SONG DIVERGENCE BY SENSORY DRIVE IN AMAZONIAN BIRDS

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Visual signals are shaped by variation in the signaling environment through a process termed sensory drive, sometimes leading to speciation. However, the evidence for sensory drive in acoustic signals is restricted to comparisons between highly dissimilar habitats, or single-species studies in which it is difficult to rule out the influence of undetected ecological variables, pleiotropic effects, or chance. Here we assess whether this form of sensory drive—often termed "acoustic adaptation"—can generate signal divergence across ecological gradients. By studying avian communities in two Amazonian forest types, we show that songs of 17 "bamboo-specialist" bird species differ in predictable ways from their nearest relatives in adjacent terra firme forest. We also demonstrate that the direction of song divergence is correlated with the sound transmission properties of habitats, rather than with genetic divergence, ambient noise, or pleiotropic effects of mass and bill size. Our findings indicate that acoustic adaptation adds significantly to stochastic processes underlying song divergence, even when comparing between habitats with relatively similar structure. Furthermore, given that song differences potentially contribute to reproductive isolation, these findings are consistent with a wider role for sensory drive in the diversification of lineages with acoustic mating signals.

KEY WORDS: Acoustic adaptation, birdsong, deterministic selection, ecological selection, phenotypic divergence, sensory drive, signal evolution.

Divergent signals play a key role in the establishment and maintenance of premating reproductive isolation, and the processes driving signal divergence are therefore clearly relevant to studies of speciation and species co-existence (West-Eberhard 1983; Endler 1992; Panhuis et al. 2001; Boughman 2002; Rundle et al. 2005). Several routes to divergence have been hypothesized, but three are particularly prominent. The first and most parsimonious is that divergent traits reflect random genetic drift and mutation (Lewontin 1974; Kimura 1983; Lynch and Hill 1986). The second is that sexual selection drives continuous but otherwise unpredictable divergence because of the attractiveness of novel signals and the absence of well-defined optima (Fisher 1930; Iwasa and Pomiankowski 1995). The third possibility is that ecological selection causes adaptive divergence in signaling traits by "sensory drive," a term coined by Endler (1992) for the process by which signals evolve to minimize degradation and maximize conspicuousness against background noise. It is widely recognized that each of these factors contributes to trait divergence, but their relative influence is poorly understood, particularly in the case of acoustic signals (Mundinger 1982; Martens 1996; Irwin et al. 2008; Amézquita et al. 2009).

There is abundant evidence that visual signals and associated sensory systems are to some extent "tuned" to match characteristics of the environment in a range of animals, including fish (Endler 1980), birds (Marchetti 1993), and reptiles (Leal and Fleishman 2002). The potential influence of sensory drive as a force determining the tempo and direction of signal evolution is further highlighted by evidence that signal design is shaped by variation in lighting, turbidity, or background color (Endler and Basolo 1998; Gomez and Théry 2004; Engström-Ost and Candolin 2007; Uy and Stein 2007). These findings have attracted attention because they suggest that habitat heterogeneity will cause divergent selection on signals associated with mate choice, potentially facilitating speciation (Endler 1992; Boughman 2002). Indeed, empirical studies have clearly demonstrated the link between sensory drive, signal divergence, and reproductive isolation (Boughman 2001; Seehausen et al. 2008), a two-step mechanism of speciation that may be widespread in animals with visual mating signals.

The same process of environmentally driven divergence may apply to acoustic mating signals. If habitats vary in their sound transmission properties, and if individual fitness is linked to long-distance communication, then local adaptation should theoretically result in habitat-dependent acoustic signals with properties that maximize the reliability of transmission from signalers to receivers. Although this concept is nested in the broader framework of sensory drive, it is often referred to as the acoustic adaptation hypothesis (Morton 1975). It is widely proposed that acoustic adaptation plays a role in speciation (Ryan and Wilczynski 1988; Slabbekoorn and Smith 2002a; Förschler and Kalko 2007), but this has never been demonstrated. Moreover, it remains unclear whether habitat-mediated selection has a consistent effect on acoustic signals except when habitat differences are very large.

Dense habitats are associated with greater reverberation and increased attenuation at higher frequencies (Marten and Marler 1977; Marten et al. 1977; Slabbekoorn et al. 2002). The sensory drive hypothesis therefore predicts that acoustic signals will have lower frequency, simpler structure, and slower pace in densely foliated habitats compared to open habitats. Evidence of these effects is reported in numerous single-species studies (Hunter and Krebs 1979; Slabbekoorn et al. 2002; Slabbekoorn and Smith 2002b; Patten et al. 2004; Nicholls and Goldizen 2006; Dingle et al. 2008; Derryberry 2009; Kirschel et al. 2009a), as well as comparative analyses (Wiley 1991; Badyaev and Leaf 1997; Bertelli and Tubaro 2002; Tubaro and Lijtmaer 2006). However, there are also a considerable number of studies that either refute or only weakly support sensory drive (e.g., Payne 1978; Lemon et al. 1981; Daniel and Blumstein 1998; Tubaro and Mahler 1998; Hylton and Godard 2001; Blumstein and Turner 2005), including a recent meta-analysis (Boncoraglio and Saino 2007).

Further uncertainty about the role of sound transmission in acoustic adaptation is generated by attributes of previous studies. In particular, most single-species and some multispecies approaches are vulnerable to the influence of confounding variables or chance. This is the case in analyses of signal phenotype over wide geographic areas, across which local conditions vary in many ways, making it impossible to be certain about the direct cause of phenotypic variation (Schluter 2000). For example, spatial variation in signals can be caused by shifts in climate, elevation, or ambient noise profiles (e.g., Slabbekoorn and Peet 2003; Kirschel et al. 2009a), or by various forms of character displacement (Seddon 2005; Grether et al. 2009; Kirschel et al. 2009b; Tobias and Seddon 2009). Similarly, it is often proposed that acoustic adaptation explains the relationship between signal structure and vegetation density, but an obvious alternative possibility is that signal variation reflects a correlated evolutionary response (sensu Nosil et al. 2008), perhaps as a byproduct of habitat-related selection on body size or traits associated with foraging (e.g., Ryan and Brenowitz 1985; Podos 2001; Podos et al. 2004; Badyaev et al. 2008).

Cultural evolution may also affect the interpretation of previous studies testing acoustic adaptation in birds, particularly as most of these—including all single-species studies—have focused on oscine passerines (Boncoraglio and Saino 2007). Oscine species develop songs by an imprinting-like process called learning (Beecher and Brenowitz 2005). In general, this increases random geographical variation and leads to the formation of repertoires and dialects. It also means that young individuals may simply learn the sounds, or parts of sounds, that they perceive most clearly in their natal habitat (Hansen 1979). For this reason, habitat-associated patterns of song variation may lack a genetic basis, reflecting phenotypic plasticity rather than acoustic adaptation (Ellers and Slabbekoorn 2003; Ripmeester et al. 2010). Moreover, the emphasis on oscines reduces the extent to which we can draw firm conclusions about sensory drive in speciation because it remains unclear whether signal plasticity promotes or delays reproductive isolation (Lachlan and Servedio 2004; Seddon and Tobias 2007; Olofsson and Servedio 2008).

These issues can be largely avoided by testing for sensory drive in systems with innate acoustic signals. However, only two comparative studies have taken this approach in nonpasserine birds, and they report conflicting results. Specifically, Bertelli and Tubaro (2002) found that the songs of open-country tinamous (Tinamidae) had a wider bandwidth than those of their relatives occurring in closed habitats, which supports the predictions of acoustic adaptation. Meanwhile, Tubaro and Mahler (1998) found that songs of open-country New World doves (Columbidae) were of lower pitch than their relatives in closed habitats, counter to the predictions of acoustic adaptation. Similarly, in a nonavian system, no evidence for acoustic adaptation was found in a study

of marmot (Sciuridae) alarm calls (Daniel and Blumstein 1998), which are assumed to be innate signals.

Previous support for acoustic adaptation almost universally derives from comparisons between habitats with radically different structural characteristics, for example, dense forest versus open grassland (Morton 1975; Wiley 1991; Tubaro and Mahler 1998; Bertelli and Tubaro 2002) or forest versus urban environments (Slabbekoorn et al. 2007). From the perspective of speciation research, this is problematic as speciation tends not to involve abrupt switches from dense forest to open grassland, or vice versa. On the contrary, sister species usually occupy relatively similar habitats. Put another way, ecological niches are phylogenetically conserved (Wiens and Graham 2005), or associated with a high phylogenetic signal (Losos 2008), and thus if acoustic adaptation plays a widespread role in speciation it should be relevant across habitat gradients rather than restricted to habitat extremes.

Set against the evidence for sensory drive, there is growing support for the role of stochasticity in the form of unpredictable sexual selection, or random drift and mutation. Some studies of visual signals conclude that patterns of signal evolution cannot be explained by adaptation to the environment (e.g., Fleishman et al. 2009); others conclude that sexual selection is the most powerful influence (e.g., Allender et al. 2003). In addition, it is often concluded that acoustic signals may diverge by purely nonadaptive or stochastic processes rather than ecological determinism (Mundinger 1982; Searcy and Andersson 1986; Martens 1996; McCracken and Sheldon 1997; Price and Lanyon 2002; Amézquita et al. 2009). As a recent example, Irwin et al. (2008) showed that variation in song and call structure in *Phylloscopus* warblers was related to geographic and genetic distance, but unrelated to habitat structure and morphology. Such findings suggest that, as an evolutionary force, acoustic adaptation is rather weak. However, the relative roles of deterministic and stochastic processes in acoustic signal divergence are rarely assessed, particularly across habitat contrasts relevant to speciation.

In this study, we focus on a bird community at a single Amazonian locality to investigate habitat-mediated variation in birdsong—a classic long-distance communication signal affected by the environment through attenuation, degradation, and interference with ambient noise (reviewed in Slabbekoorn 2004b). Specifically, we ask whether vocal divergence between pairs of closely related but ecologically divergent species—one occurring in bamboo forest and the other in adjacent terra firme (upland) forest—is explained by acoustic adaptation to the transmission properties of habitats. Although our main aim is to test the predictions of (1) the sensory drive hypothesis, we also assess the extent to which habitat-mediated vocal divergence can be explained by (2) avoidance of interference with ambient noise, (3) a correlated evolutionary response of selection on bill size and body size, or (4) neutral genetic drift. If divergence is determined by sensory

drive, we predict that song variables such as pitch and pace will vary consistently across habitats, in line with differences in sound transmission optima. Conversely, if song differentiation is shaped principally by neutral processes, the associations between vocal and ecological divergence will be weak or absent, and outweighed by a positive relationship between vocal and genetic divergence.

Our system is uniquely suited to answering these questions for three reasons. First, bamboo forest and terra firme forest are relatively similar in structure, both having tall trees and a dense understory. Moreover, phylogenetic data confirm that Amazonian "bamboo-specialist" birds evolved from terra firme species (e.g., Brumfield et al. 2007; Irestedt et al. 2009). We can therefore be certain that we are focusing on an ecological contrast relevant to speciation (i.e., potentially involved in driving cladogenesis). Second, we exclude species with learnt songs from our analysis (i.e., our sample does not include any oscine passerines, hummingbirds or parrots) to maximize the likelihood that phenotypic differences have a genetic basis (Kroodsma 1996). And third, by focusing on a single locality we effectively control for a wide range of factors known to influence song design, including climate, elevation, interspecific coevolution, and geographical distance between sampling localities.

Materials and Methods
STUDY SITE
We conducted fieldwork in August 2006–December 2007 in a 2–3 km^2 area of lowland forest (280–300 m asl) at the Centro de Investigación y Conservación de Río Los Amigos (CICRA), Madre de Dios, SE Peru (12°34′07″S, 70°05′57″W). Vegetation here consists of a mosaic of Amazonian habitats (see Fig. S1 for a Landsat image) dominated by *Mauritia* palm swamp, flood plain forest, terra firme (upland) forest, and large stands of tall *Guadua* bamboo—mainly patches of *G. weberbaueri* and *G. sarcocarpa* (Nelson and Bianchini 2005). This kind of bamboo forest is pervasive in south-west Amazonia, where it covers a total area of 120,000–180,000 km^2, typically in patches of 0.07–12.6 ha (Saatchi et al. 2000; Silman et al. 2003). We focus on a comparison between bamboo forest and terra firme forest because these habitats support a diverse assemblage of specialist bird species (Terborgh et al. 1990; Kratter 1997).

HABITAT STRUCTURE
To understand the basis of differences in the sound transmission properties of bamboo forest and terra firme forest, as well as to quantify the relative similarity of these habitats compared to adjacent manmade grassland, we conducted basic vegetation surveys at 15 points in each of these three habitat types. Survey points were positioned at least 30 m apart, and within a 10-m radius

of each point we quantified aspects of habitat relevant to birds singing within 2 m of ground level: mean canopy height ($\pm$5 m); mean visibility ($\pm$2 m) at 1.5 m above the ground; understory vegetation density ($\pm$10%); and total number of trees with diameter at breast height (dbh) in three categories ($<$20, 20–40, $>$40 cm). Understory vegetation density was an estimate, expressed as a proportion of total volume, of the amount of dense foliage within a circle of 10-m radius and below 4 m.

We conducted a principal components analysis (PCA) with Varimax rotation and Kaiser Normalization on the correlation matrix of survey points to quantify overall habitat structure (all variables log-transformed). This generated two uncorrelated principal components (PCs) accounting for 83.4% of the variation in the original dataset: $PC1_{veg}$ (Eigenvalue = 3.84) which was positively related to mean canopy height and number of large trees, and $PC2_{veg}$ (1.17) was negatively related to mean visibility and positively related to understory vegetation density (all factor loadings $>$ 0.8).

SOUND TRANSMISSION

To determine whether bamboo and terra firme forest have distinct acoustic environments, we quantified their sound transmission properties. We used the standard approach of broadcasting and rerecording artificial sound stimuli in the different habitats and quantifying the extent to which sounds are differentially degraded by attenuation and reverberation (e.g., Morton 1975; Slabbekoorn et al. 2007).

Sound stimuli

Standardized artificial sound stimuli were generated using Avisoft SASLAB Pro, version 4.15 (Specht 2006). We designed the sounds so that they matched the basic structure of songs in our community as closely as possible. The songs of rainforest passerines tend to be tonal in structure, that is, they have a restricted bandwidth (frequency range) and are relatively free of overtones (harmonics) (Morton 1975; Slabbekoorn 2004b). Accordingly, mean song bandwidth in our avian community is only 1.72 $\pm$ 0.66 kHz, and all but two species (*Hemitriccus flammulatus* and *H. griseipectus*) lack distinct overtones. We therefore used constant frequency tones as our sound stimuli. These tones were 100 msec in duration as this was close to mean note duration in our sample (117 $\pm$ 9 msec).

Tones were synthesized at six different frequencies (0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 kHz) to encompass the range of maximum avian auditory sensitivity (Dooling 1982). At each frequency, we generated two sequences of two 100-msec tones. One sequence had a relatively short interval of 150 msec, close to the mean internote interval in our sample (152 $\pm$ 74 msec). The other sequence had a longer interval of 250 msec, close to the mean maximum internote interval in our sample (283 $\pm$ 74 msec). The first

sequence reflects a fast-paced song and the second a slower paced song (sensu Slabbekoorn et al. 2007). The master file (44100 Hz/16 bit WAV) thereby consisted of a series of 12 pairs of artificial 100-ms constant-frequency tones at six different frequencies (0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 kHz).

Experiments

We conducted sound transmission experiments at 50 locations (hereafter, sound points), 25 in bamboo forest and 25 in terra firme forest, with at least 30 m between points. Experiments took place at 0600–0800 h (i.e., during the period of peak vocal activity for Amazonian birds) in March–April 2007. We used a Sony HI-MD device to broadcast the master sound file through a SME-AFS loudspeaker (Saul Mineroff Electronics, Elmont, NY), and simultaneously recorded it as a 44.1kHz WAV file using a unidirectional Sennheiser ME66-K3U microphone and an Edirol R-09 digital recorder (Roland). At each sound point, we broadcast the master file at two transmission heights (0.1 and 2.0 m) and recorded it at two distances (5.0 and 20.0 m). All transmission pathways passed through uninterrupted vegetation on flat ground. Volume levels for playback and recording of stimuli were standardized at 60 dB SPL at 10 m to approximate natural songs (see Seddon and Tobias 2006) using an Adastra handheld analogue sound level meter (set at "C" weighting and fast response).

Analysis

Transmission experiments produced a total of 2400 sound files for analysis. To determine levels of degradation of transmitted stimuli in respective habitats, these files were redigitized at a sampling rate of 25 kHz, after which data were extracted automatically using a preprogrammed procedure in SIGNAL (Engineering Design, 1997). Specifically, three amplitude measurements (root-mean-squared, RMS, values of sound pressure deviations in volts) were taken during fixed 100-ms periods relative to the onset or offset of a tone: measurement A was taken after the onset of the first tone; measurement B after the offset of the first tone; and measurement C after the onset of the second tone (see Fig. S2). These measures were used to calculate three transmission characteristics as follows: (1) Attenuation (i.e., loss of amplitude) was A at 20 m divided by A at 5 m; (2) reverberation (i.e., tail-to-signal ratio) was B at 20 m divided by A at 20 m; and (3) distortion (i.e., signal-to-signal ratio) was C at 20 m divided by A at 20 m. We pooled fast- and slow-paced stimuli to calculate attenuation and reverberation, as these were quantified solely from the first note and its echoes. We used data from both notes to calculate distortion in relation to signal pace, and therefore fast- and slow-paced stimuli were not pooled. Distortion was a measure of the extent to which reverberations from a preceding note compromised signal fidelity, as these may increase (i.e., distort) the amplitude of successive notes.

TESTING THE SENSORY DRIVE HYPOTHESIS

The next step was to investigate whether the sound transmission properties of habitats influence the acoustic structure of signals. To test this idea, we determined whether there were any consistent differences in the acoustic structure of the songs of bamboo and terra firme birds and, if so, whether the differences correlated with the sound transmission properties of the two habitats.

Study species

Approximately 25 species regularly, if not exclusively, occur in bamboo at CICRA (Kratter 1997; Tobias et al. 2008; Tobias et al. 2009). Of these, we studied 17 species with a close relative in adjacent terra firme forest. We excluded species that were only observed vocalizing higher than 2 m above ground level (e.g., *Drymophila devillei*) because we only assessed sound transmission properties of the understory (i.e., at 0.1 and 2.0 m only). We also limited the sample to sedentary, territorial nonpasserines or suboscine passerines, and excluded any species from families

known to develop songs by learning (i.e., parrots, hummingbirds, or oscine passerines). All study species had simple, stereotyped vocalizations, without repertoires or dialects, and come from families without evidence of song learning (see Kroodsma 1984; Kroodsma and Konishi 1991; Seddon and Tobias 2007; Seddon et al. 2008). The total sample contained 33 species (one species was compared twice). For details of these species, and their phylogenetic relationships, see Figure 1 and Table S1.

Species pairings were typically not true sister species. This did not matter because we were not attempting to directly test speciation hypotheses, but rather to assess the role of ecological selection on song divergence. Instead, we paired "bamboo specialists" with their closest terra firme relative at the study site. The only exceptions were two ambiguous cases (pairs 5 and 6 in Fig. 1) in which we paired bamboo species and terra firme species according to similarity in their acoustic signals. Song similarity was quantified on the basis of overlap in peak frequency and note pace, as determined through spectrographic analysis
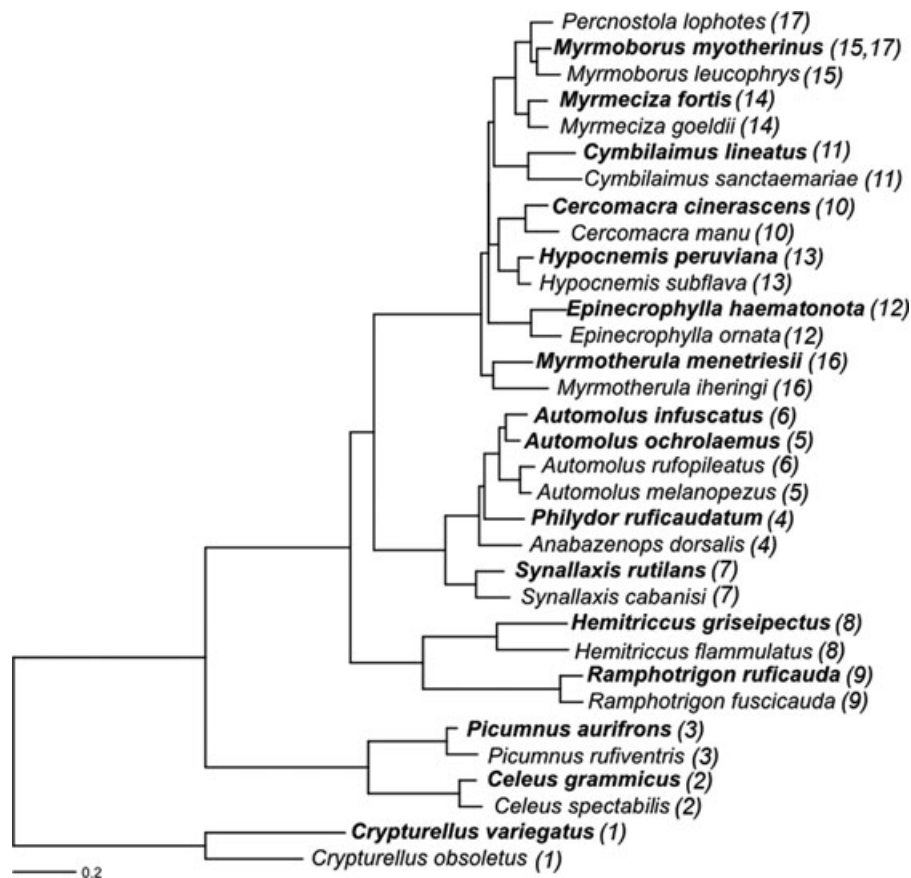

Figure 1. A maximum-likelihood tree from an analysis of the ND2 gene depicting evolutionary relationships among the 33 species used in acoustic analysis (voucher data in Table S2). Acoustic comparisons were conducted on 33 pairs of species, one of which was found primarily in *Guadua* bamboo at our study site (depicted in gray), and the other found primarily in terra firme habitat (depicted in black). Species were identified as occurring primarily in bamboo or terra firme following Kratter (1997) and Tobias et al. (2009). Pairwise comparisons between bamboo birds and their closest relatives in terra firme are numbered (1–17). All species are compared once, except *Myrmoborus myotherinus* which is compared twice.

(see below for details). Combining these two species pairs on the basis of song similarity rested on the assumption that vocally similar species are more likely to be closely related.

Song divergence

We used a Sennheiser ME67 directional microphone and a Sound Devices 720 digital recorder (file format: WAV; sampling frequency: 44.1 kHz) to record male songs from at least three different individuals for all species (see Table S1 for sample sizes). Songs were defined as the loudest, most structurally complex vocalization in the repertoire of each species; in all cases, they were assumed to have a territorial function because they elicited aggressive responses when played to conspecifics (Tobias and Seddon 2009; J. A. Tobias and N. Seddon, unpubl. data). Territorial signals are typically broadcast over long distances, and are therefore more likely than short-range signals (e.g., contact calls or alarm calls) to meet the assumptions of the acoustic adaptation hypothesis.

We used Avisoft SASLab Pro 4.50 (Specht 2006) to generate spectrograms from high-quality recordings (i.e., low background noise). The final sample contained 347 songs (mean $= 10.2 \pm 5.9$ songs per species), which were described quantitatively in terms of seven basic acoustic characteristics: maximum frequency (kHz), minimum frequency (kHz), peak frequency (kHz; frequency in the song with the greatest amplitude), bandwidth (kHz; maximum frequency minus minimum frequency), song duration (s), number of notes, and pace (number of notes s^{-1}). Peak frequency was automatically measured from amplitude spectra using Avisoft; all other measures were taken manually from spectrograms using on-screen cursors. The amplitude of each recording was adjusted to a standard level prior to analysis to minimize the impact of recording volume on our measurements. To maximize resolution, spectral characters (in kHz) were taken from narrowband spectrograms (bandwidth $= 55$ Hz), and temporal characters were taken from broadband spectrograms (323 Hz). For each acoustic character, we generated mean values per individual and also per species (see Table S1). We then conducted a PCA on the correlation matrix of species means (log-transformed) to quantify overall song structure. Three uncorrelated PCs were extracted with Varimax rotation and Kaiser Normalization, one of which reflected song pitch (PC1$_{song}$), one of which reflected song pace (PC2$_{song}$), and one of which reflected song duration and note number (PC3$_{song}$); together these accounted for 91.7% of the variance in the original acoustic dataset (Table 1).

ALTERNATIVE HYPOTHESES

We explored the role of three alternative drivers of song divergence between bamboo and terra firme forest birds: adaptations to minimize interference with ambient noise, byproducts of morphological adaptation, and neutral genetic divergence.

Table 1. Factor loadings on three principal components for acoustic measures taken from the songs of bamboo and terra firme forest specialists.

	Factor loadings		
	PC1	PC2	PC3
Eigenvalue	3.316	1.708	1.392
% of variance	47.375	24.398	19.886
Maximum frequency	0.929	0.329	−0.093
Minimum frequency	0.954	−0.094	0.037
Peak frequency	0.969	0.193	−0.059
Duration	−0.042	−0.593	0.777
Note number	−0.039	0.196	0.977
Pace	−0.002	0.938	0.086

Ambient noise

The hypothesis that habitat-mediated vocal divergence reflects adaptation to avoid or minimize interference with noise (Slabbekoorn 2004b) predicts consistent differences in the ambient noise profiles of bamboo and terra firme forest habitats. To test this idea, we took digital recordings of ambient noise at dawn, starting at nautical dawn (i.e., when the sun is 12° below the horizon, as determined using US Naval Observatory data: http://aa.usno.navy.mil). Each recording lasted 120 min, and was therefore timed to coincide with peak vocal activity in Neotropical birds (Berg et al. 2006). Digital recordings (file format: WAV; sampling frequency: 44.1 kHz) were made using a Sennheiser ME62 omnidirectional microphone attached to an Edirol R09 digital recorder with an 8 GB Lexar sound card. The microphone was suspended 1.5 m from the ground, and the same settings were used for all recordings to ensure that amplitude was consistent. We collected an initial sample of 30 recordings in bamboo, and 30 recordings in terra firme forest, with all recording sites located at least 200 m apart. However, we removed any recordings heavily affected by rain, leaving a final sample of 17 recordings in bamboo and 18 in terra firme forest. Rain generates noise across a broad frequency, and may conceal habitat-dependent patterns of ambient noise (Slabbekoorn 2004a).

From these 120-min recordings, we sampled 1-s cuts every 5 min, producing a total of 24 cuts per recording, and 840 cuts in total. From these, we calculated RMS-values in Matlab 7.5 (Mathworks, Natick, MA) by filtering sound files in 50-Hz bins in the range of 0–10.0 kHz (resulting in 200 bins per 1-s cut). RMS-values were then averaged across time for each 50-Hz bin, to produce a mean ambient noise spectrum per recording site. RMS-values were then averaged across all sites to produce mean ambient noise spectra for the focal habitats.

To test whether song divergence reflects adaptation to minimize interference with ambient noise, we first determined whether

there were any consistent differences in the ambient noise profiles of the two habitats. We then plotted the mean peak frequency of the songs of bamboo and terra firme birds against the mean ambient noise spectra of the two habitats. Finally, we used chi-square tests to ask whether the peak frequencies of birdsongs in each habitat avoided prominent bands of ambient noise more than expected by chance. We defined prominent bands as all noise above a cut-off of 0.003 RMS-values (i.e., ∼50 dB).

Correlated evolution

Habitat-mediated vocal divergence may reflect a correlated evolutionary response to selection on bill size and body size, in which case there should be an association between vocal divergence and morphological divergence. In theory, lower pitched songs should be associated with larger body mass, and slower paced songs should be associated with larger bill size (Podos et al. 2004). To test these predictions we collated data on body mass and bill size and quantified morphological divergence between species pairs. Data on body mass (to the nearest 0.1 g) were either collected from birds caught in 12×4 m mistnets at CICRA in 2004–2008, or from the literature (Dunning 1993; Zimmer and Isler 2003). Similarly, bill measurements were taken from a minimum of three males per species for all 17 species, mainly from mistnetted individuals, with gaps filled using specimens housed at the Museum of Natural Science at Louisiana State University (LSU), Baton Rouge, USA. Dial calipers were used to measure (to the nearest 0.01 mm) bill length from the tip to the meeting of the culmen and skull, and bill depth and bill width at the anterior end of the nares. We used PCA to reduce bill measures into a single component representing bill size ($PC1_{bill}$), which explained 84.1% of the variation (Eigenvalue = 2.52) and with which all three variables had high correlation coefficients (>0.8).

Neutral divergence

If neutral genetic divergence explains song divergence in our study, there should be a positive relationship between song divergence and genetic divergence. To estimate genetic distance between species pairs, we sequenced the mitochondrial protein-coding gene (ND2). Studies of ND2 and other mitochondrial protein-coding genes in birds have shown genetic variation that is consistent with neutral evolution (Arbogast et al. 2006; Weir 2006). Data for 11 species were obtained from GenBank (see Table S2). For the remaining species, we performed DNA sequencing. Total DNA was extracted from 25 mg of pectoral muscle using the DNeasy kit following the manufacturer's protocol. In a 20 mL total volume, PCR amplifications contained approximately 60 ng of genomic template DNA, 50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl, 0.5 mM dNTPs, 0.75 μM of each external primer, and 0.08 U Promega *Taq*. The thermocycling program consisted of an initial denaturing step (94°C for 2 min) followed

by 35 cycles of 94°C for 1 min, 50°C annealing step for 30 s, and a 72°C extension step for 1 min. The program ended with a final 72°C extension step for 3 min. PCR primers and internal sequencing primers included H6313 (Johnson and Sorenson 1998), L5215 (Hackett 1996), H5766 (Brumfield et al. 2007), and L5758 (Johnson and Sorenson 1998). Amplicons were purified using PEG precipitation, eluted in 12.5 mL 10mM Tris, and sequenced using the ABI Prism cycle sequencing protocol (Applied Biosystems Inc., Carlsbad, California) and an ABI 3100 Genetic Analyzer. Sequencing reactions were purified using Sephadex®️ G-50 and 400 mL 96-well filter plates.

Sequences were edited and aligned manually using Sequencher 4.6 (Gene Codes Corporation, Ann Arbor, MI) and translated into amino acids to verify the absence of stop codons or any anomalous residues. All new sequences were deposited into GenBank (see Table S2). The final dataset consisted of the entire ND2 gene (1,041 base pairs) for 33 taxa. Using PAUP* 4.0, we calculated the corrected *p*-distance between each species pair based on a finite-sites DNA substitution model. The best-fit model with the fewest number of estimated parameters was determined using the AIC test implemented in ModelTest (Posada and Crandall 1998). Likelihood scores for input into ModelTest were calculated on a neighbor-joining tree using PAUP* 4.0. We used maximum-likelihood methods implemented in RAxML 7.0.4 on the Cipres Portal version 1.5 (http://www.phylo.org/sub_sections/portal/) (Stamatakis et al. 2008) to infer the best tree using the GTR + Γ + I model with the data partitioned by codon position (three partitions) with joint branch length optimization.

Individual values for all acoustic variables (log-transformed; Table 1) were entered into a PCA, from which we calculated song distance between bamboo bird species and their closest sympatric terra firme relative as the Euclidean distance between group (i.e., species) centroids.

STATISTICAL AND PHYLOGENETIC COMPARATIVE ANALYSES

PC scores for vegetation structure ($PC1_{veg}$ and $PC2_{veg}$) were compared between habitat types using univariate General Linear Models (GLM) followed by Tukey's HSD posthoc pairwise comparison tests. The effect of habitat, frequency, and transmission height on attenuation and reverberation was tested using GLM with main effects and first-order interactions; and we used the same GLM approach to analyze the effect of habitat, frequency, and note-pace on distortion. To test for habitat differences in the spectral distribution of ambient sound energy, we used a GLM in which habitat and frequency (i.e., 50-Hz bin) were entered as fixed effects and log-transformed RMS-values as the dependent variable.

Song structure (separate acoustic measures and PC_{song} scores) and morphology were first compared between bamboo specialists and their closest relative in adjacent terra firme forest

Table 2. Phylogenetic signal of acoustic and morphological traits.

Variable	λ	LR P^1	AIC λ	AIC Brownian
Maximum frequency	1.00	1.00	118.75	116.75
Minimum frequency	1.00	1.00	102.18	100.18
Peak frequency	1.00	1.00	116.08	114.08
Bandwidth	0.67	**0.01**	67.44	72.20
Duration	1.00	1.00	132.21	130.21
Pace	0.49	**<0.0001**	205.96	343.15
Note number	0.00	**<0.0001**	269.76	1141.38
$PC1_{song}$	1.00	1.00	90.89	88.89
$PC2_{song}$	0.88	**0.04**	89.98	92.17
$PC3_{song}$	0.86	**0.01**	92.90	96.93
Body mass	0.91	**<0.0001**	5350.14	5635.57
$PC1_{bill}$	1.00	1.00	91.79	89.79

[1]Denotes the *P* value on a likelihood ratio (LR) test comparing λ and Brownian models of evolution; *P*<0.05 (marked in boldface) indicates that λ is a better fit than a Brownian model.

using a paired, nonparametric approach (Wilcoxon signed-rank tests). However, this approach assumes that species pairs are equally related to one another and that the phylogenetic signal of acoustic and morphological characters is weak. To test this assumption, we estimated lambda (λ), which measures the degree to which traits vary/covary across a tree in line with Brownian motion (Freckleton et al. 2002). A λ of 1 corresponds to the Brownian model, λ of 0 indicates a lack of phylogenetic structure, and λ values between 0 and 1 indicate the degree of trait lability (Pagel 1999). To determine whether λ values departed significantly from a Brownian model, we compared the fit of the two models using a likelihood ratio test. About half of the variables departed from a strict Brownian model (Table 2). The implication is that the evolution of these traits may have been influenced by shared ancestry.

To correct for this, we used two types of comparative models. First, we used generalized estimating equations (GEEs) for comparative data as described in Paradis and Claude (2002). We then used the generalized least squares (GLS) phylogenetic comparative method as described in Freckleton et al. (2002). We present results from both approaches because the GEE models are robust to analysis of repeated measures (Overall and Tonidandel 2004) but assume a Brownian motion model of evolution, whereas the modified GLS approach simultaneously estimates and uses λ to adjust the phylogenetic correction to reflect trait lability (Freckleton et al. 2002). To work around the assumption of a Brownian model in the GEE analyses, we transformed tree branch-lengths using the estimated λ for each trait and used the transformed trees in analyses. We used the maximum likelihood tree (Fig. 1) as our phylogenetic hypothesis for comparative analyses.

Prior to parametric analyses, all variables were log-transformed to meet assumptions of normality and homogeneity of variance. Nonparametric tests were used wherever the distribution of variables did not meet parametric assumptions. Means are reported $\pm$ SD, unless stated otherwise; *P*-values are two-tailed and corrected for ties where appropriate. Phylogenetic analyses were conducted in *R* (R-Development-Core-Team 2008) using the Ape and Geiger libraries (Harmon et al. 2008) as well as code written by R. P. Freckleton; all other tests were conducted using SPSS version 17.0 (SPSS 2008).

Results
HABITAT STRUCTURE
We found highly significant variation in habitat structure between stands of *Guadua* bamboo, terra firme forest and adjacent manmade grassland ($PC1_{veg}$: $F_{2,44} = 56.3$, $P < 0.0001$; $PC2_{veg}$: $F_{2,44} = 73.04$, $P < 0.0001$). Grassland had a highly uniform structure with an open understory and few trees, and showed no overlap in overall structure with either bamboo or *terra firme* forest (Fig. S3). Conversely, the principal component scores were more variable and partially overlapping for bamboo and *terra firme* (Fig. S3). Nonetheless, we found that the former had a significantly denser understory, lower mean visibility and fewer large trees than the latter (all posthoc pairwise comparisons: $P < 0.0001$; Fig. S4). In summary, all three habitats had a distinctive structure, but bamboo and *terra firme* forest were much more similar to one another than either was to grassland.

HABITAT SOUND TRANSMISSION PROPERTIES
We found a significant effect of signal frequency, height of transmission and habitat type on attenuation (Table 3, Fig. 2). Transmission height explained most variance in attenuation, and the interaction between transmission height and signal frequency had the strongest effect. At 0.1 m above the ground there was a prominent attenuation window between 2.0 and 4.0 kHz in both habitats (Fig. 2A), with bamboo attenuating high frequency signals (4.0 and 5.0 kHz) significantly more than terra firme forest. This mid-frequency attenuation window, a common feature of forested habitats, is caused by high attenuation for low-pitched signals close to the ground (Fig. 2A; Morton 1975). At 2.0 m, the window was absent and instead we found a strong linear increase in attenuation with increasing frequency (Fig. 2B), with bamboo attenuating high frequency signals significantly more than terra firme forest.

Signal frequency, transmission height, and the interaction between frequency and height had a strong significant effect on the way in which the artificial sound stimuli were degraded through reverberation. Specifically, we found that signal reverberation was

Table 3. Effects of signal frequency, habitat type, and transmission height on the (A) attenuation, and (B) reverberation of artificial sound stimuli. Statistics are from GLMs with main and two-factor interaction effects. Habitat refers to the habitat (bamboo or terra firme forest) in which the transmission experiment took place; height refers to the height (0.1 or 2.0 m) of the microphone and loudspeaker above ground level; n=25 sound points per habitat type.

Sources of variation		df	MS	F ratio	P[2]
(A) Attenuation					
Main effects	Frequency	5	1.556	16.227	**<0.0001**
	Habitat	1	1.351	14.091	**<0.0001**
	Height	1	3.585	37.395	**<0.0001**
Two-way interactions	Frequency×Habitat	5	0.315	3.287	**0.006**
	Frequency×Height	5	3.155	32.909	**<0.0001**
	Habitat×Height	1	0.002	0.023	0.881
Final model[1]	r^2=0 .35	17	1.769	18.59	**<0.0001**
(B) Reverberation					
Main effects	Frequency	5	2.283	34.593	**<0.0001**
	Habitat	1	0.112	1.704	0.192
	Height	1	26.276	398.229	**<0.0001**
Two-way interactions	Frequency×Habitat	5	0.096	1.458	0.202
	Frequency×Height	5	0.321	4.871	**<0.0001**
	Habitat×Height	1	0.104	1.581	0.209
Final model[1]	r^2=0.51	11	3.572	53.9	**<0.0001**

[1]Excludes nonsignificant terms and interactions.

[2]Bold denotes significant terms and interactions (P < 0.05).

significantly greater at 0.1 than 2.0 m, and increased linearly with increasing signal frequency (Table 3, Fig. 2C,D). There was no overall effect of habitat on reverberation, although post-hoc tests indicate a trend toward greater reverberation in higher pitched signals in bamboo than in terra firme forest (Fig. 2D).

Both signal pace and signal frequency had significant effects on distortion, with pace explaining most of the variation (Table 4). Specifically, we found that fast-paced, higher pitched signals suffered more distortion than did slow-paced, low-pitched signals. In other words, reverberations significantly increased the amplitude of the second tone relative to the first for faster paced, higher pitched signals. There was no overall effect of habitat type on distortion, nor was there any significant effects of interactions between pace and frequency, or pace and habitat (Table 4). Indeed, the value of r^2 for the final model was very low (0.07), in line with the idea that our target habitats are relatively similar. However, there was a significant effect of the interaction between habitat and frequency. Exploring this interaction more closely, we found a strong significant effect of habitat on amplitude distortion within specific frequency bands. Most importantly, 3 kHz signals transmitted 2 m above ground in terra firme forest suffered greater distortion when they were faster paced (Fig. 3). Conversely, there was no significant difference in levels of degradation between fast and slow paced 3 kHz signals in bamboo. This finding is notable because 3 kHz is close to the mean peak frequency of songs in both habitats (Table 5), and a transmission height of 2 m

is within the normal range of song perch height for all species in this study except *Crypturellus* (pair 1), which habitually sing at ground level.

To summarize, our results show that higher frequency signals suffer greater attenuation in bamboo than terra firme, whereas faster paced songs at key frequencies suffer greater distortion in terra firme than bamboo. Thus, the sensory drive hypothesis predicts that bamboo songs should be lower in pitch and faster in pace than terra firme songs.

TESTING THE SENSORY DRIVE HYPOTHESIS

These predictions were tested with acoustic analyses, which revealed significant differences in the spectral and temporal properties of bamboo and terra firme forest birdsongs (Figs. 4 and 5; Table 5). Specifically, we found that the songs of bamboo birds had significantly lower peak, maximum and minimum frequencies, and significantly higher pace, than did the songs of their close relatives in terra firme forest (Table 5). They also consisted of a significantly greater number of notes. The effect sizes (Cohen's d) for all but two acoustic measures (duration and bandwidth) were >0.2 (mean unsigned effect size = 0.25 ± 0.11), indicating a strong directional trend for bamboo songs to have lower pitch and higher pace. Moreover, when we assessed overall song structure using PCA we found that PC1$_{song}$ (representing song pitch), PC2$_{song}$ (song pace), and PC3$_{song}$ (song duration and note number) differed significantly between the two habitat types (Fig. 5,
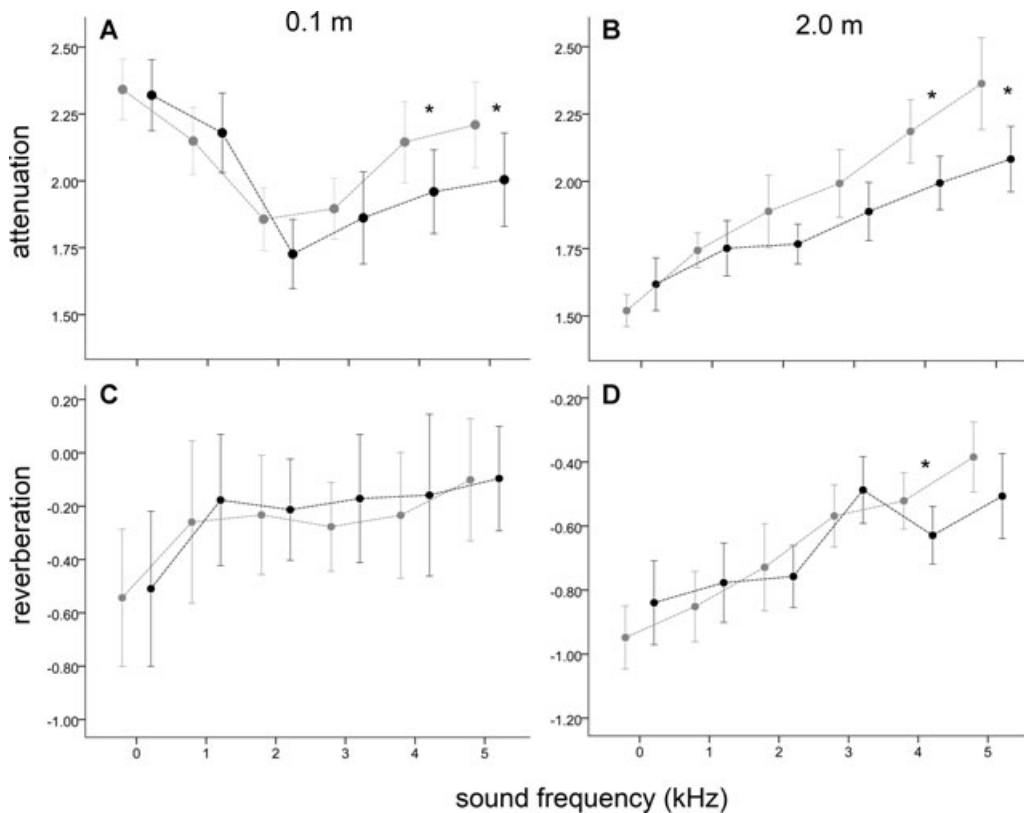
Figure 2. Relationship between the frequency of artificial sounds transmitted and the degree of attenuation (A, B) and reverberation (C, D). Data are presented for transmission heights of 0.1 m (A, C) and 2.0 m (B, D) in both bamboo (gray) and terra firme forest (black). The *x*-axis in all cases is the frequency of the sound transmitted; with the position of bamboo and terra firme points displaced slightly either side of each sound frequency to avoid overlap and facilitate interpretation. The *y*-axis in (A) and (B) is 1-LG10attenuation, such that higher values indicate higher levels of attenuation, and a value of 2 signifies that the signal is 10 times weaker at 20 m when compared to 5 m. In (C) and (D) the *y*-axis is LG10reverberation, with a value of zero signifying that tail and signal are of equal amplitude. Bars show 95% CIs; *n* = 25 sound points per habitat type. Asterisks denote significant differences between habitat types in levels of signal degradation (unpaired Bonferroni-corrected *t*-tests, *P* < 0.008).

Table 5). In other words, the songs of bamboo birds produced songs of significantly lower pitch but higher temporal complexity than their closest relatives in adjacent terra firme forest. This significant effect of habitat on song structure held when controlling for phylogeny using both the GEE and GLS approaches (Table 6).

ALTERNATIVE HYPOTHESES

Ambient noise

We found a significant effect of frequency on ambient noise characteristics, but no effect of habitat or the interaction between frequency and habitat (Table 7). This was corroborated by visual inspection of ambient noise profiles, which showed that both habitats were characterized by continuous noise at a number of discrete frequency bands (Fig. 6). These bands are typical of tropical forest and are mainly generated by calling insects, in particular different species of cicada (Cicadidae; Slabbekoorn 2004a). In our recordings, two bands (at ~2.0 and ~5.0 kHz) were par-

ticularly prominent; these appear as two conspicuous peaks in the mean ambient noise spectra shown in both bamboo (Fig. 6B) and terra firme forest (6C). We found that the peak frequency of songs fell within these bands of noise in 8 of 17 (47%) of bamboo species (Fig. 6D) and 8 of 16 (50%) of terra firme forest species (Fig. 6E). Thus, the proportion of songs in our community with peak frequency overlapping with noise bands did not differ from that expected by chance (chi-square tests: $\chi^2_1 < 0.029$, ns).

Correlated evolution

Bamboo and *terra firme* forest birds did not differ consistently or significantly in body mass (Fig. 7A) or bill size (Fig. 7B), suggesting that habitat-dependent song divergence is not a byproduct of morphological adaptation (Fig. 7, Tables 5 and 6). In other words, we found no evidence that habitat-mediated vocal divergence reflects a correlated evolutionary response of selection on body size or bill size.

Table 4. Effect of signal pace, frequency, and habitat on signal-to-signal ratio. Statistics are from a GLM with main and two-factor interaction effects. Habitat refers to the habitat (bamboo or terra firme forest) in which the transmission experiment took place; pace refers to the pace (fast or slow) of the artificial sound stimuli; *n*=25 sound points per habitat type.

Source of variation	df	MS	*F* ratio	*P*[2]
Pace	1	0.002	6.825	**0.009**
Frequency	5	0.001	2.542	**0.027**
Habitat	1	0.000	0.113	0.737
Pace×Frequency	5	0.001	2.016	0.075
Pace×Habitat	1	0.000	0.406	0.524
Frequency×Habitat	5	0.001	2.399	**0.036**
Final model[1] (r^2=0.07)	17	0.001	2.457	**0.001**

[1]Excludes nonsignificant terms and interactions.
[2]Bold denotes significant terms and interactions ($P < 0.05$).

Neutral divergence

The best-fit model determined using ModelTest was the general time-reversible (GTR) model of nucleotide substitution with rate heterogeneity among sites modeled with a gamma + invariant

Figure 3. Effect of habitat and pace on patterns of degradation for 3 kHz signals. The *y*-axis is LG10signal-to-signal ratio at 20 m, such that higher values indicate higher levels of amplitude distortion caused by reverberations between successive tones. A value of zero signifies that the first and second notes have equal amplitude. Bars show 95% CIs; $n = 25$ sound points per habitat type. Statistics are from unpaired *t*-tests.

sites distribution ($\Gamma + I$). The maximum-likelihood analysis resulted in a single maximum-likelihood tree (log $L = 12759.2$; Fig. 1). Mean corrected *p*-distance in our sample of 17 pairs was $0.173 \pm 0.128\%$. Assuming a 2% molecular clock (Weir and Schluter 2008), we estimate that the time to coalescence for species pairs ranged from 3.41 million years ago (MA, pair 13: *Hypocnemis* spp.) to 29.7 MA (pair 1: *Crypturellus* spp.) for species pairs. We found no significant relationship between divergence in overall song structure and genetic distance (Spearman rank correlation; $r = 0.39, P = 0.122, n = 17$ pairs; Fig. 8A). There was a weak nonsignificant positive association between genetic distance and divergence in song duration ($r = 0.44, P = 0.078$). However, divergence in all other acoustic traits was unrelated to evolutionary age ($0.202 < P < 0.801$). Rerunning the analyses removing the two oldest pairs in our sample–*Crypturellus* spp. (pair 1) and *Hemitriccus* spp. (pair 2)–produced a significant relationship between overall song structure and genetic distance ($r = 0.59, P = 0.021, n = 15$ pairs; Fig. 8B). The relationship between genetic distance and divergence in song duration was also strengthened ($r = 0.56, P = 0.030$). However, divergence in all other acoustic traits remained unrelated to evolutionary age ($0.232 < P < 0.820$).
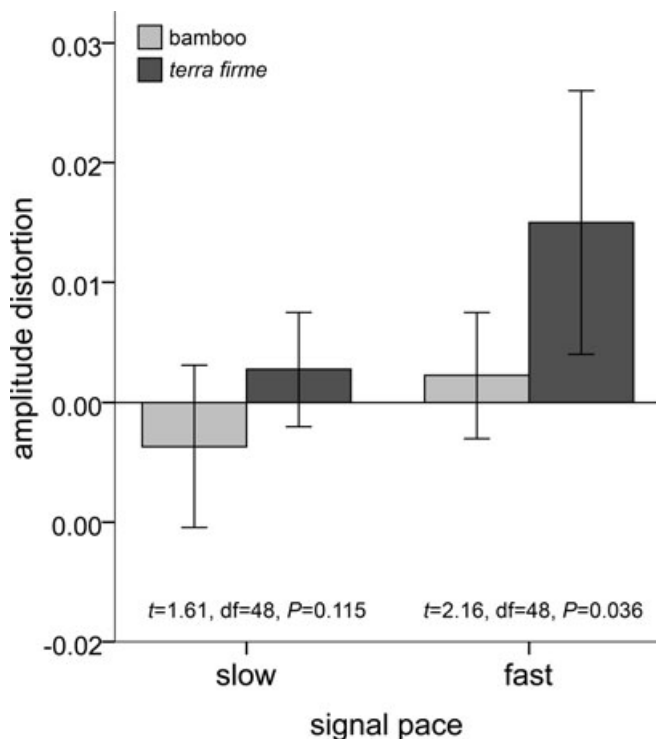
Discussion

Divergence in mating signals may be driven by deterministic processes (e.g., natural selection) or stochastic processes (e.g., genetic drift; Coyne and Orr 2004). Uncertainty about which of these factors best explains the patterns of phenotypic variation between species has fuelled a long-standing debate in evolutionary biology. The relative influence of determinism and stochasticity has proved particularly difficult to resolve in the case of acoustic signals, which are known to be under a degree of environmental control (Kirschel et al. 2009a), but which may also be strongly influenced by phylogeny or genetic distance (Price and Lanyon 2002; Irwin et al. 2008). Based on variation between Amazonian habitats, we show that the extent and direction of song divergence between closely related bird species are only weakly related to stochastic genetic divergence yet consistent with sensory drive. These results suggest that selection on birdsong may outweigh the contribution of neutral processes, even when environmental differences are relatively minor (Fig. S3).

EVIDENCE FOR SENSORY DRIVE IN AMAZONIAN BIRDSONG

Previous studies have focused on the sound transmission properties of terra firme forest either at single localities (e.g., Marten et al. 1977; Nemeth et al. 2001; Ellinger and Hodl 2003), or across different localities (e.g., Slabbekoorn and Smith 2002b; Dingle et al. 2008). The current study provides the first quantification

Table 5. Comparison of the song properties and morphology of bamboo and terra firme forest birds.

	Bamboo	Terra firme	z	P^1	Cohen's d (95% CIs)
Separate acoustic measures:					
Maximum frequency (kHz)	3.427±1.654	3.873±1.766	−2.249	**0.025**	**−0.26** (−1.05–0.58)
Minimum frequency (kHz)	1.754±1.468	2.131±1.499	−2.627	**0.009**	**−0.25** (−0.95–0.46)
Bandwidth (kHz)	1.623±0.572	1.737±0.746	−0.355	0.723	−0.17 (−0.44–0.18)
Peak frequency (kHz)	2.746±1.629	3.198±1.732	−2.012	**0.044**	**−0.27** (−1.04–0.55)
Pace (notes per sec)	6.666±4.839	5.277±5.739	−1.728	0.084	**0.26** (−2.04–2.99)
Note number	12.962±9.601	8.817±8.491	−2.201	**0.028**	**0.46** (−4.11−4.49)
Duration (sec)	2.710±2.432	2.500±2.163	−1.254	0.210	0.09 (−1.06–1.12)
Overall song structure[2]:					
$PC1_{song}$ (pitch)	−0.210±1.034	0.210±0.949	−2.343	**0.019**	**−0.42** (−0.92–0.03)
$PC2_{song}$ (pace)	0.217±0.899	−0.217±1.075	−2.012	**0.044**	**0.44** (0.01–0.95)
$PC3_{song}$ (note number & duration)	0.256±0.882	−0.256±1.070	−2.627	**0.009**	**0.52** (0.11–1.03)
Morphology:					
Body mass (g)	51.060±97.992	45.930±86.996	−1.344	0.179	0.06 (−46.53–41.41)
PC1 (bill)	0.078±1.061	−0.112±0.935	−1.160	0.246	0.19 (−0.31–0.63)

[1]Statistics derive from Wilcoxon signed-rank tests; bold denotes significant differences and biologically meaningful effect sizes (i.e., d>0.20 sensu Cohen 1988).

[2]For factor loadings of song principal components (PC1, PC2 and PC3), see Table 1.

of habitat acoustics in a mosaic of two distinct tropical forest types, as well as the first assessment of the acoustic properties of Neotropical bamboo forest. Our experimental data allow three key conclusions to be drawn. First, high-pitched signals of 4–5 kHz suffered significantly greater attenuation in *Guadua* bamboo than in terra firme forest. Second, reverberation profiles also suggested that higher frequency signals are, overall, more degraded by passage through bamboo than terra firme. And third, faster paced signals at moderate frequencies suffered greater distortion in terra firme forest than bamboo. In general, high levels of distortion indicate that the echo of the first note has extended beyond the internote interval, which may affect perception by potential receivers (Blumenrath and Dabelsteen 2004; Slabbekoorn et al. 2007). The first two points suggest that there should be downward selection on signal frequency in bamboo, whereas the third point suggests that slower paced songs should be selected for in terra firme forest.

These findings make sense in the light of vegetation structure. On the one hand, higher attenuation in bamboo likely reflects the fact that *Guadua* grows in dense stands and provides multiple surfaces to scatter and absorb sound energy, whereas the understory of terra firme forest tends to be more open (Fig. S3A, B). On the other hand, greater distortion of faster paced signals in terra firme may reflect the fact that this habitat has a higher density of major reflective surfaces (i.e., many more large trees; Fig. S3C,D). Previous work has shown that internote reverberation is greatest where reflective surfaces are more varied (Slabbekoorn et al. 2007), and it seems likely that this applies more to terra firme than

bamboo. In summary, although bamboo grows in densely packed stands, resulting in greater signal attenuation, it also provides a rather more uniform and less reflective substrate than terra firme forest, apparently resulting in less distortion for fast-paced signals at key frequencies.

We have shown that signal transmission properties vary in line with vegetation structure, but the strength of selection imposed by this variation remains unclear. It is worth pointing out, however, that our experimental results were based on relatively short transmission distances (up to 20 m) whereas most Amazonian birdsongs typically travel further from signaler to receiver (up to at least 200 m). This means that habitat-related differences in sound transmission properties detected in this study are likely to be accentuated in real signaling systems. Thus, the sensory drive hypothesis clearly predicts that bamboo birds should produce songs with lower peak frequency and faster pace than terra firme birds.

We tested for these effects using acoustic analyses. The results revealed consistent habitat-dependent spectral and temporal differences in birdsong, and in both cases these went in the direction predicted by sensory drive. First, bamboo species used songs with significantly lower maximum, minimum, and peak frequency than their terra firme counterparts. This was reflected in the fact that songs with peak frequency under 3 kHz were used by 88% of bamboo birds, but only 37% of terra firme birds. Second, bamboo birds used signals with significantly higher note-number and note-pace. These differences are in line with greater attenuation of high frequency signals, and reduced distortion of faster paced signals,
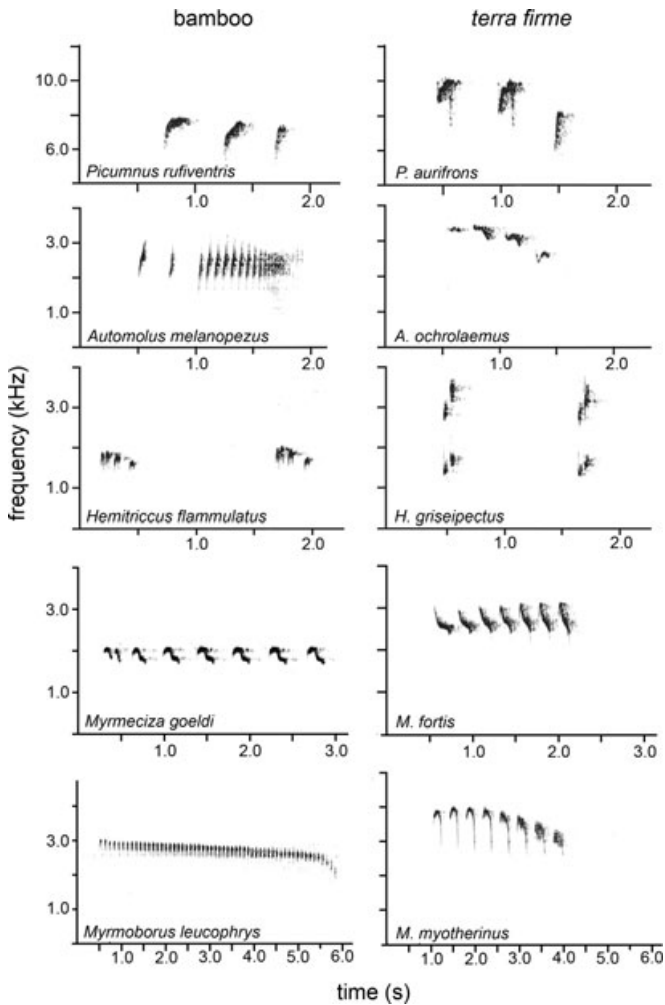
Figure 4. Spectrograms of the songs of five of the 17 species pairs, with songs of bamboo-specialist species shown on the left, and those of their closest terra firme relative shown on the right. In all cases spectrograms compare single songs of each species, except for *Hemitriccus* where two songs are given per species. Spectrograms were generated from digital (wav) recordings (sampling rate = 44.1 kHz) in Avisoft SASLabPro Version 4.50 using narrowband (55 Hz) filter settings (FFT = 1024). All recordings were made at the study site (CICRA, Peru).

Figure 5. Song structure in bamboo and terra firme forest birds. Each point plots mean trait values for species occurring in terra firme forest (*y*-axis) against the mean of their closest relative occurring in bamboo forest (*x*-axis). Trait values are (A) song pitch (PC1$_{song}$); (B) song pace (PC2$_{song}$); and (C) note number and song duration (PC3$_{song}$). To facilitate comparison, a reference line depicts the 1:1 relation between bamboo and terra firme forest values. Note that 15 of 17 points fall above the line in (A), and 12 of 17 points fall below the line in both (B) and (C), showing that the songs of terra firme birds are, on average, of higher pitch and lower temporal complexity than the songs of their closest relative in the bamboo (for quantitative analyses, see Tables 5 and 6).

in bamboo. Hence, our results strongly suggest that adaptation to the sound transmission properties of different forest types has shaped the design of long-distance signals in Amazonian birds.

Consistent acoustic adaptation seems to contradict the view that selection is often dampened or unpredictable as a result of environmental fluctuation over time. The paradigm of unpredictability has gained ground in recent years, partly because long-term studies of bird populations have revealed significant oscillations in phenotype—including song structure—in response to changing environments (e.g., Grant and Grant 2002; Derryberry 2009). However, these studies are focused on grasslands or shrublands, which are both low-stature habitats predisposed to temporal
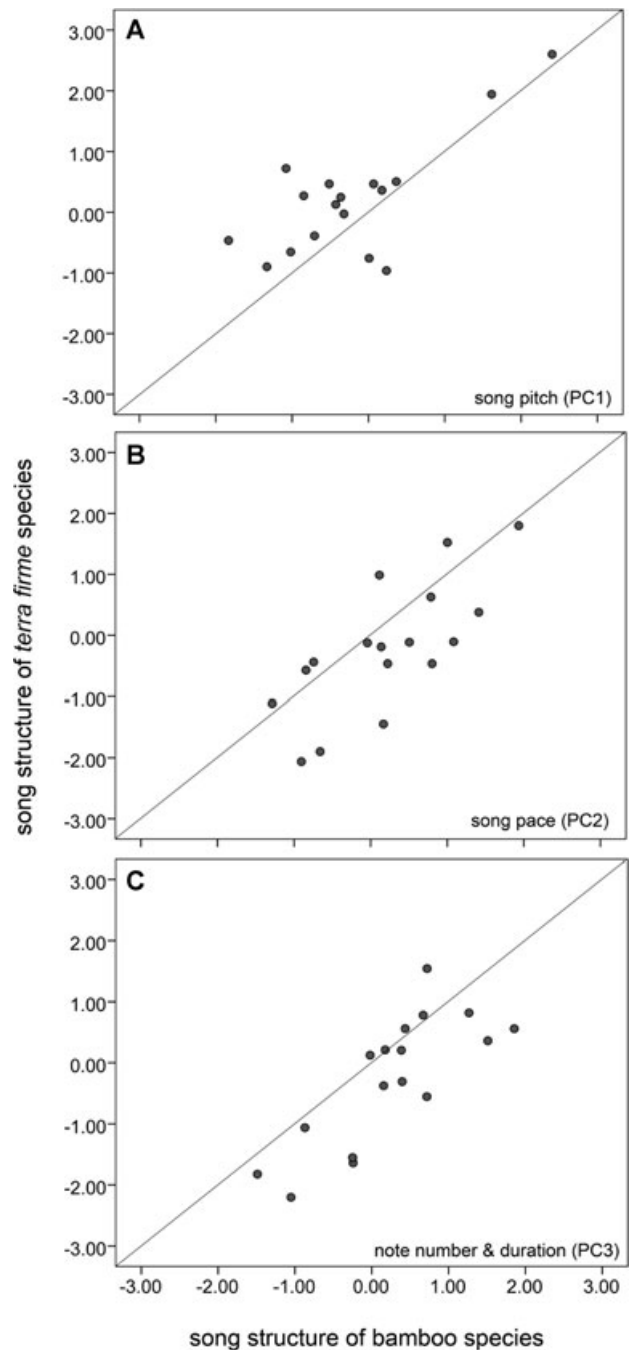
Table 6. Effect of habitat on song structure, controlling for phylogenetic effects.

	GEE with λ tree		PGLM (incorporates λ)	
	F	P[1]	F	P[1]
Maximum frequency	6.276	0.075	1.864	0.182
Minimum frequency	17.274	**0.019**	4.005	0.054
Bandwidth	0.037	0.850	0.001	0.975
Peak frequency	12.235	**0.031**	3.060	0.090
Pace	8.266	**0.022**	4.843	**0.035**
Note number	11.263	**0.007**	6.499	**0.016**
Duration	1.712	0.270	0.714	0.405
$PC1_{song}$	18.027	**0.017**	4.584	**0.040**
$PC2_{song}$	6.763	**0.036**	4.142	**0.050**
$PC3_{song}$	10.920	**0.012**	5.340	**0.027**
Mass	0.441	0.548	0.841	0.366
$PC1_{bill}$	8.980	**0.047**	2.482	0.125

[1]**Bold denotes a significant effect of habitat on a trait after correcting for phylogenetic inertia.**

variation in species composition and vegetation structure. By contrast, mature tropical forests are highly heterogeneous but nonetheless stable in structure over longer time frames (Terborgh 1992; Tuomisto et al. 1995). We speculate that such habitats may drive relatively predictable evolution over time, and that this may reinforce the divergent selection underlying our results.

ALTERNATIVE ADAPTIVE EXPLANATIONS

A range of alternative explanations for song divergence needs to be considered. For example, the acoustic adaptation hypothesis predicts that song divergence may be driven, not only by sound transmission, but by habitat-related differences in noise (Slabbekoorn and Smith 2002b; Slabbekoorn and Peet 2003). Acoustic analyses of both songs and ambient noise revealed that peak song frequency tended to fall well below a conspicuous upper band created by insect calls, whereas two avian genera—

Table 7. Effect of frequency and habitat on levels of ambient noise. Statistics are from a GLM with main and two-factor interaction effects. Habitat refers to the habitat (bamboo or terra firme forest) in which the ambient noise recordings were made.

Source	df	MS	F ratio	P
Frequency	1	102.064	203.170	<0.0001
Habitat	1	1.625	3.234	0.073
Frequency×habitat	1	0.149	0.296	0.587
Final model[1] (r^2=0.35)	2	51.844	103.202	<0.0001

[1]**Excludes nonsignificant terms and interactions.**

Picumnus and *Epinecrophylla*—communicate using frequencies above this band (Fig. 6). This suggests a role for noise in setting the upper limit of song frequencies in Amazonian birds. However, the finding that in both habitats peak frequency of songs often fell within a conspicuous lower band of noise suggests that avoidance of interference by noise does not have a major influence over spectral properties. Moreover, we also find that bamboo and terra firme do not differ in their ambient noise profiles, and hence it is unlikely that the songs of bamboo birds have diverged by this mechanism.

Another possible explanation for our results is that signals may have diverged as a byproduct of morphological adaptation to the environment (Podos 2001; Nosil et al. 2008). In this case, we found no consistent difference in body mass or bill size of birds in bamboo and terra firme forest, suggesting that habitat-dependent song divergence is not due to correlated evolution. Indeed, there is little evidence of habitat-dependent selection on ecological traits, as related bamboo and terra firme species typically show very minor or inconsistent divergence in size and bill morphology, even after millions of years of reproductive isolation (Fig. 7).

A final issue to consider is predation pressure. Many studies have shown that the imperatives of crypsis or mimicry exert divergent selection on visual signals, such as when pigmentation varies across ecotones in tandem with background coloration (e.g., Mullen and Hoekstra 2008; Robertson and Rosenblum 2009), or when populations mimic different aposematic models (e.g., Jiggins et al. 2001). Both of these mechanisms can lead to ecological speciation when color patterns are involved in mating decisions (e.g., Nosil 2004; Jiggins 2008). The influence of predation on acoustic signals is more diffuse, but there are cases of mortality-driven signal divergence, such as when local pressure from predators or parasitoids causes signals to become less conspicuous (e.g., Tuttle and Ryan 1981; Zuk et al. 2006). However, this is again unlikely to explain our results because the relevant predators—diurnal raptors like *Accipiter* and *Micrastur*—commonly hunt in both bamboo and terra firme. Moreover, it is not clear how a lower song frequency would help bamboo birds to avoid predation.

THE NEUTRAL PERSPECTIVE

Stochastic processes such as genetic drift are often proposed as alternative, nonadaptive routes to signal divergence. In support of this view, we find that song divergence is weakly associated with the level of mitochondrial sequence divergence (when the two oldest species pairs are removed from analyses). This finding suggests a degree of stochasticity in the tempo of song evolution, in line with Brownian models that predict a linear relationship between phenotypic and genetic divergence across multiple species. However, the relationship between genetic divergence and song divergence is highly variable, with the significant
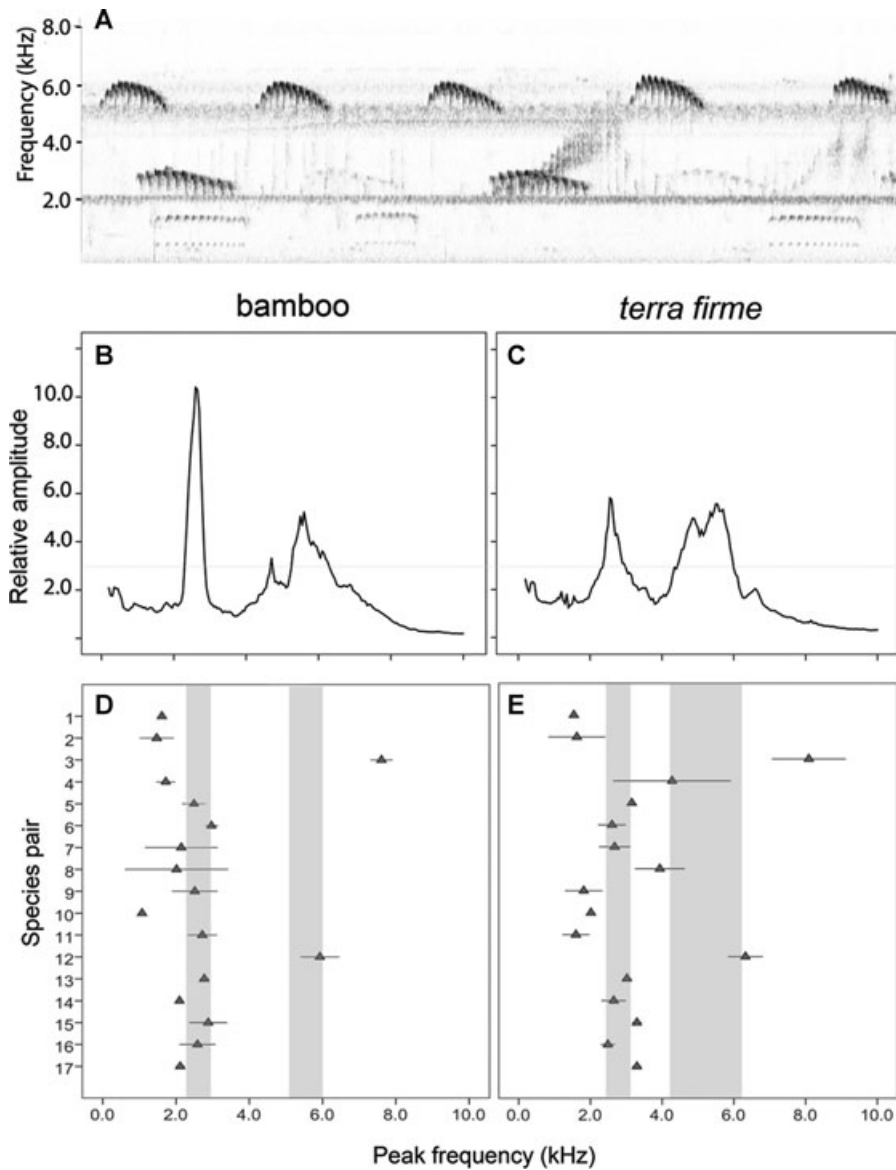
Figure 6. (A) Spectrogram of a 25-sec dawn recording made in terra firme forest at CICRA showing the songs of three antbird species (a: *Epinecrophylla haematonota*; b: *Myrmoborus myotherinus*; c: *Thamnophilus schistaceus*), plus two prominent horizontal bands of noise made by insect and frog choruses. (B) and (C) show mean ambient noise levels for bamboo and terra firme, respectively, with relative amplitude (root mean squared [RMS]-values x 10^{-3}) on the *y*-axis and frequency (kHz) on the *x*-axis. The lines are based on 432 and 408 1-s sound files, respectively, sampled at 5 min-intervals from standardized 120-min recordings made at 18 sites in bamboo (B) and 17 in terra firme (C). Bands of ambient noise shown in (A) are represented by steep peaks in both habitats at ∼2.0 and ∼5.0 kHz in (B) and (C); note that the lower band is louder in bamboo, but both bands are broader in terra firme. The cutoff point for prominent background noise (0.003 RMS-values) is indicated by a horizontal line. (D) and (E) show peak frequency of songs (mean ± 95% CIs) for bamboo and terra firme birds, respectively (see Fig. 1 for details of species pairs given on *y*-axis). Note the overall lower pitch of bamboo songs compared to terra firme songs, and the fact that the songs of several species overlap with the bands of insect noise (shaded in gray).

correlation largely driven by an association between genetic distance and the level of divergence in one temporal trait: song duration. Divergence in spectral traits (e.g., peak frequency), or indeed in other aspects of temporal structure (e.g., note number and note pace), was not significantly related to genetic distance.

Widely varying rates of divergence are difficult to explain by gradual genetic drift or mutation, but there are other stochastic processes to consider, namely sexual selection and cultural evolution. Both these factors may alter rates of song divergence, the former because signals are thought to evolve more rapidly under strong sexual selection (Fisher 1930), and the latter because
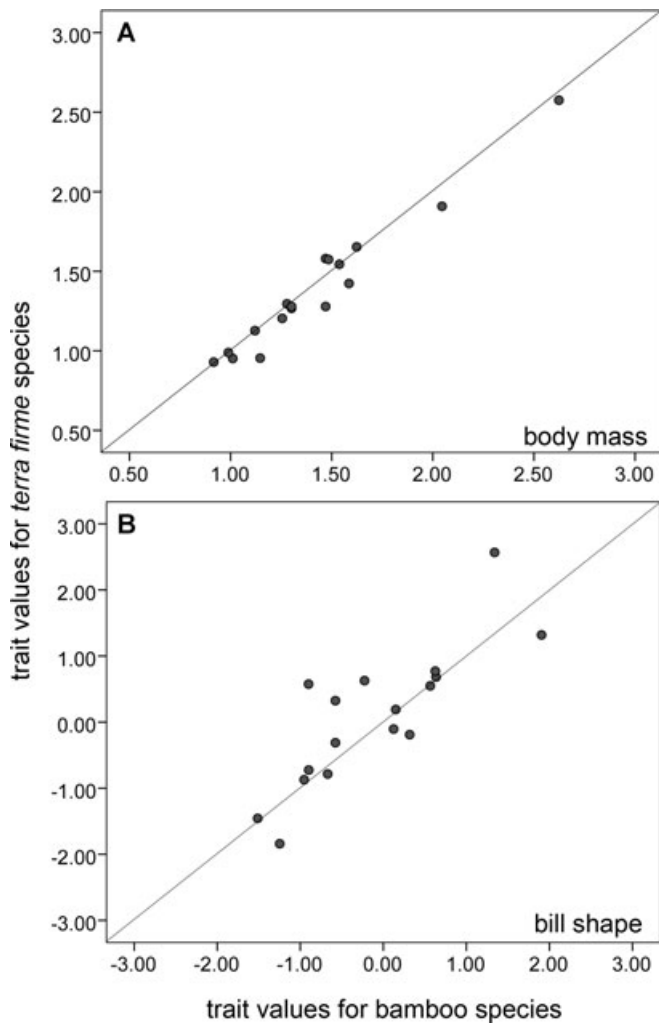
Figure 7. Body mass (A) and bill size (B) of bamboo and terra firme forest birds. Each point plots mean trait values for species occurring in terra firme forest (*y*-axis) against the mean of their closest relative occurring in bamboo forest (*x*-axis). To facilitate comparison, a reference line depicts the 1:1 relation between bamboo and terra firme forest values. Note that in contrast to the situation for song structure (Fig. 5), there is no consistent morphological divergence between bamboo and terra firme forest birds in either mass or bill size (see Table 5).

song learning leads to increased plasticity and the accumulation of random copying errors from one generation to the next (Lachlan and Servedio 2004). However, all species included in this study are apparently monogamous with long-term pair bonds indicating weaker overall levels of sexual selection, and none is thought to develop songs by learning. In other words, our sample contains no obvious biases with respect to sexual selection and phenotypic plasticity. Overall, we do not find a strong signature of genetic drift, and in any case the predictable direction of song divergence allows us to reject all purely stochastic explanations.

LINKING ADAPTATION, SPECIATION, AND TROPICAL DIVERSITY

We have shown that two major Amazonian habitat types, bamboo forest and terra firme forest, have distinct sound transmission properties, and that birds living in these habitats produce songs with correspondingly divergent spectral and temporal traits. It seems reasonable to assume that stochastic processes always contribute to signal divergence in reproductively isolated populations, but they cannot fully account for the predictable direction or the variable magnitude of divergence reported here. Instead, our findings support the view that physical characteristics of the environment influence how effectively acoustic signals are broadcast and
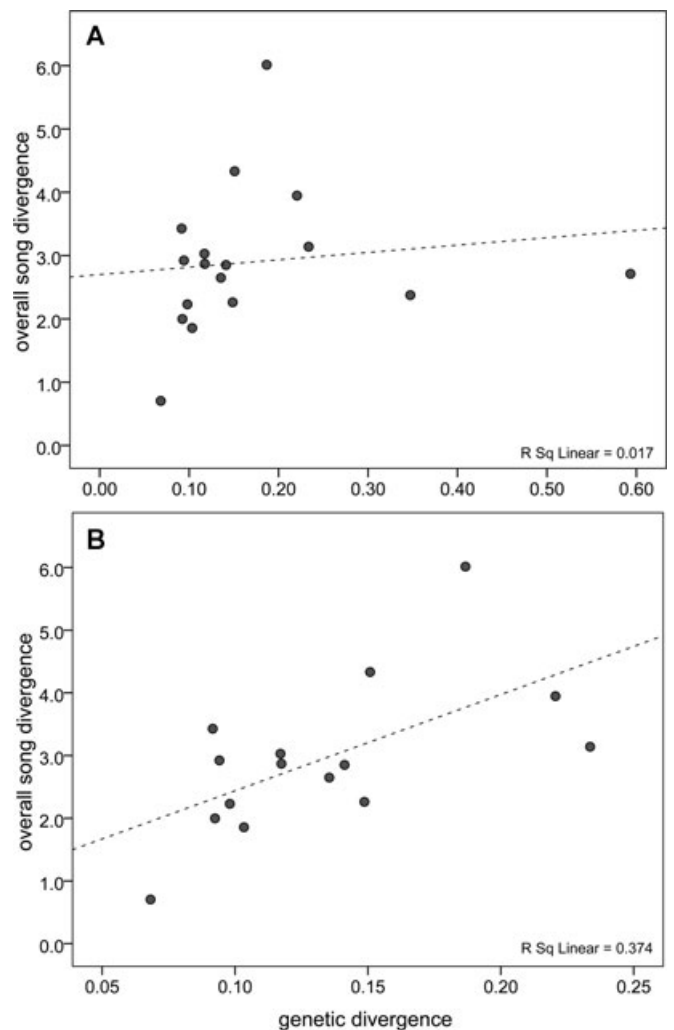

Figure 8. Relationship between the genetic divergence (corrected *p*-distance) between pairs of bamboo and terra firme species, and the overall divergence in song structure (Euclidean distance) within each species pair. Data are shown for (A) all 17 species pairs; and (B) a reduced sample with two outliers excluded. There is a weak relationship between genetic divergence and song divergence which is only significant when the two oldest pairs in the sample (*Crypturellus* spp. and *Hemitriccus* spp.) are removed.

perceived, thereby exerting deterministic selection (Bradbury and Vehrencamp 1998; Patten et al. 2004).

We cannot be sure that this mechanism has driven speciation in our system, yet our findings shed important light on the potential role for song in the speciation process. In particular, by revealing that sensory drive can promote phenotypic divergence across habitat gradients relevant to species formation, this study provides the strongest evidence to date that ecological adaptation of acoustic signals offers a route to reproductive isolation, as proposed by many previous studies (e.g., Ryan and Wilczynski 1988; Slabbekoorn and Smith 2002a; Förschler and Kalko 2007; Seddon and Tobias 2007). Overall, our results are consistent with a role for habitat heterogeneity and sensory drive in generating high levels of diversity in tropical birds.

ACKNOWLEDGMENTS

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Supporting Information

The following supporting information is available for this article:

Figure S1. LANDSAT image illustrating the heterogeneous mix of habitat types found in the lowland forests of SE Peru: *Guadua* bamboo (yellowish patches), terra firme forest (pale green), floodplain forest (dark green), *Mauritia flexuosa* palm swamp (purple).
Figure S2. Diagram of an artificial signal used in transmission experiments.
Figure S3. Scatterplot showing vegetation structure at survey points in stands of *Guadua* bamboo (red), terra firme forest (blue) and adjacent manmade grassland (green).
Figure S4. Comparison between terra firme forest, stands of *Guadua* bamboo and adjacent manmade grassland in (A) understory density (% volume), (B) mean visibility at 1.5 m above the ground, (C) number of trees with diameter at breast height (dbh) 20–40 cm, and (D) number of trees with dbh > 40 cm.
Table S1. Morphology and basic song structure of all species included in this study.
Table S2. Tissue samples used in the genetic analysis.

Supporting Information may be found in the online version of this article.

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