



Original Article

Bill morphology and neutral genetic structure both predict variation in acoustic signals within a bird population

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Adaptive evolutionary divergence within a population can be facilitated by associated divergence in mating signals. Acoustic signals are often involved in mate choice and are also known to diverge spatially in response to a variety of processes. In birds, for instance, variation in bill size and shape can result in correlated changes in vocalizations due to functional constraints on sound production. Acoustic signals can also vary spatially in relation to neutral genetic structure (due to cultural drift) and/or habitat structure (due to acoustic adaptation for optimal sound transmission). Here, we test these alternative hypotheses as causes of variation in acoustic signal structure in the Island Scrub-Jay (*Aphelocoma insularis*), a species that is restricted to one small island (Santa Cruz Island, CA) and exhibits spatial genetic structure and microgeographic divergence in bill morphology across short distances and habitat types. We find that bill morphology is related to the structure of the female “rattle” call, a vocalization associated with territorial disputes and male–female interactions. Females with longer, shallower bills produced calls that were more rapid, and those with shallower bills also produced calls that were lower in frequency. In addition, rattle rapidity varied across the island in accordance with neutral genetic structure. Vocal characteristics were not related to habitat structure, suggesting that variation in rattle calls is unlikely to reflect optimization for sound transmission. Our findings indicate that selection on bill morphology and cultural drift can jointly shape variation in acoustic signal structure, even at fine spatial scales within populations.

Key words: isolation by distance, magic trait, mate choice, microgeographic divergence, vocalization.

INTRODUCTION

The evolution of mating signals has played a central role in driving evolutionary diversification (West-Eberhard 1983). Divergence in acoustic, visual, and behavioral traits that influence mating success can act as a premating isolation mechanism and facilitate speciation through the evolution of reproductive isolation (Lande 1981; Servedio 2004; Ritchie 2007). Mating signals are widely recognized as being important during the reinforcement phase of allopatric speciation (Coyne and Orr 2004). They may also be an important factor underlying the evolution of reproductive isolation and divergence-with-gene-flow when physical barriers to dispersal and gene flow are lacking (Higashi et al. 1999; Balakrishnan and Sorenson

2006; Bolnick and Fitzpatrick 2007; Servedio and Kopp 2012; Richardson et al. 2014).

Theoretical work indicates that mechanisms of nonrandom mating are critical for facilitating divergence in sympatry (Dieckmann and Doebeli 1999; Gavrillets 2004). Even when divergent natural selection is present within a population, recombination and gene flow are predicted to hinder divergence and the generation of nonrandom mating by preventing the build-up of linkage disequilibrium between traits under natural selection and those related to mate choice (Mayr 1963). The problem of linkage disequilibrium can be bypassed if 1) an ecological trait under natural selection contributes directly to mate choice (“magic” trait; Servedio et al. 2011) or 2) an ecological trait is genetically linked with a trait related to mate choice (“non-magic” trait complex; Servedio and Kopp 2012). Traits involved in mate choice can also diverge independently of traits under natural selection and in turn be co-opted to promote evolutionary divergence in sympatry (Thibert-Plante and Gavrillets

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2013). Although this body of theory was developed in the context of sympatric speciation, it is also relevant to understanding how local adaptation can occur at microgeographic scales when populations are subject to the competing effects of gene flow and disruptive or divergent selection (Bolnick 2006; Richardson et al. 2014).

The bird bill is a model trait for studying how divergent selection can lead to nonrandom mating (Podos 2001; Servedio et al. 2011). The bills of Darwin's finches (*Geospiza* spp.), for example, are adapted to foraging on specific food resources (Boag and Grant 1981; Grant and Grant 2011). At the same time, bill morphology is correlated with the structure of species-specific songs (Podos 2001; Huber and Podos 2006). Podos (2001) attributes this finding to the observation that gape width can modify the resonance properties of the vocal tract (Westneat et al. 1993). Larger-billed birds cannot move their bill as quickly to alter gape width (Hoese et al. 2000), a constraint that places an upper limit on the pace and frequency bandwidth of vocalizations that involve frequency modulations within individual notes (e.g., trilled songs; Podos 1997). Since Podos (2001) first described this pattern, other studies on New World sparrows (Ballentine 2006; Derryberry 2009), finches (Badyaev et al. 2008), and woodcreepers (Derryberry et al. 2012) have found evidence that the bill may be acting as a magic trait in those species as well. However, work testing for a relationship between bill morphology and acoustic signal structure has largely been limited to species that sing trilled songs (but see: Seddon 2005; Medina-Garcia et al. 2015) and the degree to which similar physical constraints apply for other types of vocalizations is unclear.

Avian vocal signals can also diverge independently of bill morphology, particularly as a function of dispersal and habitat (reviewed in Podos and Warren 2007). In songbirds, vocalizations are learned from tutors, so geographic variation can arise through the vagaries of cultural transmission (i.e., imperfect copying), especially in species that have limited dispersal (Slater 1986; Slater 1989; Podos and Warren 2007; Fayet et al. 2014). Cultural transmission is predicted to result in an association between neutral genetic structure (e.g., population connectivity) and acoustic signal structure but empirical studies have found mixed support for such correlations (e.g., Loughheed and Handford 1992; MacDougall-Shackleton and MacDougall-Shackleton 2001; Soha et al. 2004; Wright et al. 2005; Nicholls et al. 2006; Ortiz-Ramírez et al. 2016). Alternatively, geographic variation in acoustic signals might reflect acoustic adaptation to different habitats. The acoustic adaptation hypothesis predicts that vocal signals are adapted for optimal transmission in a given environment (Morton 1975; Boncoraglio and Saino 2007). Sounds degrade (i.e., change in structure) as they are transmitted from sender to receiver and vocalizations that have lower frequencies and longer internote intervals are expected to experience the least amount of degradation in densely-vegetated habitat (reviewed in Slabbekoorn and Smith 2002). For example, Nicholls et al. (2006) reported convergence in vocalizations across similar habitats despite on-going gene flow in Satin Bowerbirds (*Ptilonorhynchus violaceus*), which resulted in a mismatch between acoustic signal structure and neutral genetic structure.

Here, we test alternative hypotheses for variation in acoustic signals in the Island Scrub-Jay (*Aphelocoma insularis*), a species with striking variation in neutral genetic structure, bill morphology, and habitat use within its narrow (250 km²) geographic range (Santa Cruz Island, CA). The species is not panmictic and exhibits a continuous pattern of isolation by distance across the longest (east–west) axis of the island. This neutral genetic structure is likely driven by limited dispersal, as individuals have not been observed establishing breeding

territories more than 4 km from their natal territory (Langin et al. 2015). Island Scrub-Jays are also found in 2 different habitat types (oak and pine) and exhibit adaptive divergence in bill morphology at a microgeographic scale: individuals in pine habitat have longer, shallower bills than those in oak habitat (Langin et al. 2015). Interestingly, the morphological differences are not abrupt at the transition between habitat types; instead, bill length declines gradually with distance from pine habitat, consistent with a scenario of divergence-with-gene flow. Although we had no prior knowledge of the acoustic properties of habitats occupied by Island Scrub-Jays, we expected that sound transmission could be spatially variable because the density of woody vegetation is heterogeneous across Santa Cruz Island (e.g., Supplementary Material Figure S1) and oak and pine habitats have different leaf and branch structure. Thus, variation in location (longitude), bill morphology, and habitat structure all have the potential to be related to variation in acoustic signal structure in the Island Scrub-Jay, despite the species' small geographic range.

Our study focused on the “rattle” call, a rapid, broad-band vocalization only produced by female corvids (Goodwin 1976; Woolfenden and Fitzpatrick 1996; Curry and Delaney 2002). Our focus on a female-specific vocalization is unconventional, given that most work on avian acoustic signaling has been conducted on male advertisement songs (e.g., Catchpole 1987, Podos and Warren 2007). Island Scrub-Jays—like other Corvidae—differ from most oscine passerines in that males do not produce an advertisement song (Goodwin 1976). The female rattle call includes a stereotyped visual display and is associated with pair behavior (Goodwin 1976; Woolfenden and Fitzpatrick 1996; Curry and Delaney 2002). Many corvids have a species-specific rattle call, leading to speculation that this vocalization is homologous within the Corvidae (Goodwin 1976). We have only observed *A. insularis* rattle calls in conjunction with territorial defense and male-female interactions. We hypothesize that it could be important in the pair-formation and bonding process, despite it being a female-specific vocalization, because 2 lines of evidence suggest that mate choice in Island Scrub-Jays does not follow the conventional female-choice model (Catchpole 1987): 1) breeding is habitat limited and both sexes compete fiercely for territory vacancies (Collins and Corey 1994) and 2) during the pair-formation process, females feed males; we have never observed the reverse (authors' personal observation).

We measured the structure of rattle calls (rapidity, frequency) and tested for variation in accordance with 3 nonmutually exclusive hypotheses. First, if vocal signals are learned from local neighbors/tutors (cultural drift hypothesis), then we predicted that the structure of rattle calls would vary along the east–west axis of the island in concert the observed pattern of neutral genetic structure. Second, if bill morphology places physical constraints on the production of vocal signals (functional constraints hypothesis), then we predicted that bill length and/or depth would be correlated with the structure of rattle calls. Third, if habitats have different selective pressures for optimal sound transmission (acoustic adaptation hypothesis), then we predicted that vegetation density and architecture would be correlated with the structure of rattle calls and lower frequencies and longer internote intervals should be observed in more densely-vegetated habitat.

METHODS

Recordings

We recorded rattle calls produced by pair-bonded, territorial females ($n = 75$ individuals) in a range of locations and habitat types (see

Figure 1) using a Sennheiser ME67 directional microphone and a Marantz PMD661 digital recorder (digitized with a 48k sample rate). All vocalizations were recorded from September to December 2011 during clear weather (no rain or fog); occasionally recordings were made during breezy conditions. Island Scrub-Jays maintain pair bonds and defend territories year-round (Curry and Delaney 2002), enabling us to collect rattle-call data outside the breeding season. We assume that the structure of rattle calls would be consistent throughout the year, although we did not explicitly test this assumption because we only collected data during one season. Most females were individually identifiable by a unique combination of colored and aluminum leg bands ($n = 48$; see Langin *et al.* 2015 for capture methods); the remainder were unbanded but they could be distinguished from one another via either their mate's leg bands ($n = 18$) or differences in territory location ($n = 9$). Females produce rattle calls most frequently during territorial disputes (e.g., in response to the female's mate chasing an intruder; authors' personal observation). Thus, to facilitate recording, disputes were encouraged by feeding peanuts to neighboring jays to coax them onto the focal female's territory. Nearly all of the recorded rattle calls were produced when the female's mate was 1) chasing a territory intruder, 2) entering her vicinity (within 10 m), or 3) flying away with a peanut. Supplemental feeding may have altered the structure of the rattle calls, although the metrics we measured (see below) did not differ when comparing rattles between the first 2 stimuli and the third (rapidity: $F_{1,53} = 1.5$, $P = 0.23$; center frequency: $F_{1,25} = 0.6$, $P = 0.44$).

Vocalization measurements and analyses

We used Program Audacity (Audacity Team 2013) to remove low-frequency background noise (by applying a high pass filter set at 400 Hz) and to normalize the amplitude of all rattle calls to 0 db. Two spectrograms were then generated for each rattle call using Program Raven 1.4 (Bioacoustics Research Program 2011), one for temporal measurements (Hann window type, 256 window size, 2.67 ms time resolution) and one for frequency measurements (Hann window type, 512 window size, 98.3 Hz frequency resolution). For each rattle call, we selected ten consecutive notes that had the least amount of overlap with other background noise (e.g., vocalizations from conspecifics and heterospecifics). We used those notes to measure: 1) rattle rapidity, defined as the average number of notes produced per second and 2) center frequency, defined as the frequency that separates the sound energy into 2 frequency intervals of equal energy (see Figure 2 for spectrogram example).

The program calculated center frequency within a "box" we drew around the ten notes selected for analysis. Frequency data were not collected if the rattle notes overlapped with vocalizations produced by other birds. We also measured the maximum amplitude of the ten consecutive rattle notes (within the "box") and the maximum amplitude of a representative section of background noise immediately prior to or following the rattle call. Most background noise was caused by wind. Frequency data were excluded from analyses if the background noise was greater than 10% as loud as the rattle call. Thus, the sample size for analyses involving center frequency is less than for analyses involving rattle rapidity.

We calculated the repeatability of our vocalization measurements following Lessells and Boag (1987). Duplicate measurements were collected for 20 rattle calls at least 7 days after the original measurements were collected (i.e., we measured the same spectrograms twice). The repeatability of those measurements was high for both rattle rapidity ($r = 0.85$) and center frequency ($r = 0.95$). We also collected data for multiple rattle calls produced by the same female (rattle rapidity: $n = 44$ females, with an average of 3 recordings per female; center frequency: $n = 18$ females, with an average of 3 recordings per female) and we used those data to estimate a composite measure of repeatability that reflected measurement repeatability and within-female repeatability (the 2 could not be disentangled because measurements on multiple rattle calls from the same individual inevitably involve some amount of measurement error). This composite measure was 0.60 for rattle rapidity and 0.51 for center frequency, indicating that differences between individuals accounted for more than half of the variance in those measurements. These values are in the midrange of repeatability estimates that have been documented for behavioral traits in other studies (Boake 1989; Bell *et al.* 2009). We did not consider interquartile range—that is, the frequency range that encompasses the middle 50% of the sound energy—because repeatability was low ($r = 0.12$). Average measurements were used for all subsequent analyses in cases where multiple rattle calls were recorded for the same female.

We treated rattle rapidity (measured in notes per second, Hz) and center frequency (measured in sound waves per second, Hz) as separate independent variables because they were not correlated (Supplementary Material Figure S2). For both vocalization variables, we used 3 general linear models and the program JMP (SAS Institute 2013) to test whether the structure of rattle calls (rapidity, frequency) varied with 1) longitude (cultural drift hypothesis), 2) bill length and depth (functional constraints hypothesis), and

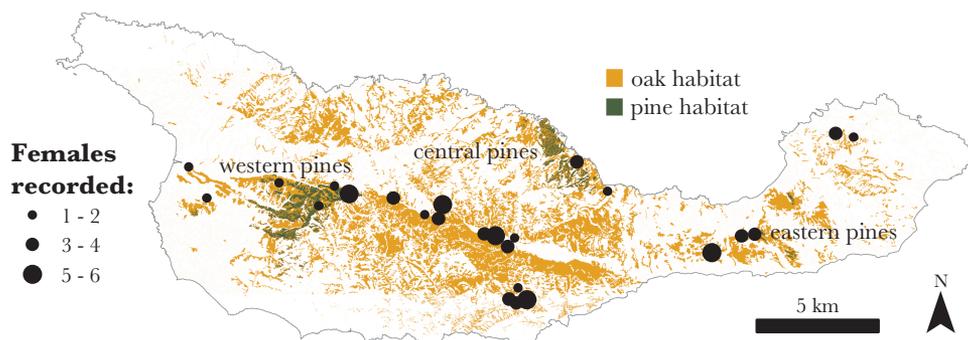


Figure 1

Recording locations for rattle calls produced by female Island Scrub-Jays ($n = 54$ in oak habitat, 21 in pine habitat) on Santa Cruz Island, CA. The baselayer is colored according to habitat type and shows locations where vegetation height was greater than 0.5 m (habitats without oak or pine trees were excluded). The size of the dots corresponds to the number of individuals recorded in that location. Individuals recorded within 500 m of each other were combined for visualization purposes.

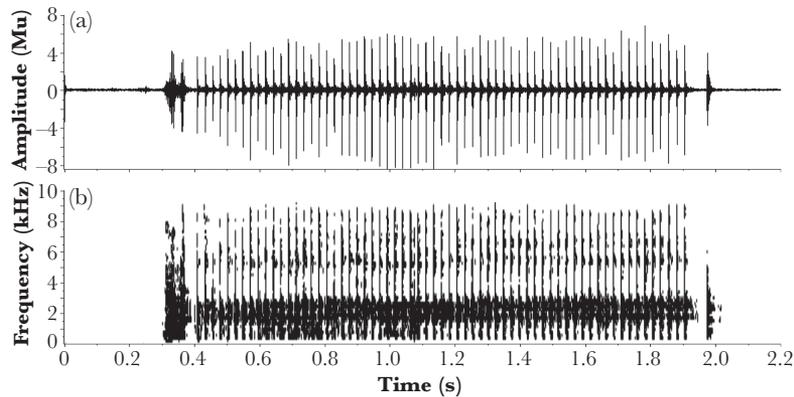


Figure 2
Waveform (a) and spectrogram (b) for a rattle call produced by a female Island Scrub-Jay.

3) vegetation/habitat (acoustic adaptation hypothesis). In models that included metrics of bill morphology, we also included tarsus length—a standard metric of body size in birds—as a covariate because syrinx size can constrain the production of acoustic signals (Wallschläger 1980; Laiolo and Rolando 2003). We confirmed that the data met the assumptions of general linear models. Further details about the bill morphology and habitat analyses are detailed below.

Bill length, bill depth, and tarsus length were measured on 34 (of 75) females captured during the fall from 2007 to 2011. All individuals were measured according to the methods in Langin et al. (2015). Six individuals were measured in the fall after they hatched but their vocalizations were recorded in later years. In Island Scrub-Jays, bill length varies between hatch-year and after-hatch-year birds captured in the fall (paired t -test, $t_{14} = -4.3$, $P < 0.0001$, $n = 15$); bill depth ($t_{14} = -0.5$, $P = 0.59$, $n = 15$) and tarsus length ($t_{14} = -0.7$, $P = 0.49$, $n = 15$) do not. We therefore applied a correction to the bill length data (0.77 mm, representing the average difference between age groups in the paired analysis) for birds that were captured in their first year (that way, all data reflect after-hatch-year bill length). The morphological variables were not correlated (Supplementary Material Table S1), so we included all of them in models testing for an effect of morphology on vocalization structure (i.e., on rattle rapidity and, in a separate model, center frequency). In a separate analysis, longitude was found to be a significant predictor of rattle rapidity (see Results for details), so we included that variable as a covariate in the rapidity model. Bill depth was moderately correlated with longitude (Supplementary Material Table S1) but we included both predictors in the rattle rapidity model because the variance inflation factors for the model effects were all less than 1.2 (Quinn and Keough 2002 recommend worrying about collinearity if variance inflation factors > 10).

Metrics of habitat structure were generated using LiDAR data collected in 2010 (Channel Islands National Park 2010) and a map of 2005 vegetation communities (U.S. Geological Survey 2010). We used ArcGIS (ESRI 2015) to calculate the following variables within 100 m buffers around each focal female's location (the average radius of Island Scrub-Jay territories; Caldwell et al. 2013): 1) mean vegetation height (range: 1.2–5.1 m, $n = 74$) and 2) the proportion of Island Scrub-Jay habitat covered by vegetation > 0.5 m in height (range: 0.01–0.93, $n = 74$). We only included vegetation communities that had oak and pine trees and excluded man-made structures, grasslands, rocky slopes, and other open and non-native habitat types. One female was excluded from this analysis because

she was not recorded within 100 m of oak or pine habitat. The 2 vegetation variables were correlated (Supplementary Material Table S1), so we summarized them using the first axis of a principal components analysis (PCA1, hereafter referred to as vegetation structure) that explained 75% of the variation. We also used the map of vegetation communities to determine whether oak or pine habitat dominated within a 100 m buffer around each focal female's location. Vegetation structure (PCA1) did not differ between habitat types (t -test, $t_{72} = 0.4$, $P = 0.72$, $n = 53_{\text{oak}}, 21_{\text{pine}}$), so we included both variables in models testing for an effect of habitat structure. We tested for an interaction but did not include it in the final models because, for both rattle rapidity and center frequency, there was no evidence for an interaction between vegetation structure and habitat type. Longitude was included as a covariate in the rattle rapidity model.

RESULTS

We tested the cultural drift hypothesis by analyzing spatial variation in the structure of calls produced by female Island Scrub-Jays. We found that rattle rapidity varied along the east-west axis of the island ($r^2 = 0.07$, $F_{1,74} = 5.3$, $P = 0.02$, $n = 75$; Figure 3), the same axis along which neutral genetic divergence has been previously documented (Langin et al. 2015). Center frequency, however, was not related to longitude ($r^2 = 0.01$, $F_{1,45} = 0.6$, $P = 0.43$, $n = 46$).

To test the functional constraints hypothesis, we examined the relationship between bill morphology and acoustic signal structure. Controlling for longitude, bill length and bill depth were both significant predictors of rattle rapidity (model $r^2 = 0.37$, $n = 34$; bill length effect: $F_{1,33} = 11.0$, $P = 0.003$, Figure 4a; bill depth effect: $F_{1,33} = 5.0$, $P = 0.03$, Figure 4b; tarsus length effect: $F_{1,33} = 0.8$, $P = 0.38$; longitude effect: $F_{1,33} = 5.6$, $P = 0.02$), such that females with long, shallow bills (characteristic of pine habitats) produced faster rattles than females with short, deep bills (characteristic of oak habitats). Bill length was not a significant predictor of center frequency but deeper-billed individuals produced higher-frequency rattles (model $r^2 = 0.46$, $n = 21$; bill length effect: $F_{1,20} = 0.9$, $P = 0.36$, Figure 4c; bill depth effect: $F_{1,20} = 13.8$, $P = 0.002$, Figure 4d; tarsus length effect: $F_{1,20} = 0.1$, $P = 0.83$). Tarsus length, an index of body size, was not a significantly predictor of either acoustic variable.

To test the acoustic adaptation hypothesis, we examined the relationship between habitat variables (vegetation structure, habitat type) and rattle characteristics. We found no significant effect on

rattle rapidity ($model\ r^2 = 0.11, n = 74$; vegetation structure effect: $F_{1,73} = 0.9, P = 0.35$; habitat type effect: $F_{1,73} = 2.3, P = 0.14$; longitude effect: $F_{1,33} = 3.8, P = 0.06$) or center frequency ($model$

$r^2 = 0.08, n = 45$; vegetation structure effect: $F_{1,44} = 3.3, P = 0.08$; habitat type effect: $F_{1,44} = 0.6, P = 0.46$).

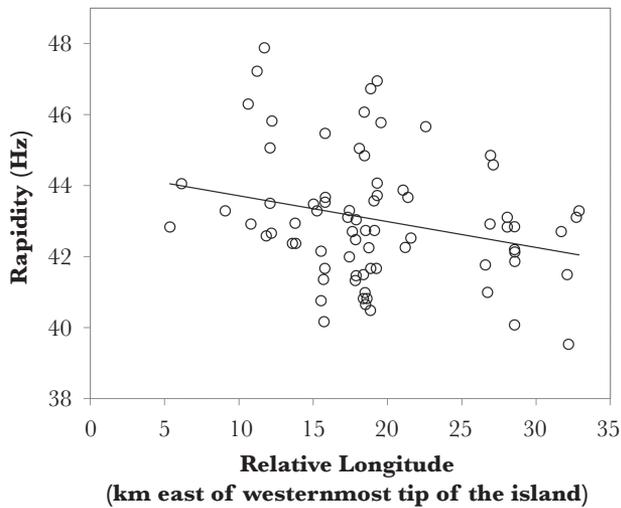


Figure 3

Female Island Scrub-Jays on the western side of Santa Cruz Island had faster-paced rattle calls than females on the eastern side of the island ($n = 75$). Rapidity is presented as notes per second (Hz). A longitude value of zero corresponds to the westernmost tip of the island. The line gives the best linear fit to the data.

DISCUSSION

Spatial variation in acoustic signals can be caused by different mechanisms (Podos and Warren 2007), which has implications for mate choice, local adaptation, and even speciation (West-Eberhard 1983). The environment can shape variation in acoustic signals (Slabbekoorn and Smith 2002), either through 1) direct selection on vocal structure (acoustic adaptation hypothesis) or 2) indirect selection on morphological traits that influence the production of vocalizations (functional constraints hypothesis). Vocal differences can also be a by-product of the vagaries of cultural transmission (cultural drift hypothesis; Slater 1986). Our findings are consistent with 2 of these hypotheses, the functional constraints hypothesis and the cultural drift hypothesis, as the structure of rattle calls was correlated with variation in both bill morphology (length and depth) and longitude (a proxy for neutral genetic structure). These results indicate that selection on bill morphology and cultural drift can jointly shape variation in acoustic signal structure, even at fine spatial scales within populations.

The mechanisms that underlie the relationship between bill morphology and acoustic signal structure in female *A. insularis* are unclear. Bill depth may influence the frequency of rattle calls because of constraints on maximum gape width (Westneat et al. 1993). However, a hypothesized explanation is more difficult to develop for the relationship we document between bill morphology

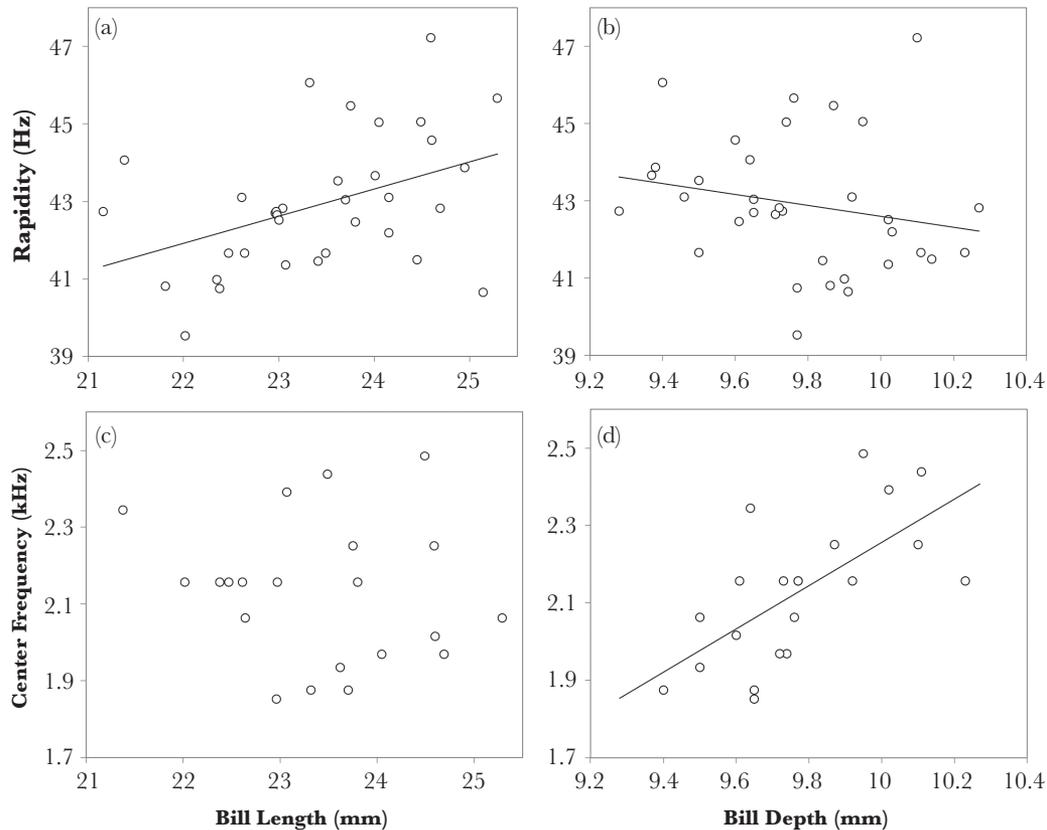


Figure 4

The correlation of bill morphology with rattle rapidity (in notes per second, Hz; panels a and b; $n = 34$) and center frequency (in thousands of sound waves per second, kHz; panels c and d; $n = 21$) in Island Scrub-Jays. Females with longer, shallower bills produced faster-paced rattles and females with shallower bills produced lower-frequency rattles. The lines give the best linear fit to the data for significant relationships.

and rattle rapidity. Previous work has shown that larger-billed birds are constrained in terms of the pace with which they can produce trilled songs because they cannot move their bill as rapidly to modulate frequency changes (Podos 1997; Podos 2001). The rattle calls produced by Island Scrub-Jays are similar to trilled songs in that they are also rapid but they differ from those vocalizations in one important respect: frequency modulations do not occur within individual notes (see Figure 2). Therefore, the same constraint does not apply. Further study will be needed to determine if the relationship between bill morphology and rattle rapidity is reflective of a functional constraint or another factor.

One alternate possibility derived from theoretical models—which does not involve the bill acting as a functional constraint—is genetic linkage (i.e., a nonmagic trait complex; Servedio and Kopp 2012). The regulation of genes underlying variation in bill morphology is well-studied and upregulation of calmodulin and *Bmp4* has been shown to account for a substantial amount of variation in bill length and bill depth, respectively (Abzhanov et al. 2004; Abzhanov et al. 2006). Physical linkage between a gene that regulates bill morphology and a gene involved in the production of rattle calls could, therefore, at least partially account for the association between bill morphology and acoustic signal structure. This is a plausible alternative to functional constraints given that vocal features like the rapidity of note production have been shown to have a genetic basis, even in species that exhibit plasticity due to learning (Marler and Sherman 1985; Slabbekoorn and Smith 2002).

Consistent with the cultural drift hypothesis, rattle rapidity also varied along the east–west axis of the island in accordance with spatial patterns of gene flow (Langin et al. 2015). Females on the western side of the island had faster-paced rattles than females on the eastern side of the island (Figure 3), although longitude only explained 7% of variation in rattle rapidity. Little is known about the timing of vocal learning in Island Scrub-Jays, but in a well-studied congener—the Florida Scrub-Jay (*A. coerulescens*)—observations suggest that a homologous female call, the “hiccup,” does not appear to change following natal dispersal (J.W. Fitzpatrick and R. Bowman, personal communication). Therefore, Island Scrub-Jays likely produce rattles that were learned at or near their natal location. An association between neutral genetic structure and acoustic signal structure has been documented in a number of other studies (e.g., MacDougall-Shackleton and MacDougall-Shackleton 2001; Wright et al. 2005), although we are unaware of an example at the fine spatial scale we document for *A. insularis*. This pattern of cultural evolution could be a by-product of locally restricted dispersal and gene flow in Island Scrub-Jays (Langin et al. 2015). However, if long-distance dispersers are less likely to attract mates and/or establish territories because they have an abnormal rattle call (MacDougall-Shackleton et al. 2002), then local dialects could also play an active role in further restricting the spatial scale of gene flow—thus helping to foster the conditions necessary to generate fine-scale adaptive divergence in traits like bill morphology.

Our findings do not support the acoustic adaptation hypothesis. Vegetation structure was marginally related ($P = 0.08$) to center frequency in the predicted direction (lower frequency rattles in more dense habitat) but the r^2 -values were low for both habitat models (0.11 and 0.08 for rattle rapidity and center frequency, respectively) and none of the effects were significant. Spatial autocorrelation in vegetation structure may be too fine-scale to allow for the evolution of vocal differences within the population (Richardson et al. 2014). Alternatively, selection also may not be operating in accordance with variation in the acoustic environment. Rattle calls are usually

produced when females are in close proximity to their mate (typically within 30 m) and this distance may not be substantial enough to degrade the structure of rattle calls (Boncoraglio and Saino 2007). Selection also may not be acting to minimize signal degradation if male Island Scrub-Jays use the structure of rattle calls to estimate distance to females during territorial disputes (McGregor and Krebs 1984; Fotheringham et al. 1997). The latter explanation seems less likely given that rattle calls are produced when males are nearby but distance estimation could be advantageous in the more densely vegetated locations on the island (e.g., Supplementary Material Figure S1a) where visibility is limited.

The correlation between acoustic signal structure and bill morphology may have important consequences for reducing gene flow between adaptively-divergent Island Scrub-Jays in oak and pine habitats. We have evidence for nonrandom mating for bill length at the scale of the island (Langin et al. 2015), a pattern that could be due to jays 1) actively selecting mates based on a visual cue like bill morphology (Ratcliffe and Grant 1983) or an acoustic cue like rattle calls (Ratcliffe and Grant 1985; Snowberg and Benkman 2007) or 2) simply pairing with nearby and therefore morphologically similar individuals. We also have evidence for a genetic discontinuity across the boundary between the largest pine stand and adjacent oak habitat (Langin et al. 2015; unpublished genomic data), which could be due to assortative mating. Our results provide a tantalizing hint that vocal cues may contribute to nonrandom mating and a reduction in gene flow between habitats, and thus may be acting as a magic trait. However, limited information is available on the process of mate selection and territory acquisition in Island Scrub-Jays (Curry and Delaney 2002), so future research is needed to address how individuals select mates and whether they can distinguish between rattle calls produced by birds with different bill morphologies.

In summary, an increasing body of evidence suggests that adaptive evolutionary divergence in the face of gene flow is more common in nature than is generally appreciated (Richardson et al. 2014). Divergent natural selection on magic traits or on loci in non-magic trait complexes could be an important mechanism for reducing gene flow between adaptively-divergent groups that are in close proximity (Servedio and Kopp 2012). The link between bill morphology and acoustic features is one mechanism that holds great promise for explaining microgeographic divergence in birds (Huber and Podos 2006; Badyaev et al. 2008) but similar mechanisms likely exist in other taxa. For instance, assortative mating could evolve as a by-product of divergent natural selection on wing coloration in Lepidoptera (Jiggins et al. 2001; Fordyce et al. 2002) and on body size and shape in freshwater fishes (Nagel and Schluter 1998; Langerhans et al. 2007) because those traits are used as cues during mate selection. Ultimately, more work is needed on a broad array of taxa to determine whether linkages between ecological traits and mating signals are an important factor driving microgeographic divergence within populations.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Langin et al. (2017).

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