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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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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; Koscinski 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; Lougheed 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

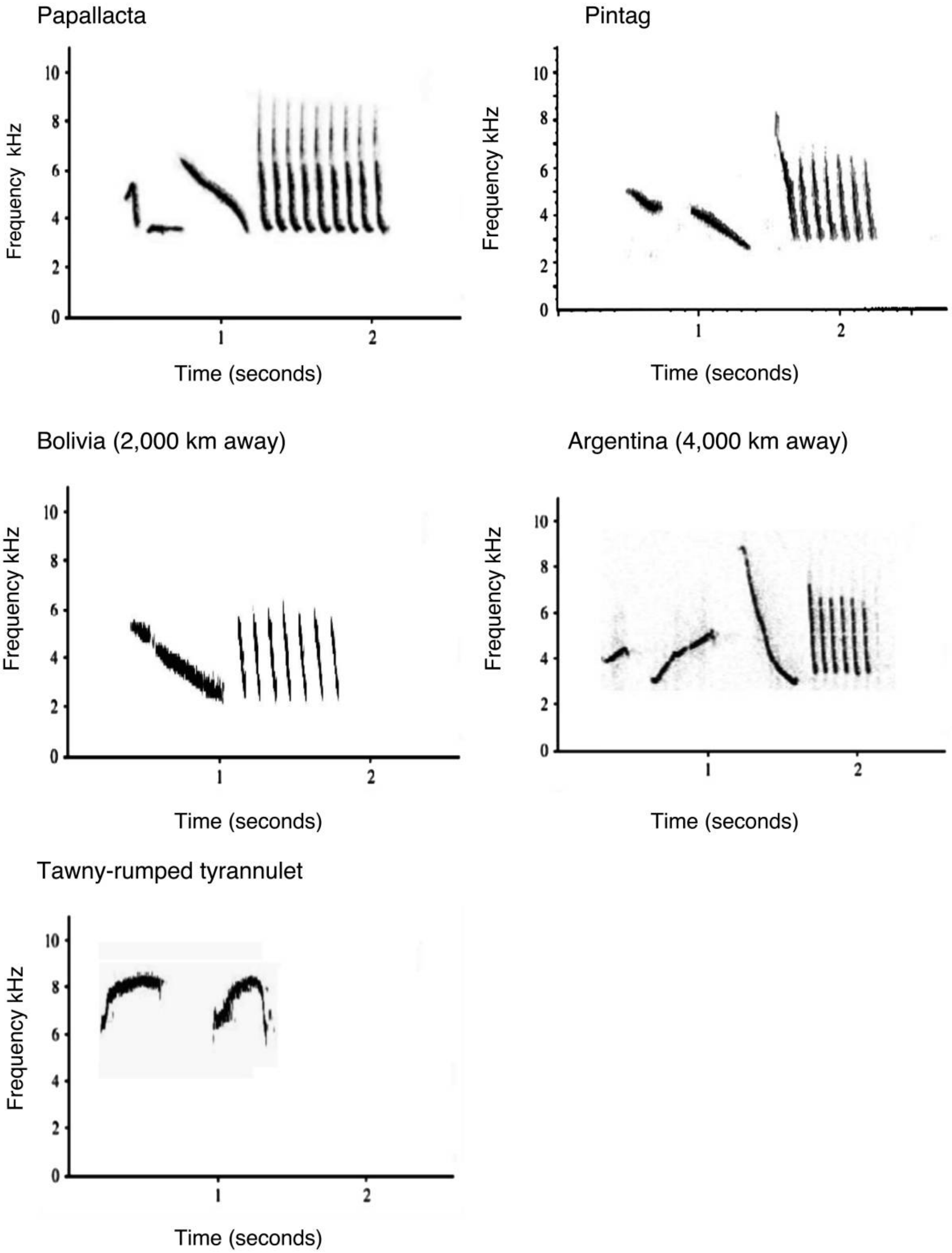
Zonotrichia capensis is a widespread Neotropical emberizid sparrow that breeds from southern Mexico to Tierra del Fuego and occupies a wide range of elevation (from sea level to 4,000 m). We conducted song-playback experiments on females and males from each of two allopatric equatorial populations of *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 17β-estradiol (length $=$ 12 mm, inner diameter $=$ 1.47 mm, outer diameter $=$ 1.96 mm; Dow Corning; O'Loghlen 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 17β-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 × 50 cm × 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-


Papallacta

Pintag

Bolivia (2,000 km away)

Argentina (4,000 km away)

Tawny-rumped tyrannulet

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 nonlocal 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 behavioral 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; Zeleis 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; Zeleis 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 number 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 (±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-

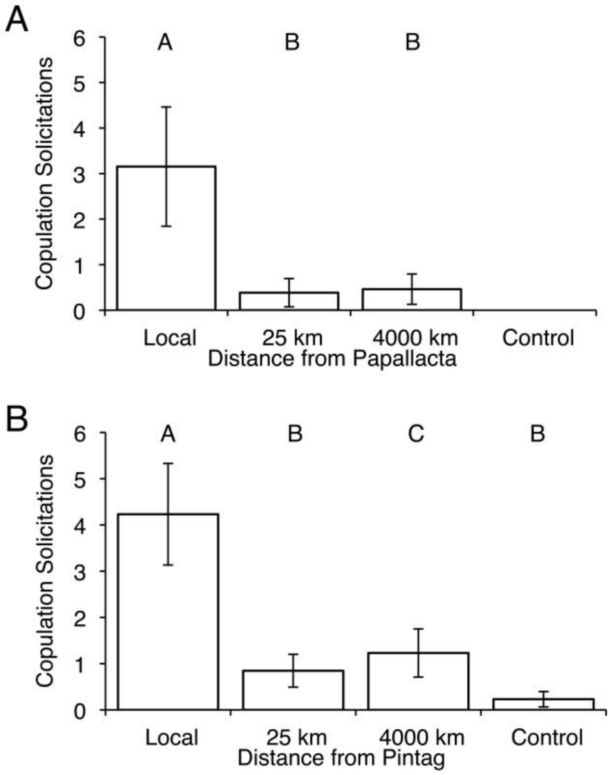


Figure 2: Number of female copulation solicitations in response to each song treatment from each population (mean ± SE). *A*, Papallacta population. *B*, Pintag population. In both populations, female *Zonotrichia capensis* displayed significantly more copulation solicitations in response to their local 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

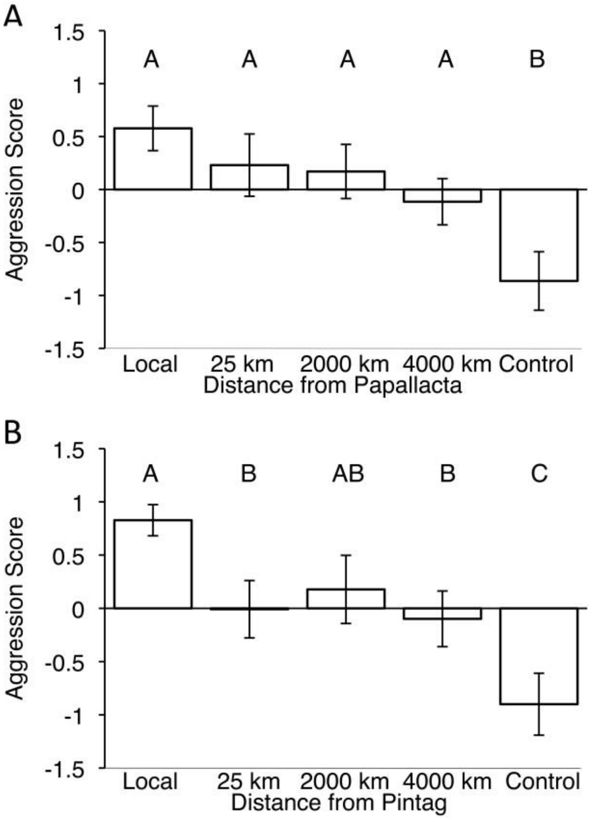


Figure 3: Aggression scores for male *Zonotrichia capensis* (mean ± SE). *A*, Papallacta population ($n = 15$). *B*, Pintag population ($n = 16$). In both populations, male *Z. capensis* showed a similar aggressive response to all conspecific song treatments. The aggression scores are derived from a 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 premating 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 neighbor'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 (Lougheed 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; Lougheed and Handford 1992; Lougheed 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 observed 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.

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