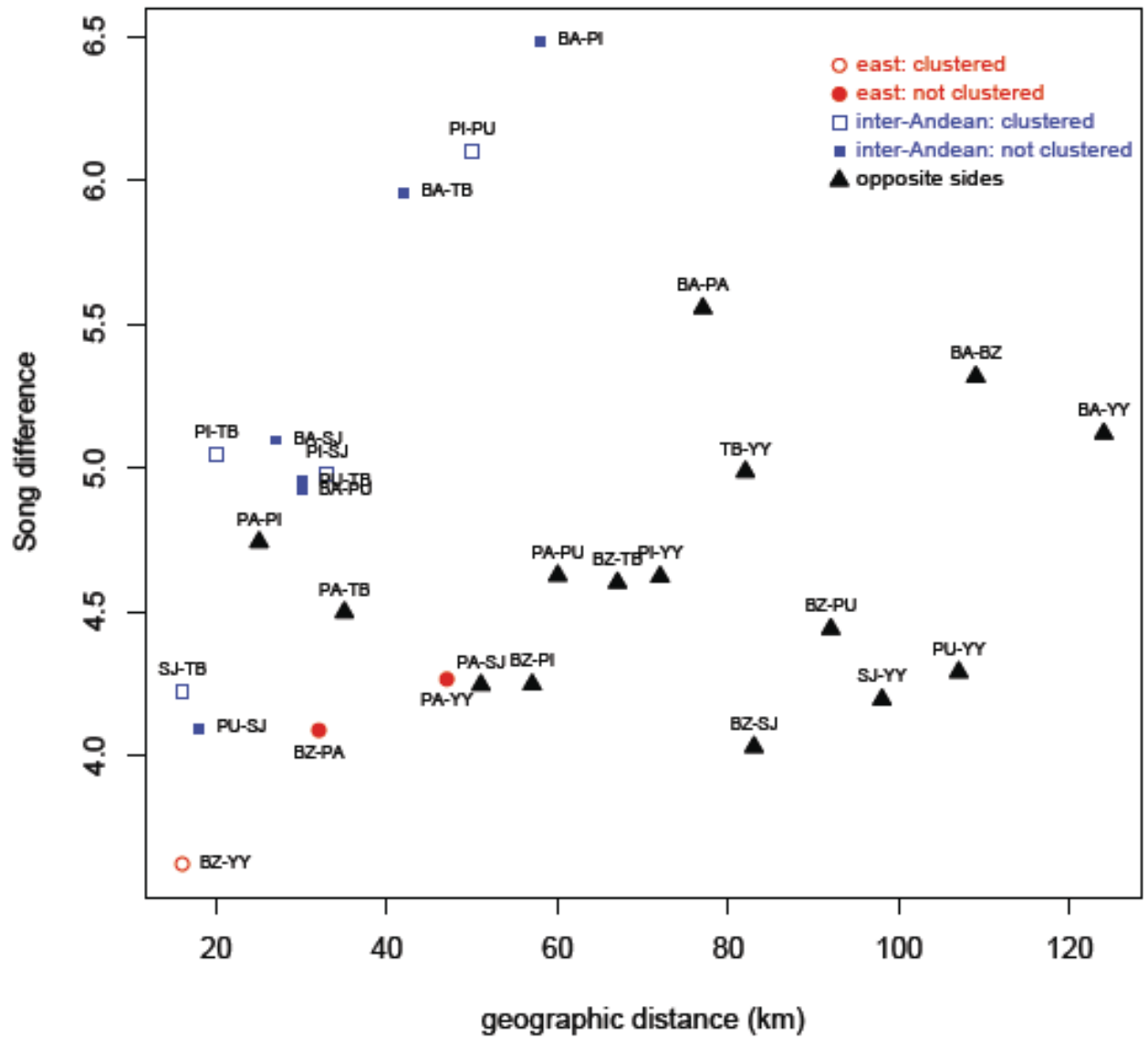


Figure 4c

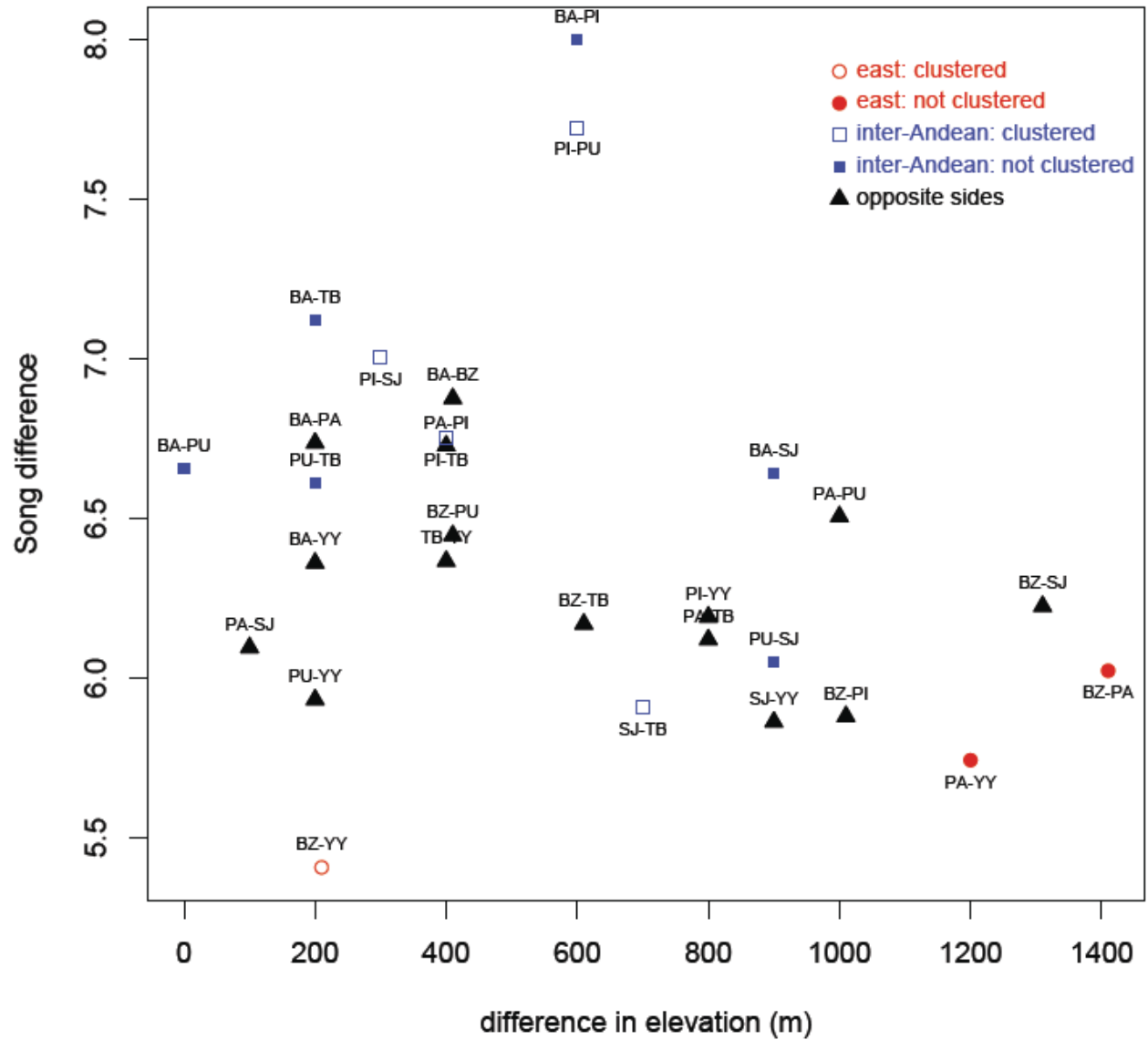


Figure 5a

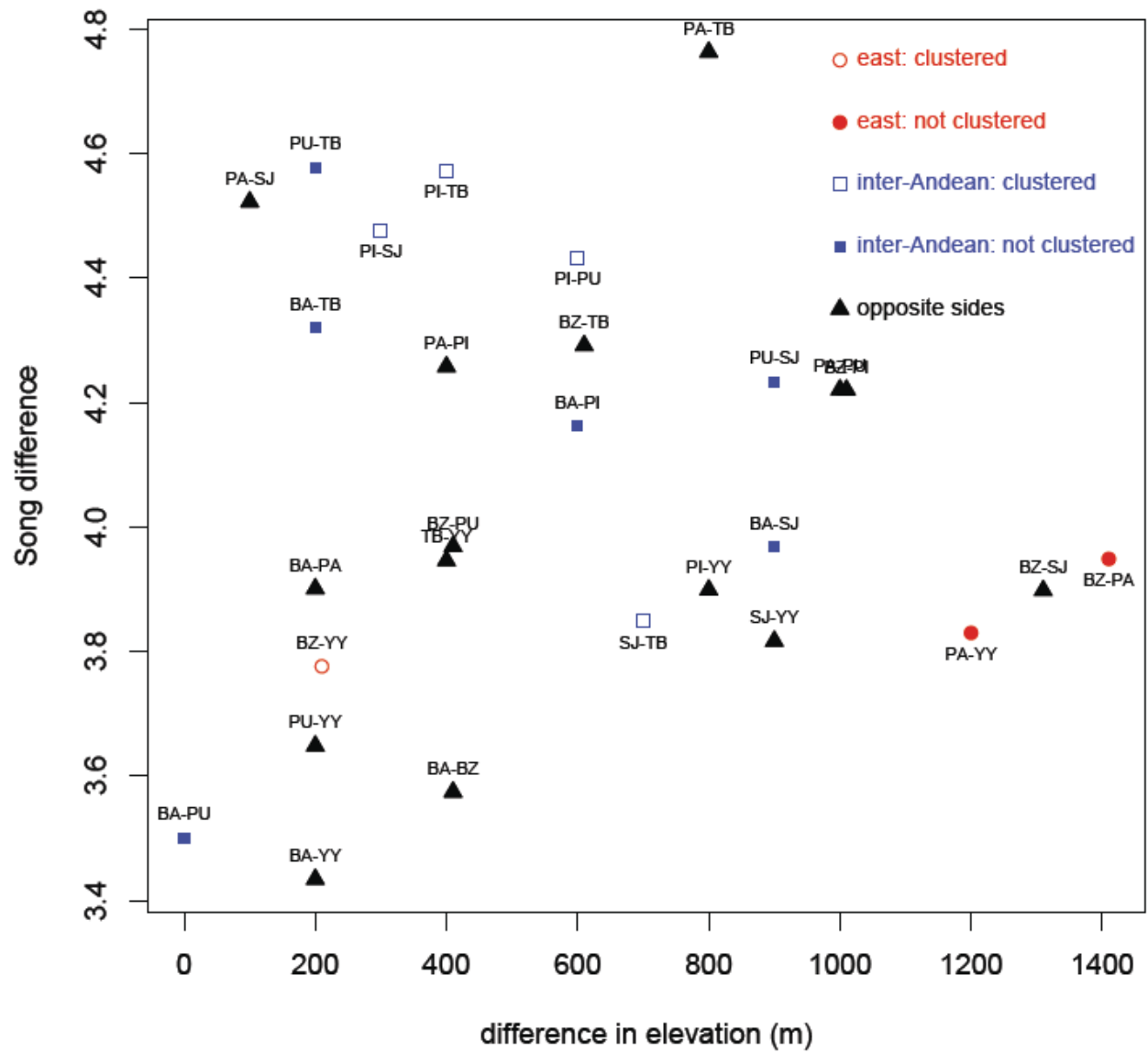


Figure 5b

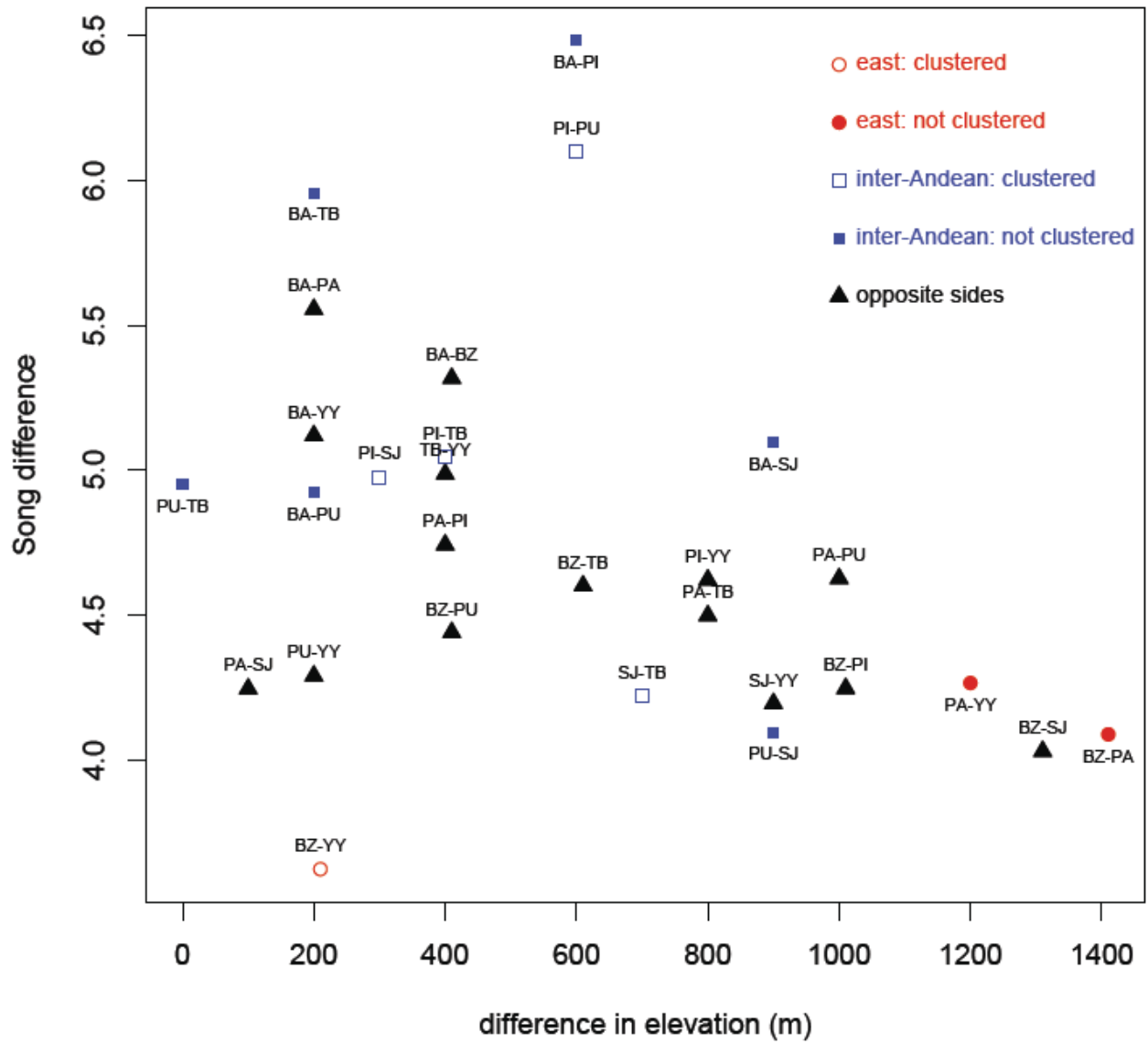


Figure 5c

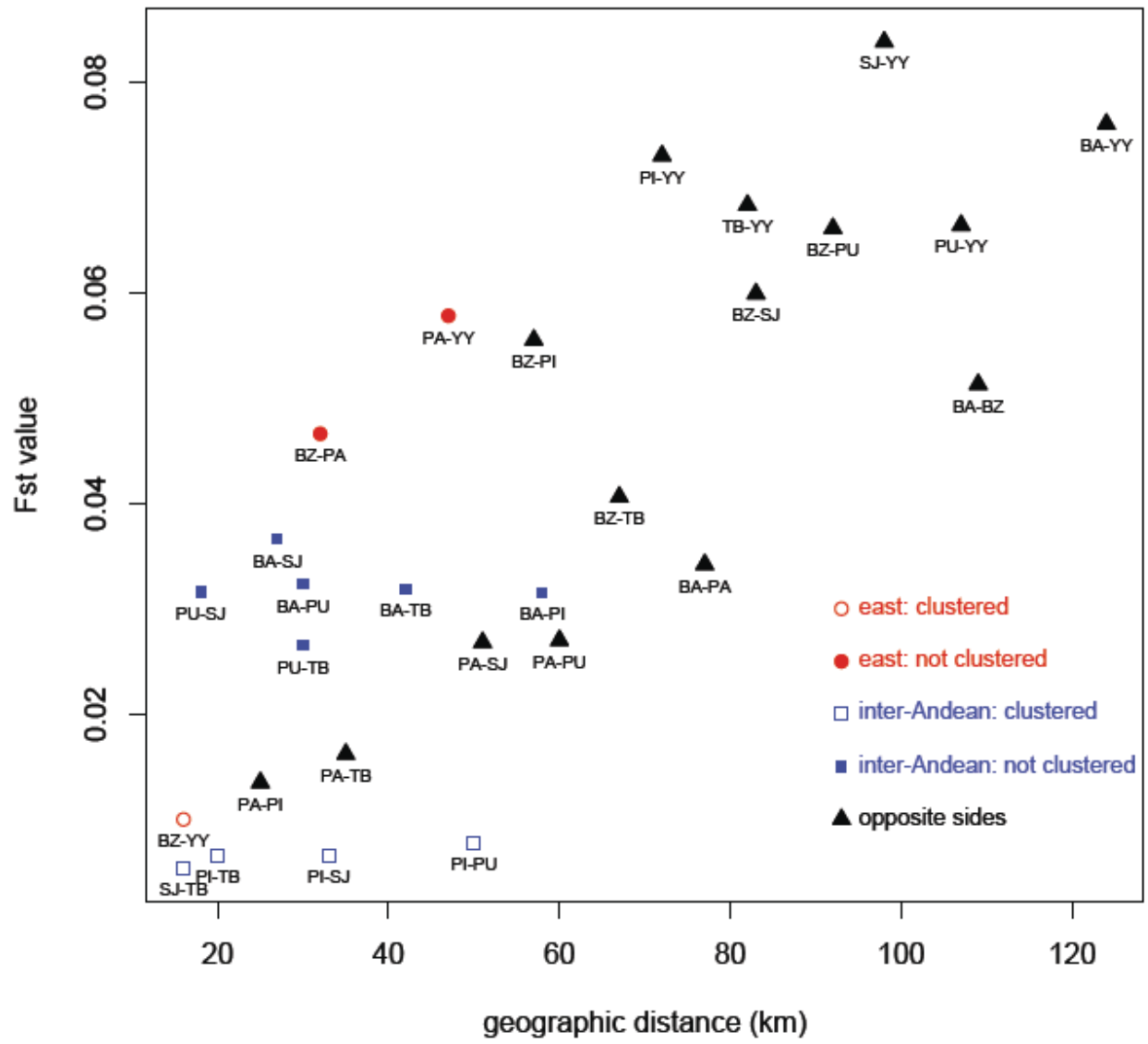


Figure 6

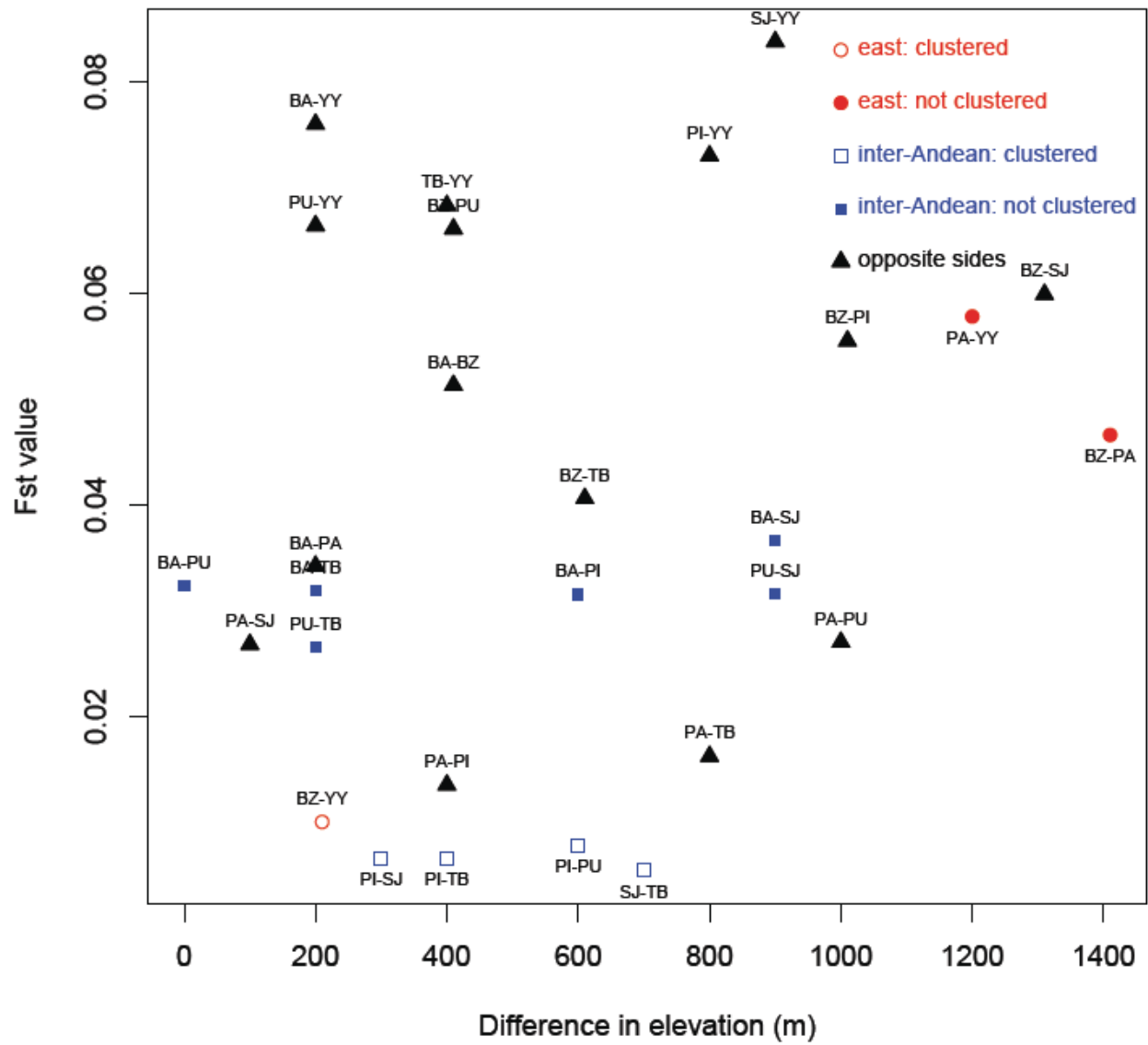


Figure 7

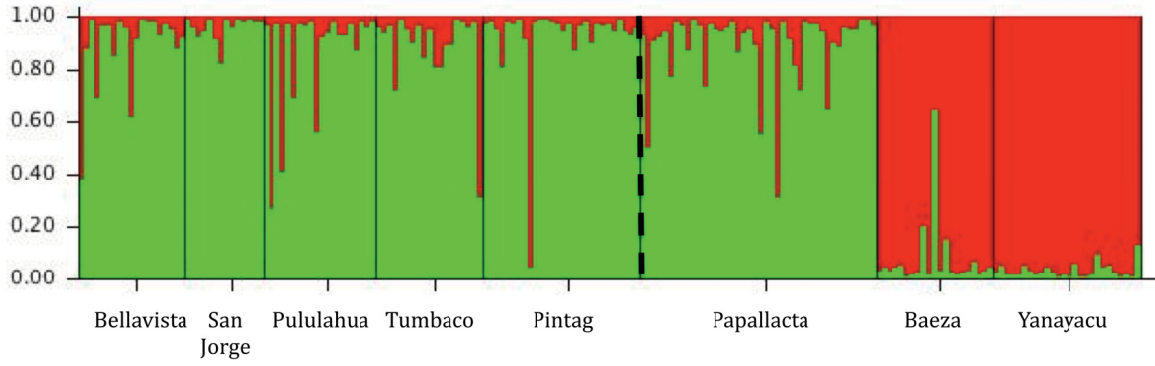


Figure 8a

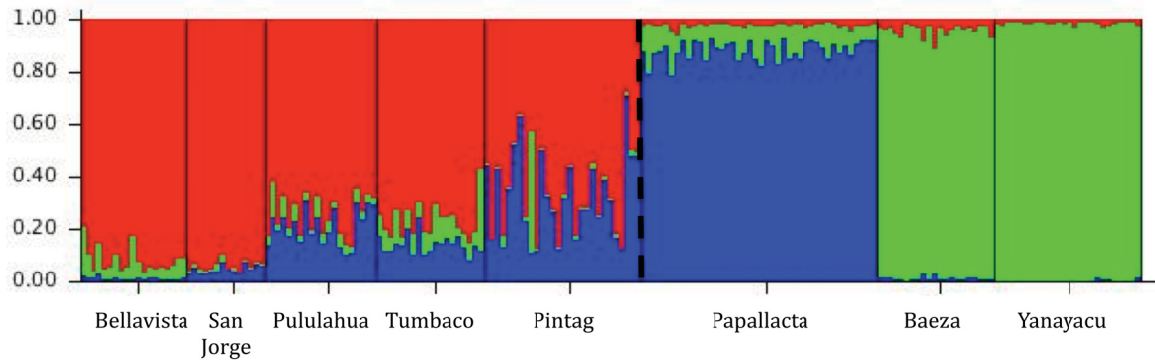


Figure 8b

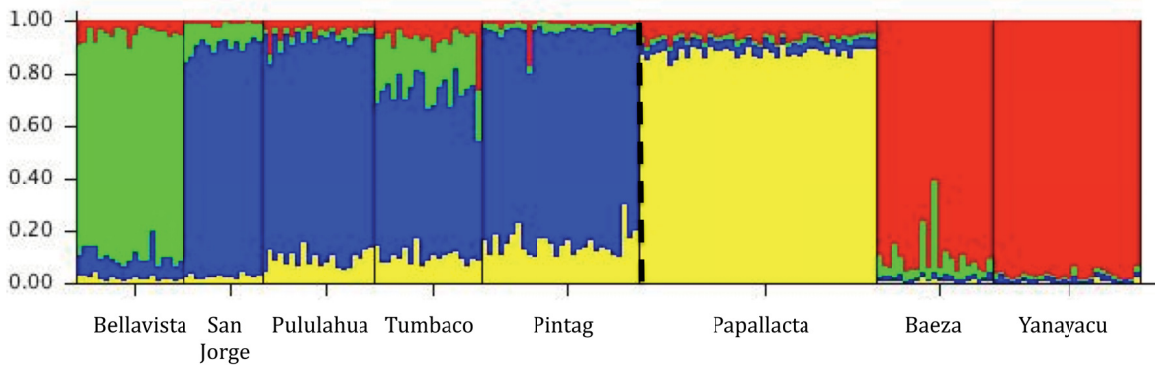


Figure 8c

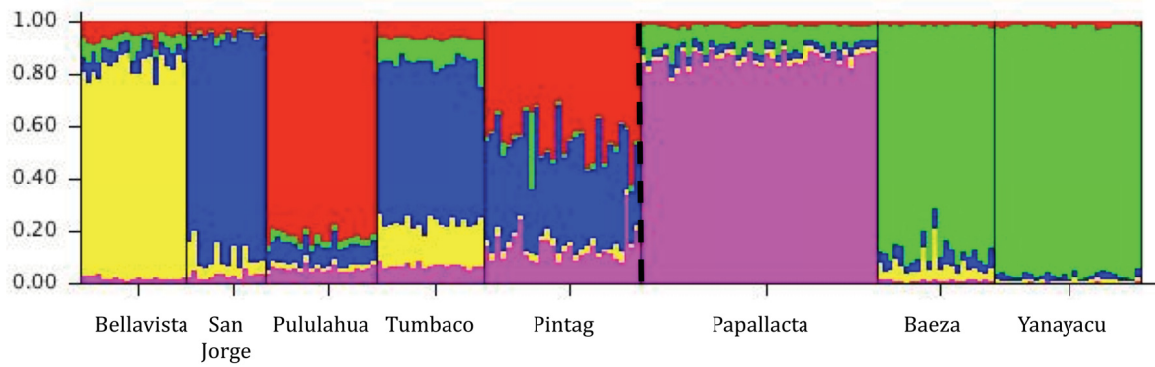


Figure 8d

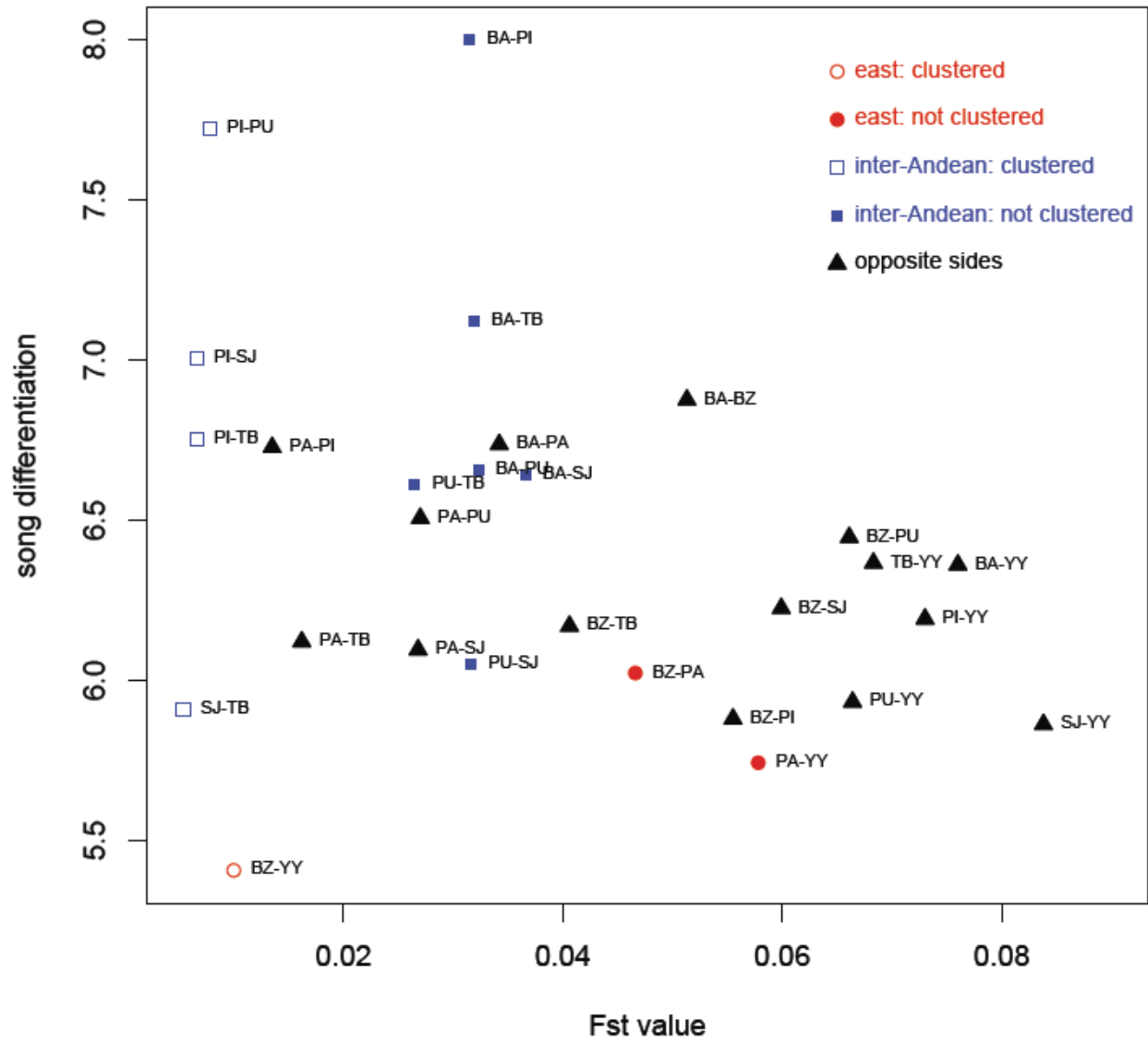


Figure 9a

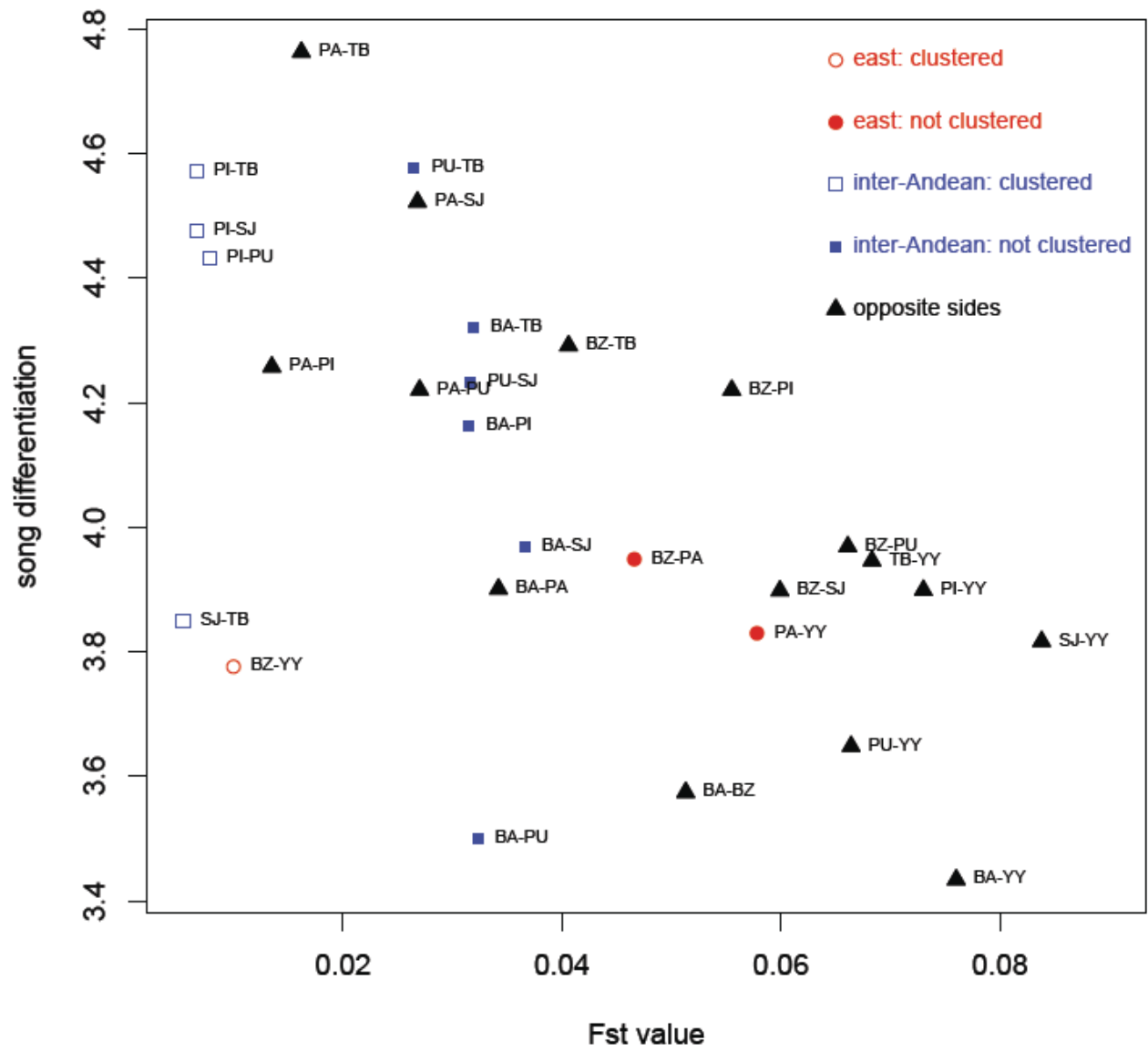


Figure 9b

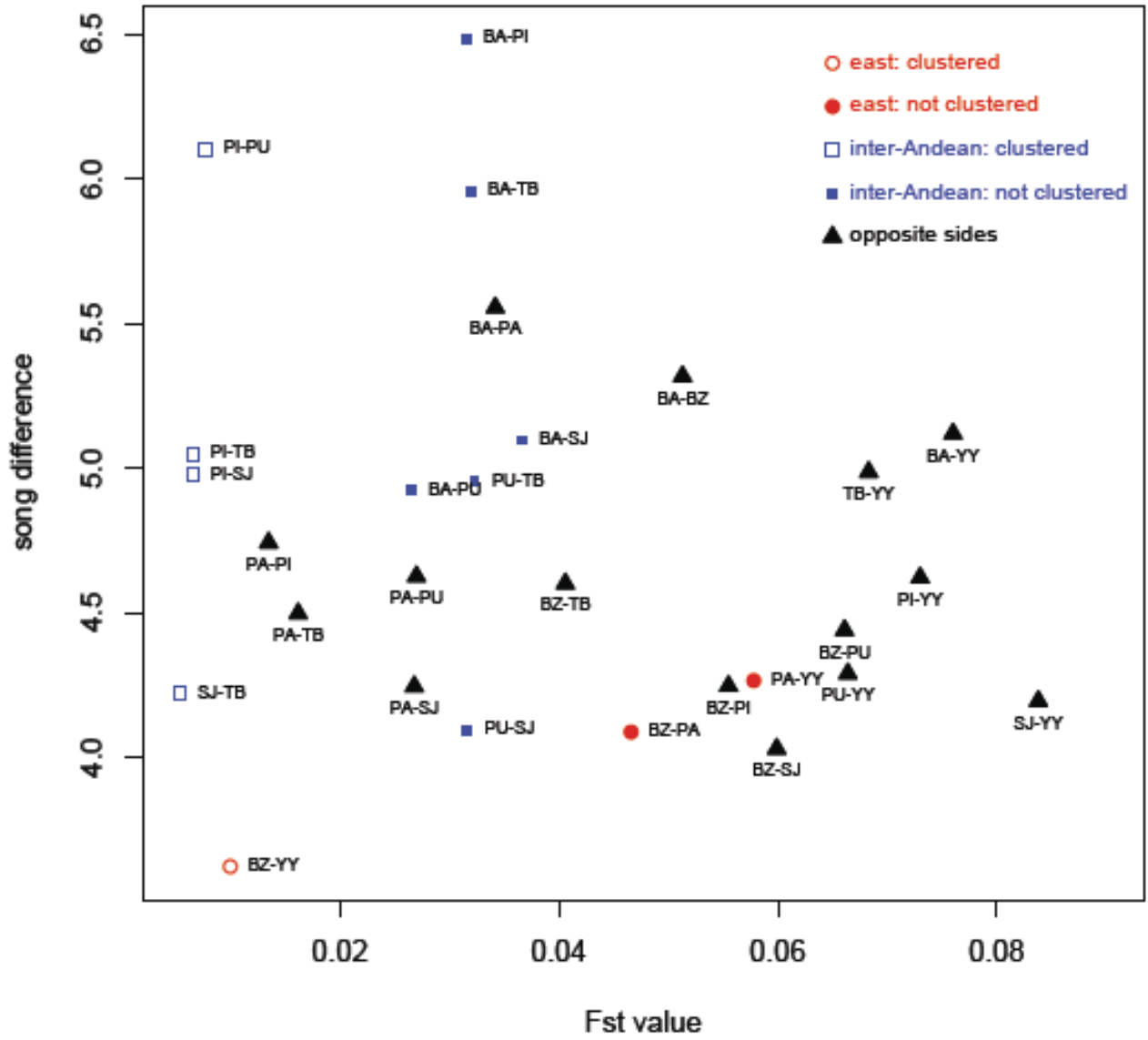


Figure 9c

Appendix

Table Legend:

Table 1: Thirteen microsatellite loci that failed to consistently amplify in *Z. capensis*. Forward and reverse primer sequence and original reference listed.

Locus	Primer sequence forward	Primer sequence reverse	Reference
Zole_A01	TATCGAGCATTGCCCTCCC	GCAGAGTATGAGGTTTTCTTCC	Poesel et al. 2009
Zole_A02	GCAGCCATTTGCTGTCATTC	CCATCTGTCTGTCTTTCTGTCTG	Poesel et al. 2009
Zole_B01	GGACTGTGTTTCACTTCCTATC	ACAGATGTTGCATTGCCG	Poesel et al. 2009
Zole_B03	GCCAAACTCAGTGACCTGC	AGTTCCTGCACGGTTCTTC	Poesel et al. 2009
Zole_B04	CCCATCCTTGCTGAAGTTCTC	GACTCTGGTGTGGTAATGAAGG	Poesel et al. 2009
Zole_C02	TCTGATATTTCAACAGCATGCAC	GCGCAACATTTCCATGCAC	Poesel et al. 2009
Zole_C03	CCTTAGGTAATTGAAAATGTGTGC	CTGTCAGTGTAATAAATCAAAAACACTCTG	Poesel et al. 2009
Zole_E11	AGAATGCTCTGGAACCGGC	AGGACCTGTGTGCCAATTAAG	Poesel et al. 2009
Zole_F09	CAGCCTGTTCCATGCATCC	GCTCGGTTCTTGCTCACAG	Poesel et al. 2009
Zole_G10	TCAGAGTCCCATGTCTCACAG	ACTCTTCCATAAGGGTTGAAATGG	Poesel et al. 2009
Zole_H05	ACTAGACAGAACAACATCATTGC	AGTAGCAGACAGTAAACTTAGAATCC	Poesel et al. 2009
Zole_H11	ACTGTAGCCATCTCATTAGCC	GAGAGAAAGCAAGAAAGCAAGC	Poesel et al. 2009
Dpu16	ACAGCAAGGTCAGAATAAA	AACTGTTGTGTCTGAGCCT	Griffith et al. 2007

Table 1

Appendix Figure Legend:

Figure 1: We used Structure Harvester (Earl and vonHoldt 2011) to calculate ΔK , a measure of the second order rate of change in the likelihood of K (Evanno et al. 2005). ΔK calculations give high weight to the presence of 5 population clusters.

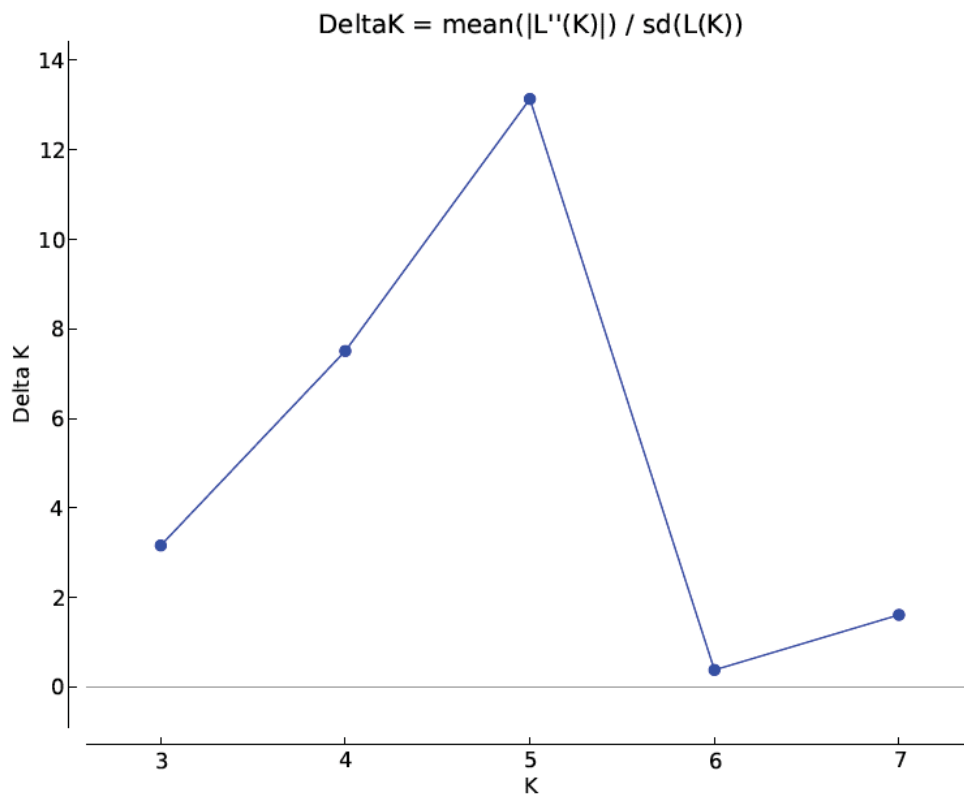


Figure 1

Chapter IV: Conclusions

The tropics are the most biodiverse area on earth, but the mechanisms responsible for the observed diversity are not well understood. The interaction between abiotic factors including relative aseasonality, constant photoperiod, geographic barriers, and varying climatic conditions, may give rise to biotic mechanisms such as breeding asynchrony, competition, mate recognition, and mate choice, which may facilitate speciation. Genetic differentiation, a critical early step in the process of speciation, is typically attributed to geographic barriers. However other mechanisms, such as culture, may facilitate population divergence.

My dissertation is an investigation of the influence that cultural evolution may have on reproductive isolation among populations of tropical sparrows, *Zonotrichia capensis*. I examined four aspects of reproductive isolation: (1) female preference for and (2) male territorial response to song dialects, (3) song dialectal differences among populations, and (4) inter-population genetic structure. In summary, my research shows that song may have an important role in reproductive isolation; however, song appears to be only a partial barrier to breeding and does not currently drive population divergence among contiguous populations. Without the presence of a geographic barrier, it is unlikely that contiguous populations will diverge based solely on song. It appears that female preference for song may be an important secondary barrier for populations with incomplete barriers to dispersal.

Chapter II: Danner, J. E., R. M. Danner, F. Bonier, P. R. Martin, T. W. Small, and I. T. Moore. 2011. Female, but not male, tropical sparrows respond more strongly to the local song dialect: Implications for population divergence. *The American Naturalist* 178: 53–63.

I demonstrated that females from two allopatric populations prefer the local dialect to all other dialects tested, including those from only 25km away. Female preference for local song may lead to assortative mating creating the opportunity for reproductive isolation. The role of female mate choice in driving reproductive isolation among tropical populations has largely been unexplored. In this dissertation, I investigated female and male behavioral responses to songs because traditionally, only male territorial response to songs has been used as a proxy for estimating reproductive isolation (Grant and Grant 2002; Podos 2007; Uy et al. 2009). The results shown in Chapter II demonstrate that female response to songs, not male territorial response, may have important predictive power in estimating prezygotic reproductive isolation among populations.

Bases for female choice

There are several hypotheses for why females may prefer the local song dialect. Songs may provide females with clues about male quality including adaptation to the local environment (Nowicki and Searcy 2005; Slabbekoorn and Smith 2002; MacDougall-Shackleton et al. 2002). MacDougall-Shackleton et al. (2002) found that song dialect is a reliable index of male parasite load and may be a reliable cue with which females select mates. Another hypothesized basis for female choice in birds is predicated on vocal performance limits. Podos (1997) demonstrated that the trill in emberizid sparrows have a performance limit, where bandwidth decreases as syllable repetition rate increases and that birds with larger bills may be more constrained (Podos 2001). Variation in bill morphological features has been demonstrated among dialectal populations of Argentinean *Z. capensis* (Lougheed and Handford 1992, 1993), which might be attributed to adaptation to local food resources (Podos 2001) or to variation in environmental temperatures

among populations (Greenberg et al. 2011, Greenberg at al. accepted). Further, it has been shown in swamp sparrows (*Melospiza georgiana*) that females prefer high performance songs (birds with smaller bills) (Ballentine et al. 2004).

Future Directions

Future research might include investigating the bases for female choice. Examining the prevalence and lineage of blood parasites in *Z. capensis* among the study populations may provide insight on why females prefer the local dialect; a local male might be better adapted to the local environment. Further, population comparisons of *Z. capensis* performance limits and morphological measures, correlated to habitat, might provide an example of different selection pressures influencing song dialects. Female choice trials that examine different levels of song performance and distinguish which parts of the song (theme or trill) elicit responses may provide insight into female choice patterns and how these patterns influence the evolution of song and premating isolation.

Chapter III: Cultural evolution and genetic structure in a tropical bird, the rufous-collared sparrow (*Zonotrichia capensis*)

I verified that cultural evolution is not driving genetic population structure in eight populations of *Z. capensis* because there was no consistent relationship between dialects and genetic differentiation. Populations separated by the ridge did not show more increased song or genetic differentiation than distance would predict, indicating that the Andean ridge is not driving population divergence. This lack of a relationship is interesting because many bird populations separated by the Andes Mountains are recognized as distinct taxa (Brumfield and

Capparella 1996; Burney and Brumfield 2009). Despite the Andean ridge exceeding *Z. capensis* elevational gradient, the Andean ridge appears to be a leaky barrier, allowing gene flow.

Elevation was not correlated to dialects or genetics, indicating that there is gene flow across our elevational gradient. Song differences and genetic isolation increased with geographic distance indicating a pattern of isolation by distance. The clinal variation in culture and genetics may present an opportunity for parapatric speciation among populations.

Future Directions

An analysis of the frequency at which different song types occur in *Z. capensis* might provide a more precise examination of geographic variation of dialects. The variation between song types is exhibited in the theme portion of the song; the theme may be used by other birds for individual identification and may be useful for investigating bases of female choice. Additionally, less than 25% of recorded males in Papallacta sang two song types, exhibiting variation in the first notes of the theme (Danner unpublished data). An in-depth analysis examining the prevalence of more than one song type among populations and determining if the second song type is similar to songs in that population, will provide a more accurate representation of song dialects in the study populations.

Testing mechanisms that may drive cultural evolution may provide insight into the persistence of structural characters and the shaping of birdsong. For example, proximity to anthropogenic noise may shape the evolution of birdsong (Luther and Derryberry 2012) and the majority of the inter-Andean sites were located in and around the sprawl of Quito. If a rural bird disperses into an urban environment, the male's song may not be heard over the city noise. An assessment of anthropogenic noise among sites and relating it to song features would determine

if city birds sing a different dialect than urban birds. This might provide an example of selection driving dialects in a bird that thrives in human disturbed habitats.

Since the Andean ridge was not a complete isolating barrier, a thorough assessment of landscape features that likely affect gene flow and dispersal patterns among the study populations might provide insight into other potential isolating mechanisms. Applying Wang et al.'s (2011) landscape genetics framework might provide a more accurate assessment of the relationship between song and genetics. This approach would include gene flow estimates between populations, constructing least-cost paths of dispersal and movement between sites, and a model fitting process to infer costs of traversing each habitat type. Related to this, future directions might include an investigation of *Z. capensis* dispersal patterns to provide information on colonization pathways and movement between dialectal groups.

Conclusion

Identifying the role that sexual selection plays in reproductive isolation has been a major yet unresolved topic in evolutionary biology. My dissertation project takes steps to address the role that song dialects and female mate choice play in population divergence of tropical oscines. I have found that female choice of song dialects may be an important secondary reproductive barrier among populations; however, other mechanisms, specifically geographic distance, appear to play a larger role in population divergence. Still many questions remain about mechanisms of reproductive isolation in the tropics, and this dissertation provides a useful foundation.

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