ECOLOGICAL ADAPTATION AND SPECIES RECOGNITION DRIVES VOCAL EVOLUTION IN NEOTROPICAL SUBOSCINE BIRDS

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Abstract.—Given that evolutionary divergence in mating signals leads to reproductive isolation in numerous animal taxa, understanding what drives signal divergence is fundamental to our understanding of speciation. Mating signals are thought to diverge via several processes, including (1) as a by-product of morphological adaptation, (2) through direct adaptation to the signaling environment, or (3) to facilitate species recognition. According to the first two hypotheses, birdsongs diversify in different foraging niches and habitats as a product of selection for optimal morphology and efficient sound transmission, respectively. According to the third hypothesis, they diversify as a result of selection against maladaptive hybridization. In this study I test all three hypotheses by examining the influence of morphology, acoustic environment, and the presence of closely related congeners on song structure in 163 species of antbird (Thamnophilidae). Unlike oscine passerines, these Neotropical suboscines make ideal subjects because they develop their songs without learning. In other words, patterns of vocal divergence are not complicated by cultural evolution. In support of the morphological adaptation hypothesis, body mass correlates with the acoustic frequency of songs, and bill size with temporal patterning. These relationships were robust, even when controlling for phylogenetic inertia using independent contrasts, suggesting that there has been correlated evolution between morphological and acoustic traits. The results also support the acoustic adaptation hypothesis: birds which habitually sing in the understory and canopy produce higher-pitched songs than those that sing in the midstory, suggesting that song structure is related to the sound transmission properties of different habitat strata. Finally, the songs of sympatric pairs of closely related species are more divergent than those of allopatric pairs, as predicted by the species recognition hypothesis. To my knowledge, these data provide the first direct evidence that species recognition and ecological adaptation operate in tandem, and that the interplay between these factors drives the evolution of mating signals in suboscine birds.

Key words.—Antbirds, comparative analysis, ecological adaptation, mating signals, speciation, species recognition, Thamnophilidae.

Evidence in a range of animal taxa suggests that the divergence of mating signals leads to reproductive isolation, and hence to speciation (West-Eberhard 1983; Panhuis et al. 2001). However, we are far from understanding the processes by which populations diverge in male signals and female preferences (Wells and Henry 1998; Boughman 2002). Divergence may arise through adaptation to divergent ecologies (Dobzhansky 1951; Mayr 1963), perhaps as a by-product of morphological adaptation (Podos 2001) or else by direct adaptation to the signaling environment (Boughman 2002). Signaling traits and preferences might also diverge late on in the speciation process by selection against maladaptive hybridization (Dobzhansky 1951; Butlin 1995; Noor 1999). Although there is some support for these theories from a disparate array of taxa, they have never been systematically tested in the same study across a monophyletic group of animals.

Endorsement for the ecological adaptation hypotheses derives mainly from studies of one type of mating signal: birdsong (Slabbekoorn and Smith 2002). Song is a long-distance communication signal prone to modification by the environment. Different habitats impose varying acoustic effects, such as frequency-dependent reverberations, attenuations, and irregular amplitude fluctuations (Marten and Marler 1977; Marten et al. 1977; Wiley and Richards 1982), all of which can interfere with song transmission (Wiley 1991). Accordingly, songs appear to be adapted to habitats in terms of their frequency (e.g., Morton 1975; Nottebohm 1975; Ryan and Brenowitz 1985) and temporal patterning (e.g., Cosens and Falls 1984; Badyaev and Leaf 1997; Brown and Handford

2000). For example, because low-frequency sounds travel best through dense foliage and because songs containing short notes repeated at longer intervals are less effected by reverberations, forest-dwelling birds produce lower-pitched songs, (e.g., Morton 1975) with slower rates of syllable repetition (e.g., Ryan and Brenowitz 1985) compared to species living in open habitats (for an overview, see Badyeav and Leaf 1997). Similarly, because sound propagation at the forest floor differs from that in the canopy (Marten et al. 1977; Ellinger and Hodl 2003), the height from which birds sing may be adapted to optimize transmission (Lemon et al. 1981; Nemeth et al. 2001).

Adaptation to divergent ecologies can also affect song structure indirectly when there is selection for changes in phenotypic traits which are functionally related to sound production. For example, body mass has a strong negative relationship with song frequency in many species (Ryan and Brenowitz 1985; Badyaev and Leaf 1997; Tubaro and Mahler 1998; Palacios and Tubaro 2000; Bertelli and Tubaro 2002; but see Laiolo and Rolando 2003). This relationship arises because body mass correlates with the size of the syringeal membrane, the vibrating frequency of which determines the fundamental frequency of a vocalization (Nowacki and Marler 1988); the larger membranes the lower the pitch of the song produced (Wallschläger 1980; Ryan and Brenowitz 1985). Similarly, because the suprasyringeal tract has resonating properties that influence the frequency spectra of songs (e.g., Nowacki 1987), bill morphology correlates with song frequency (Palacios and Tubaro 2000; Podos et al. 2004) and harmonic content (Hoese et al. 2000). Bill morphology may

also affect the temporal patterning of songs, a negative relationship between bill size and trill rate having been demonstrated in Darwin's finches *Geospiza* spp. (Podos 2001). This is probably explained by the trade-off between force and speed which arises through the biomechanical constraints of lever-arm position and muscle architecture (Herrel et al. 2002). The result is that large powerful bills are less versatile and less able to produce sounds such as fast trills (Podos 1997; Podos et al. 2004). Therefore, because body size and bill size are under strong selection for maximizing efficiency within a foraging niche (Gibbs and Grant 1987), relationships between morphology and song structure have been cited in support of the idea that morphological constraints, and hence ecological adaptation, can shape song evolution (Podos 1997, 2001).

Despite these findings, adaptation to divergent ecologies typically only explains a small proportion of the variation in song structure (Badyaev and Leaf 1997; Buskirk 1997). Furthermore, it cannot account for song similarity among closely related species with differing ecologies (Ryan and Brenowitz 1985), or the pronounced differences in the songs of cryptic species with similar ecological requirements (e.g., *Phylloscopus* warblers: Irwin et al. 2001; tyrant flycatchers: Zimmer et al. 2001; antbirds: Bierregaard et al. 1997). Therefore, it is clear, that additional selective forces are at play, as we might expect for a functionally complex phenotype such as song (Endler 1993).

The species recognition hypothesis proposes that differences in song structure among species have evolved to reduce hybridization, just as divergent plumage is proposed to evolve to avoid costly mating errors (e.g., Sætre et al. 1997, but see McNaught and Owens 2002). We might therefore predict that there will be a shift in species-specific vocal characters when closely related birds occur sympatrically (Miller 1982). However, despite the widespread occurrence of sympatric sister species, evidence for song divergence is scarce (Irwin and Price 1999). Indeed, of the few empirical studies to test this prediction, only three provide tentative support (Wallin 1985; Doutrelant et al. 2000; Haavie et al. 2004), although others have refuted it (Irwin 2000; de Kort et al. 2002a,b). However, these studies have focused on single species or small groups of closely related species. A potentially more powerful approach is to integrate data from many different species within a large-scale comparative test, but this has not yet been attempted. Further, with the exception of some recent work on *Streptopelia* spp. doves (de Kort et al. 2002a,b; de Kort and ten Cate 2004), most studies have focused on oscine passerines (Slabbekoorn and Smith 2002). Male oscines learn their songs, and female oscines their song preferences, through an imprinting-like process and some are able to modify their repertoire during their life (Kroodsma 1982); vocal learning and cultural inheritance are thus major determinants of oscine song structure (Nottebohm 1972; Catchpole and Slater 1995). Reproductive isolation among the African indigobirds *Vidua* spp., which are host-specific brood parasites, is a consequence of learning the songs of divergent host species (Payne et al. 1998, 2000; Sorenson et al. 2003; Beltman et al. 2004), but for the majority of oscines learning may actually reduce the efficacy of song as a premating barrier. This is because heterospecific copying between close relatives (e.g., Helb et al. 1985) could lead to hybridization (e.g., Grant and Grant 1997). Therefore, although learning may promote song divergence in allopatry—because of the rapid accumulation of copying errors and the plasticity of learned songs (e.g., Slabbekoorn and Peet 2003)—it may result in song convergence in sympatry (Haavie et al. 2004). In other words, it might constrain, rather than facilitate, reproductive isolation in sympatry (Slabbekoorn and Smith 2002). Further insight could therefore be gleaned by a complementary comparative approach using species that do not learn their songs.

In view of this I carried out a large-scale comparative analysis examining the factors affecting song diversification in a speciose family of suboscine birds, the ''typical antbirds'' (Thamnophilidae). Antbirds are ideal subjects because, as suboscines, song development is not thought to be dependent on learning (Isler et al. 1998) and therefore unaffected by the confounding influence of cultural evolution. Furthermore, many antbird species have weakly differentiated plumage and live in forests where dim light conditions increase the reliance on acoustic signals. Although other isolating mechanisms are likely to operate in antbirds, these two factors make vocalizations especially important in species recognition and mate choice.

In this study I test the predictions of (1) the morphological adaptation hypothesis, (2) the acoustic adaptation hypothesis, and (3) the species recognition hypothesis. If morphological adaptation influences vocal evolution, an antbird's morphology should be correlated with the acoustic properties of its song. Specifically, body size should be correlated with the acoustic frequency and bill morphology with the acoustic frequency and/or temporal patterning of songs. If there has been correlated evolution between these traits, these relationships should hold when phylogenetic information is incorporated using independent contrasts (Felsenstein 1985; Harvey and Pagel 1991). However, if acoustic adaptation determines song structure, the forest strata in which an antbird habitually sings should affect the acoustic properties of its song. Specifically, I predict that compared to species of the relatively open midstory, those of the more densely vegetated understory and canopy should use lower frequencies (to minimize attenuation) and have low rates of noterepetition (to minimize reverberation). Finally, if divergence of antbird songs is determined by the need for unambiguous species recognition, the songs of closely related sympatric species should be more divergent than those of species living in allopatry. In testing the predictions of these three hypotheses, the present study does not exclude other explanations for vocal change. Recent molecular studies indicate that antbirds have been evolving over millions of years (e.g., Bates et al. 1999), giving sufficient time for signals to diverge as a byproduct of genetic drift in allopatry, as originally proposed by Mayr (1963). Future work examining the relationships between vocal and genetic change in the Thamnophilidae should clarify the importance of this process, relative to those explored in the present study.

METHODS

Study Species

Thamnophilid antbirds are small to medium-sized sedentary insectivores of forest, woodland, and scrub; they occur
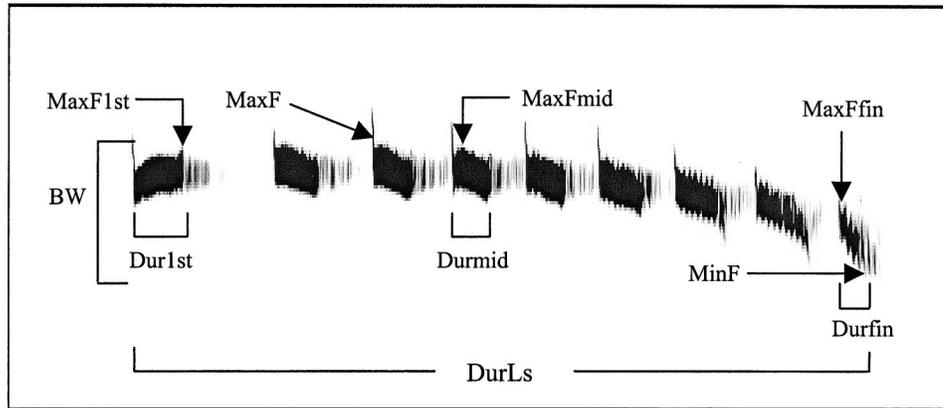
FIG. 1.   Annotated spectrogram of a single male loudsong taken from chestnut-tailed antbird *Myrmeciza hemimelaena*. The x-axis is time (in sec) and the y-axis is frequency (in kHz). Spectrograms were generated using Avisoft SASLabPro Version 4.0c with a 16-bit acquisition sound card (0 VIA [Wave] 5.10). Loudsongs were automatically filtered at half the Nyquist frequency and then digitized at a sampling rate of 44.1 kHz. Spectrograms were produced using the broad-band (323 Hz) filter settings in Avisoft (FFT, 1024; Frame, 50%; Window, FlatTop; Overlap, 88%). Loudsong structure was quantified by using on-screen cursors to measure the following time (in sec) and frequency (in Hz) traits: (1) maximum frequency of loudsong (MaxF), (2) minimum frequency of loudsong (MinF), (3) bandwidth of loudsong (BW), (4) maximum frequency of first note (MaxF1st), (5) maximum frequency of middle note (MaxFmid), (6) max frequency of final note (MaxFfin), (7) first frequency change (max frequency of fourth note divided by max frequency of first note), (8) second frequency change (max frequency of final note divided by max frequency of fourth note), (9) final note frequency change (max frequency of final note divided by max frequency of penultimate note), (10) duration of loudsong (DurLs), (11) number of notes per loudsong, (12) pace (number of notes per sec), (13) duration of first note (Dur1st), (14) duration of middle note (Durmid), and (15) duration of final note (Durfin). In addition, (16) peak frequency (frequency in the loudsong with the most energy) was automatically measured from amplitude spectra.

throughout the Neotropics but are largely confined to the lowlands (Zimmer and Isler 2003). Morphological and molecular data suggest that the family is monophyletic (Sibley and Ahlquist 1970; Ames 1971; Irestedt et al. 2002, 2004). However, antbirds are morphologically, behaviorally, and vocally diverse (Ridgely and Tudor 1994; Zimmer and Isler 2003). They are currently represented by 209 species and 45 genera, but taxonomic revisions are in progress and more taxa are likely to be identified. Antbirds form long-term pair-bonds and, with the exception of obligate ant followers and flocking species, defend permanent territories (Zimmer and Isler 2003). In most species, males and females produce loud and distinctive vocalizations consisting of multiple notes delivered in a stereotyped pattern as solos or duets. Because this differs from the traditional, rather narrow definition of song—complex male vocalizations used in mate advertisement (Catchpole and Slater 1995)—these vocalizations have been termed ''loudsongs'' (Willis 1967; Zimmer and Isler 2003). Although they are probably functionally analogous, being produced in territorial and sexual contexts, to be consistent with previous work, in this study I use the term loudsong. All vocalizations are assumed to be entirely innate in the Thamnophilids (Isler et al. 1998), largely because of their close relationship to another suboscine family, the tyrant fly-catchers (Tyrannidae) for which there is neither evidence of learning (Kroodsma 1984, 1985, 1989) nor the forebrain cell clusters that control song acquisition in oscines (Kroodsma and Konishi 1991).

Data Collation and Acoustic Analyses

Recordings of loudsongs were obtained for a total of 207 species from a commercially available audio CD (Isler and Whitney 2002) and private archives (see Appendix 2). For each species, 3–6 loudsongs were sampled from recordings of at least three individuals, where possible. For species with sexually dimorphic loudsongs, only those of the male were analyzed due to the scarcity of recordings of definite female vocalizations. Spectrograms were produced from high quality recordings and their structure was quantified using a variety of standard time and frequency measurements (see Fig. 1 for details). Body mass data were obtained from Dunning (1993) and Zimmer and Isler (2003). Using specimens housed at The Walter Rothschild Zoological Museum in Tring, United Kingdom, I measured the bills of 2–10 (mean = 3.2) individuals per species (male where possible), for 117 species. Dial callipers were used to measure (to the nearest 0.01 mm) culmen length from, and bill depth and width at, the anterior end of the nares. Although this study relies on current views of taxonomy that may change, by using a large sample of species, taxonomic revisions are unlikely to greatly affect the overall findings.

Testing the Morphological Adaptation Hypothesis

This hypothesis predicts that interspecific differences in loudsong structure will be associated with differences in species' morphology. Cross-species analyses are confounded by statistical nonindependence among species samples (Price 1997). As these effects can be partially resolved using independent contrasts analysis (Felsenstein 1985), I assessed the relationship between loudsong structure and morphology using the program CAIC version 2.0 (Purvis and Rambaut 1995). Although no comprehensive phylogeny is yet available for Thamnophilidae, I constructed an estimate of relatedness by combining data from a recent molecular study of

TABLE 1. Factor loadings for the first two principal components derived from measurements of the acoustic properties of antbird loudsongs (see Fig. 1). Statistics are derived from Wilcoxon signed-rank tests; $n = 21$ trios except for those marked with an asterisk, for which $n = 18$.

% of variance	$PC1_a$[1]	$PC2_a$	$PC1_b$	$PC2_b$	$PC1_c$	$PC2_c$
Eigenvalue	40.6	29.5	41.7	30.1	43.2	24.7
Factor loadings	5.69	4.14	5.84	4.20	3.5	2.0
Max. frequency of loudsong	**0.983**[2]	0.022	**0.976**	0.110	**0.610**	**0.623**
Min. frequency of loudsong	**0.807**	0.249	**0.794**	0.328	**0.802**	**0.429**
Bandwidth of loudsong	**0.687**	−0.259	**0.729**	0.135	−[3]	–
Max. frequency of 1st note	**0.903**	0.119	**0.884**	0.226	–	–
Max. frequency of middle note*	**0.983**	−0.033	**0.980**	0.090	–	–
Max. frequency of final note	**0.909**	0.109	**0.887**	0.284	**0.757**	**0.526**
Peak frequency	**0.958**	0.022	**0.966**	0.100	–	–
1st frequency change*	**0.245**	−0.342	0.149	−0.193	−0.594	**0.205**
2nd frequency change*	0.034	**0.235**	0.102	**0.374**	–	–
Final note frequency change*	–	–	–	–	–	–
Duration of loudsong	–	–	–	–	−0.045	−0.594
Pace	0.053	−0.936	0.278	−0.908	−0.744	0.616
Number of notes	0.066	−0.795	0.142	−0.849	−0.780	0.316
Duration of 1st note	0.045	0.826	−0.162	0.777	0.592	−0.503
Duration of middle note*	−0.010	0.942	−0.241	0.917	–	–
Duration of final note	−0.055	0.854	−0.225	0.867	–	–
n (species)		117		97		53

[1] Subscripts a–c denote the pairs of PCs derived from the three PCAs used in the analysis to test the effects of: a, morphology; b, forest strata; and c, presence of closely related species on loudsong structure.

[2] Bold denotes where variable makes an important contribution to the component (factor loading >0.2, Hedderson 1987).

[3] En-dashes (–) are given for variables that could not be included in the analyses because they violated parametric assumptions.

43 species belonging to 38 genera (R. Brumfield and J. Tello, unpubl. data) with information on intrageneric relationships based on morphology, plumage, and behavior (Zimmer and Isler 2003). Uncertain relationships within monophyletic genera were reflected as polychotomies. Because the final phylogenetic tree (see Appendix 1) was composed from a variety of sources, branch lengths could not be estimated. Independent contrasts were therefore generated with branch lengths assigned as either equal, or with the assumption that the age of a clade is proportional to the number of species it contains, that is, using Grafen's branch lengths (Grafen 1989; Purvis and Rambaut 1995). Plots of standardized contrasts against the variance of untransformed contrasts showed strong significant correlations for equal but not for Grafen's branch lengths. As significant correlations violate a key assumption of independent contrasts analysis (Diaz-Uriarte and Garland 1996), I use Grafen's branch lengths. Contrasts were calculated between nodes for body mass, bill morphology, and loudsong structure, and relationships were examined between the variables by calculating linear regressions on these standardized contrasts, with the regression forced through the origin (Garland et al. 1992; Grafen 1992). Because of correlations among loudsong characteristics and bill dimensions, I used principal components analysis (PCA) to reduce the loudsong and bill datasets into a small number of mutually independent variables. For bill measurements, PCA generated a single component explaining 89.7% of the variation ($PC1_{bill}$, Eigenvalue = 2.69), with which all three variables had correlation coefficients of > 0.9. For acoustic measures, two components were generated: $PC1_a$ which correlated with frequency-related variables and $PC2_a$ which correlated with temporal features (Table 1).

Testing the Acoustic Adaptation Hypothesis

This hypothesis predicts that interspecific differences in loudsong structure will be associated with differences in the physical properties of the habitat. To evaluate the effects of strata on antbird loudsong structure I first assigned each species with one of three codes depending on whether it habitually sang: (1) < 3 m from the ground (i.e., in the understory), (2) at 3–20 m (i.e., in the midstory), or (3) over 20 m (i.e., in the subcanopy and canopy). Species were assigned codes using field data on song-post height gathered by J. Tobias and me during trips to Peru, Ecuador, and Bolivia in 2001–2003; for species lacking field data, I used published information on antbird ecology (Ridgely and Tudor 1994; Zimmer and Isler 2003). Using species values, I first used general linear models (GLMs) to investigate the effects of strata, morphology, and their interactions on antbird loudsong structure (as defined by $PC1_b$ and $PC2_b$). Independent contrast analysis could not then be employed to control for the effects of phylogenetic inertia on the relationship between strata and loudsong structure because the method requires a linear relationship between the predictor and dependent variables (Martins and Hansen 1997; Quader et al. 2004). Because vegetation pattern and density—and hence sound reverberation and attenuation—do not vary with height in a linear fashion (Marten et al. 1977; Ellinger and Hodl 2003), nor should the relationship between strata and loudsong structure. Because of this nonlinearity I instead used a restricted maximum-likelihood model (REML), which is an algorithm of a GLM allowing fixed and random components to be fitted. Data were fitted to a normal error distribution with identity link function, with body mass, bill morphology ($PC1_{bill}$), and

strata as fixed factors, loudsong structure ($PC1_b$ and $PC2_b$) as the response variates (in two separate models). Each species was given a code (1–43) for its genus which was entered as a random factor to take account of greater similarity of the loudsongs of species within genera than between genera. Although relatedness between genera also varies, this method at least takes some account of phylogenetic inertia.

Testing the Species Recognition Hypothesis

If accurate identification of conspecifics is an important determinant of loudsong structure in antbirds, loudsongs should be more divergent between species in sympatry than in allopatry. To test this, using a recently published review of distribution and taxonomy (Zimmer and Isler 2003), I identified 21 focal species that had both a close relative in sympatry and one in allopatry (Fig. 2, Appendix 2). I tried to ensure that the species being compared occurred within the same strata in similar habitat to minimize the influence of habitat-dependent selection, and to maximize the likelihood that sympatric species were in direct contact, or had been in their recent evolutionary history. Allopatric species were defined as those with mutually exclusive breeding ranges; sympatric species overlapped by at least 25% of the breeding range of one species. When comparing the structure of sympatric species' loudsongs, in all but one trio (number 4) the recordings came from within the zone of sympatry (see Appendix 2). Where a species had a geographical subspecies defined in part by its song, I ensured that recordings were obtained from the subspecies with the appropriate pattern of sympatry or allopatry, as required. When more than one closely related species from a suitable habitat occurred allopatrically or sympatrically, I chose the one with the most similar plumage and song to the focal species (using Zimmer and Isler 2003). When choosing among very similar sympatric species, I selected the one with the greatest degree of range overlap with the focal species. Finally, when more than one subspecies occurred allopatrically, providing the recordings were available, I used loudsongs from the subspecies occurring furthest from the zone of sympatry. Spectrograms of loudsongs were compared using two complementary methods: (1) cross-correlation, and (2) on-screen measurements of time-frequency characteristics. The former takes account of all aspects of signal structure and is sensitive to complex derived features (Guyomarc'h et al. 1998); the latter allows identification of a subset of acoustic characteristics that differentiate loudsongs. In method (1) pairs of spectrograms of high-quality single loudsongs were automatically cross-correlated using the Avisoft Correlator (Avisoft-SAS Lab Pro, version 4.0 c, R. Specht, Berlin) (tolerated frequency deviation = 100–500 Hz; high-pass cut-off frequency = 500 Hz). The cross-correlation approach measures the overall similarity of pairs of sounds by sliding spectrograms across each other in time. Specifically, it calculates the normalized covariance between two time-frequency-amplitude matrices at successive time offsets (Beeman 1998) and generates coefficients of similarity, where a coefficient close to 0 indicates an orthogonal relationship between spectrograms, and coefficient close to 1 indicates that they are very similar (see Charif et al. 1995). Coefficients of similarity between sympatric and allopatric species were compared using paired t-tests. In method (2), 16 separate measures were taken from spectrograms of loudsongs comprising three or more notes, 11 from those with fewer notes. I quantified loudsong divergence between species pairs first using separate acoustic measures and then using two principal components derived from variables that did not violate parametric assumptions (Table 1). In both cases, I subtracted pair-members' loudsong values from one another and then used paired tests (Wilcoxon signed-rank and t-tests) to examine whether, across all the trios, the difference in loudsong structure (as defined by separate acoustic measures and composite PC values) was greater between sympatric than allopatric species pairs. Finally, to assess whether any loudsong divergence in sympatry was accompanied by parallel changes in morphology, where there were data I compared the body mass and bill morphology of sympatric and allopatric species pairs.

Sample Sizes and Statistics

Of the 207 antbird species whose vocalizations were analyzed, 197 (i.e., 95%) produce loudsongs with discrete phrases of at least three notes; these were comparable using the 16 acoustic measures described in Fig 1. The 10 species whose multinote loudsongs were of variable duration with irregular internote intervals were omitted from analysis, as were those species without data on body mass. When analyzing the effect of habitat (i.e., forest strata) on loudsong structure, I excluded the 100 species restricted to open country, bamboo, flooded forest, montane forest, riverine vegetation, and secondary growth; the structure and hence transmission properties of these habitats are likely to differ from those of intact lowland rainforest on which my predictions are based (following Marten et al. 1977; Ellinger and Hodl 2003). Although numerous antbird species are represented by several subspecies (Zimmer and Isler 2003), when analyzing the relationship between morphology, strata, and loudsong structure each species provided only one data point. Each species was given a mean value for each acoustic and bill measurement. The number of notes per loudsong and pace were square-root transformed; all other loudsong parameters, body mass, and bill dimensions were log-transformed prior to analysis. Variables were only included in analyses if they met parametric assumptions of error normality and constant variance, as determined by residual diagnostics. Note that when carrying out PCA, correlation matrices were used. Finally, when assessing the relationship between bill morphology and loudsong, I controlled for the confounding influence of mass by using the standardized residuals from a regression between $PC1_{bill}$ and mass. For matched-pair comparisons, when $n < 16$ the t-statistic and its associated exact P-value is given; otherwise the z statistic and its asymptotic P-value is given. All P-values are two-tailed and corrected for ties where appropriate. REML models were run using Genstat (6th ed., 2002; all other statistical tests were carried out using SPSS (vers. 11.01, 1999).

RESULTS
Morphological Adaptation Hypothesis

Using species values, I found that bill morphology was positively correlated with body mass and body mass had a

strong relationship with both the frequency and temporal features of antbird loudsongs (all P-values < 0.0001; Table 2). Controlling for the confounding influence of mass using residuals, I found that although $PC1_{bill}$ did not predict variation in the acoustic frequencies of loudsongs ($P = 0.210$), it was strongly related to their temporal patterning ($P = 0.001$; Table 2). When using independent contrasts to control for phylogenetic inertia, I found that body mass was strongly related to bill morphology and loudsong frequency. However, there was a much weaker relationship with temporal patterning ($P = 0.103$). Moreover, residuals of $PC1_{bill}$ contrasts were not significantly correlated with loudsong structure ($PC1_a$: $P = 0.121$, $PC2_a$: $P = 0.149$). However, when using phylogenetically independent linear contrasts to examine the relationships between loudsong structure and the residuals of each bill measurement, I found that bill width strongly predicted temporal features ($\beta = 0.43$, $F_{1,59} = 13.5$, $P = 0.001$; Fig. 3): birds with broad bills produced loudsongs comprising fewer notes of longer duration repeated at low rates than those with narrow bills. Bill width was unrelated to loudsong frequency ($PC1_a$: $\beta = -0.18$, $F_{1,59} = 1.88$, $P = 0.176$), and bill length and depth had no relationship with loudsong frequency or temporal patterning (length: $PC1_a$: $\beta = -0.11$, $F_{1,59} = 70$, $P = 0.408$, $PC2_a$: $\beta = 0.02$, $F_{1,59} = 0.03$, $P = 0.876$; depth: $PC1_a$: $\beta = -0.09$, $F_{1,59} = 0.46$, $P = 0.502$, $PC2_a$: $\beta = -0.18$, $F_{1,59} = 1.96$, $P = 0.167$).

Acoustic Adaptation Hypothesis

When data from species inhabiting lowland rainforest were analyzed, significant variation was found between the three forest strata in the temporal patterning of loudsongs (one-way ANOVA: $PC2_b$: $F_{2,94} = 9.45$, $P < 0.001$; Fig. 4b). However, there was no relationship between strata and frequency characteristics ($PC1_b$: $F_{2,94} = 0.96$, $P = 0.386$; Fig. 4a). Conversely, when controlling for the effects of body mass, bill morphology, and their interaction with strata (Table 3), I found that while strata was significantly related to frequency ($P = 0.005$), its effect on temporal patterning was removed ($P = 0.194$), and the relationship was largely explained by body mass ($P = 0.002$). Even when greater relatedness of species within genera was taken into account (Table 3), the same pattern emerged: frequency characteristics of antbird loudsongs were predicted by strata ($P = 0.024$) and the interaction between strata and bill morphology ($P = 0.009$); temporal patterning of loudsongs was predicted by mass ($P < 0.001$), but not strata ($P = 0.398$).

In summary, I found that within each forest stratum there was a great deal of variation in the structure of the loudsongs of forest-dwelling antbirds. Nonetheless, when correcting for morphological effects and factoring in greater relatedness within than between genera, antbirds that habitually sing in densely vegetated strata (i.e., the ground/understory and subcanopy/canopy) produced lower-pitched loudsongs than those of the more open midstory.

Species Recognition Hypothesis

Method (1) revealed that the mean coefficient of similarity for the allopatric species pairs was significantly greater than that for the sympatric pairs (0.46 ± 0.03 vs. 0.35 ± 0.03 SE,

paired t-test: $t = -6.87$, df $= 19$, $P < 0.0001$; Fig. 5). Method (2) showed that for 15 of the 16 acoustic properties measured, the mean difference between values was greater between the loudsongs of sympatric than allopatric pairs. These differences were significant for five of the nine frequency variables and two of the five the temporal variables (Table 4). The large number of comparisons meant that only one of these acoustic variables (final note frequency change) was significantly different ($P_{(0.006)} \leq 0.04$) after correcting for multiple comparisons using the false discovery rate method (Benjamini and Hochberg 1995). However, all the tests went in the same direction and a Fisher's combined probability test showed that overall, loudsongs were more divergent in sympatry than allopatry ($P < 0.001$). This was confirmed using principal components to describe loudsong structure: the mean difference in PC values was greater between the loudsongs of sympatric than allopatric pairs (Fig. 6), the difference being significant for both $PC1_c$ ($t = 2.4$, df $= 17$, $P = 0.028$) and $PC2_c$ ($t = 3.2$, $P = 0.006$).

Although it could be argued that a species is likely to be more closely related to an allopatric congener than a sympatric one, only nine of 21 allopatric pairs are considered to be members of superspecies (mainly on the basis of morphology, voice, and distribution, see Appendix 2). This suggests that the patterns of vocal congruence detected in this study are not confounded by consistent differences in evolutionary divergence. This was confirmed by the finding that when these nine trios were removed from the analyses, the pattern of greater loudsong divergence in sympatry remained intact. Specifically, five of the 16 acoustic measures were significantly more different in sympatry than in allopatry (Table 4), and the mean coefficient of similarity was greater for allopatric than sympatric species pairs (0.48 ± 0.14 vs. 0.34 ± 0.14; $t = 78$, $P < 0.0001$, $n = 12$ pairs; Wilcoxon signed-rank test). This pattern remained intact even when excluding all 13 trios containing closely related allopatric species pairs (0.49 ± 0.17 vs. 0.38 ± 0.14; $t = 36$, $P = 0.008$, $n = 8$ pairs).

Overall, these analyses indicated that antbird loudsongs are more divergent in sympatry than in allopatry in both temporal and frequency characteristics. This is corroborated by visual inspection of spectrograms, which shows no consistent patterns of loudsong divergence between the pairs of sympatric species: while some pairs diverge mainly in frequency characteristics (e.g., trio 1, Fig. 2a) or temporal patterning (e.g., trio 12, Fig. 2e), others diverge with respect to both (e.g., trio 6, Fig. 2c). Interestingly, loudsong divergence was not accompanied by morphological divergence: overall bill morphology, bill width and body mass were no more divergent between sympatric than between allopatric species-pairs (mean $\pm$ SD difference between sympatric versus allopatric pairs: $PC1_{bill}$: 0.52 ± 0.41 vs. 0.58 ± 0.54; $z = 0$, $n = 16$ pairs, $P = 1.0$; bill width: 2.91 ± 2.93 vs. 2.51 ± 2.77 mm; $z = -0.40$, $n = 16$ pairs, $P = 0.691$; body mass: 2.15 ± 2.36 vs. 3.44 ± 2.88 g; $z = -1.18$, $n = 19$ pairs, $P = 0.071$; Wilcoxon signed-rank tests).
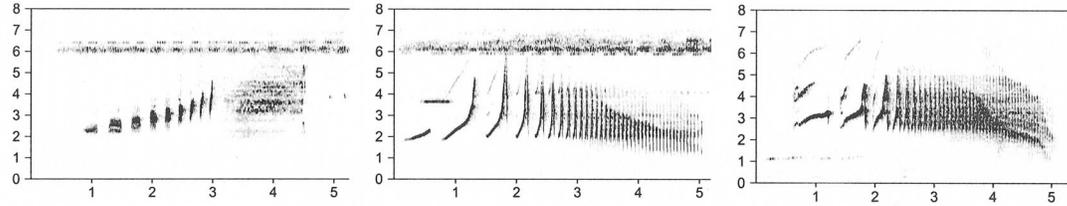
DISCUSSION

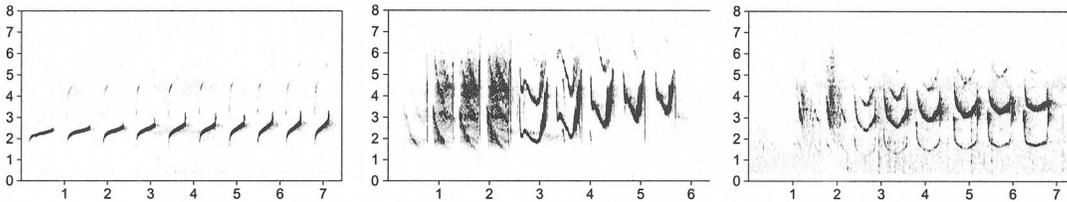In this study I have examined the effects of body mass, bill morphology, forest strata, and presence of closely related
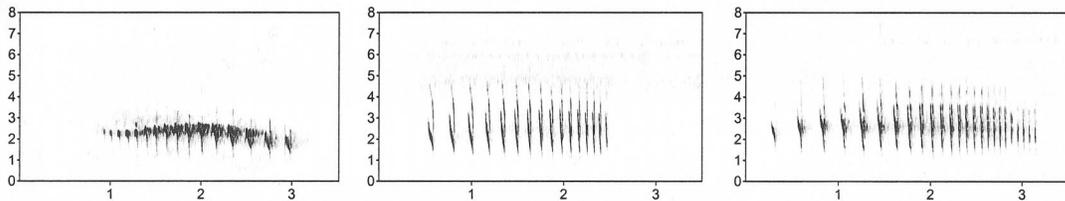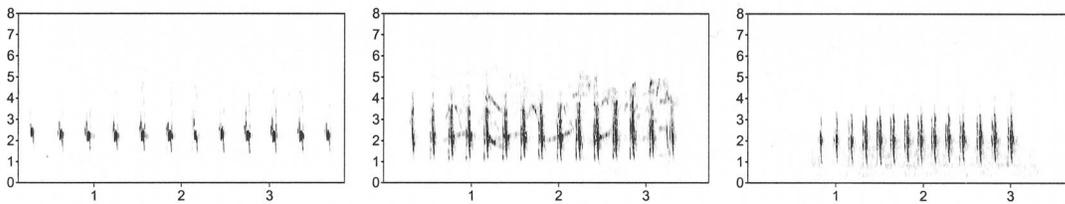
(a) Trio 1
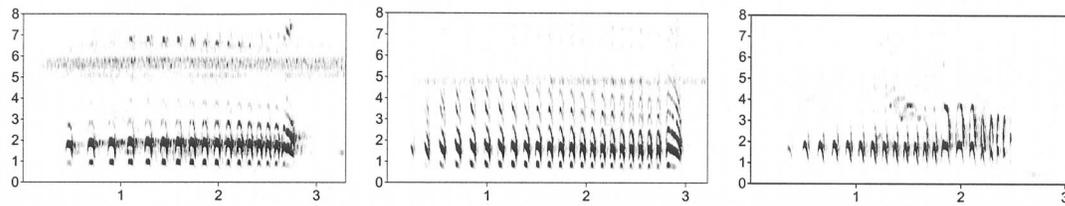
(b) Trio 2
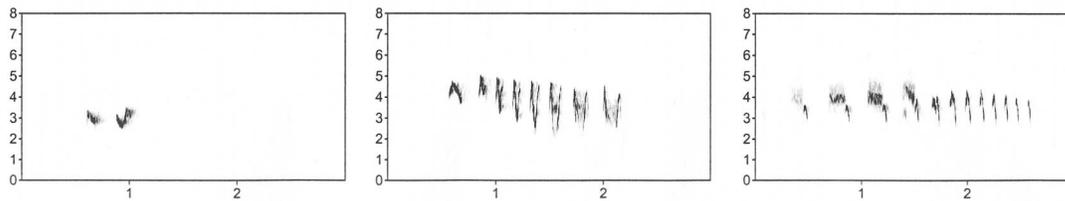
(c) Trio 6

(d) Trio 9

(e) Trio 12

(f) Trio 13

FIG. 2. Spectrograms of single loudsongs from nine of the 21 species trios used to test the species recognition hypothesis; each trio represents one of the nine genera used in the analysis. The x-axis is time (in sec) and the y-axis is frequency (in kHz). The focal species is represented by the middle spectrogram; its closest sympatric relative by the left-hand spectrogram and its closest allopatric relative
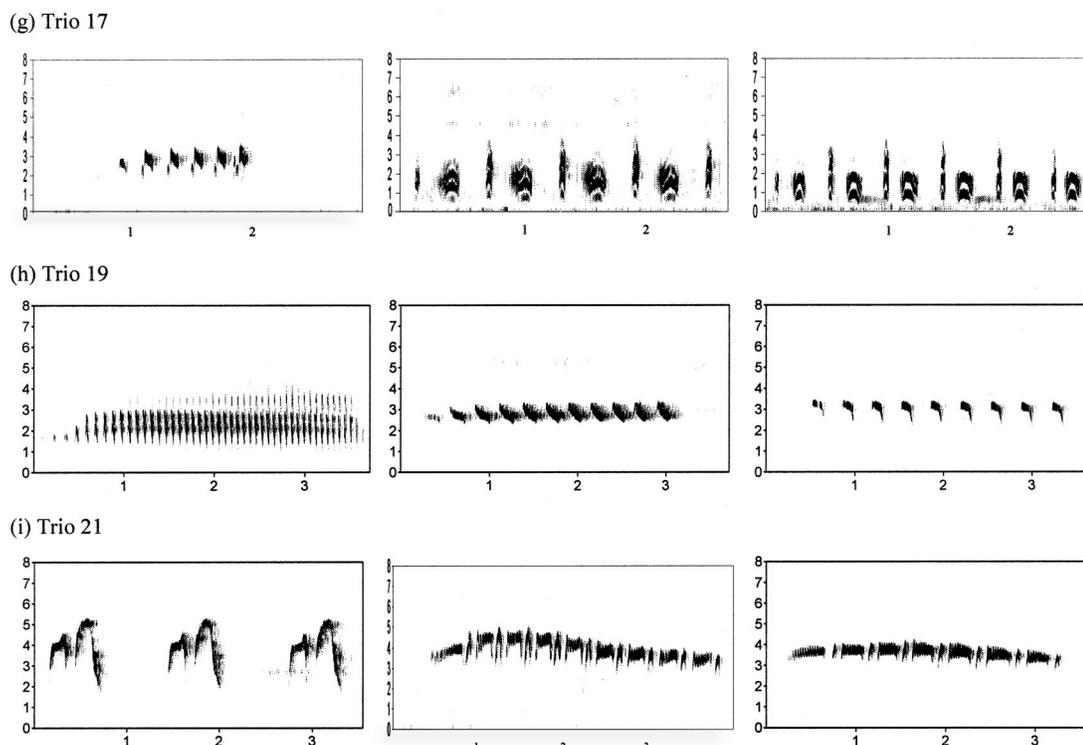
(g) Trio 17

(h) Trio 19

(i) Trio 21

FIG. 2. (continued) by the right-hand spectrogram (see Appendix 2). Coefficients of similarity of the loudsongs of species pairs, as determined using cross-correlation analyses, are as follows: (a) sympatric, 0.53; allopatric, 0.54; (b) 0.20, 0.30; (c) 0.09, 0.33; (d) 0.38, 0.53; (e) 0.26, 0.39; (f) 0.25, 0.45; (g) 0.14, 0.43; (h) 0.36, 0.54; (i) 0.44, 0.54.

conspecifics on the acoustic structure of loudsongs in a large number of Thamnophilid antbird species. The resultant evidence implies that a combination of morphological adaptation, acoustic adaptation, and species recognition has driven loudsong evolution in this diverse family of suboscines. Specifically, in support of the morphological adaptation hypothesis, the study revealed strong relationships between body mass, bill width, and loudsong structure. Controlling for these effects, the study also found support for the acoustic adaptation hypothesis: birds which habitually sing in the understory and canopy produce higher-pitched songs than those that sing in the midstory, suggesting that song structure is related to the sound transmission properties of different habitat strata. Finally, I found that closely related sympatric species have more divergent loudsongs than those of closely

related allopatric species, as predicted by the species recognition hypothesis. To my knowledge, this is the first test of all three hypotheses in the same study, and the first large-scale comparative analysis to indicate that both ecological adaptation and species recognition drives song divergence in birds.

Evidence for Morphological Adaptation

Traditional speciation theory regards mating signal divergence as a by-product of genetic differentiation in allopatry, through drift and/or adaptation to divergent ecologies (Dobzhansky 1951; Mayr 1963). Consistent with the ecological adaptation hypothesis are data showing correlated evolution among morphology and mating signal characteristics. Such

TABLE 2. Nonphylogenetic and phylogenetic regressions of body mass and bill morphology with the frequency and temporal properties of antbird loudsongs (defined by $PC1_a$ and $PC2_a$, respectively[1]).

		Nonphylogenetic (using species values)				Phylogenetic (using independent contrasts)			
Independent	Dependent	β	F	df	P	$β^2$	F	df	P
Body mass	PC1bill	0.84	285	1, 115	**<0.0001**	0.63	38.5	1, 59	**<0.0001**
	PC1[3]	−0.37	26.1	1, 161	**<0.0001**	−0.26	5.34	1, 75	**0.024**
	PC2[3]	0.34	20.5	1, 161	**<0.0001**	0.19	2.27	1, 75	0.103
$PC1_{bill}$[4]	$PC1_a$	−0.12	1.59	1, 115	0.210	−0.23	2.47	1, 59	0.121
	$PC2_a$	0.29	10.8	1, 115	**0.001**	0.19	2.13	1, 59	0.149

[1] See Table 1 for factor loadings of separate acoustic measures against each component.
[2] Slope of regression forced through the origin.
[3] PCs derived from loudsong data from all 163 species with data on body mass; factor loadings are approximately equal to those given for $PC1_a$ and $PC2_a$ (Table 1) and so are not shown separately.
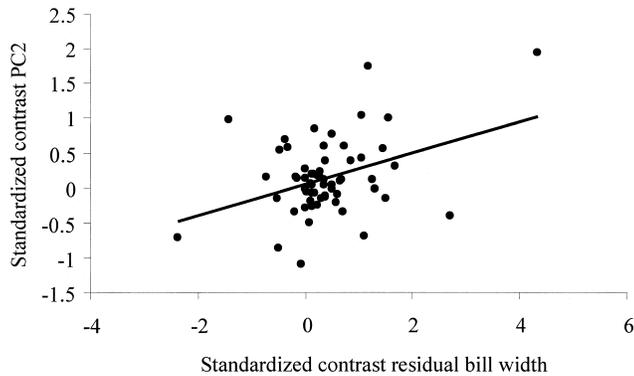[4] Standardized residuals of bill morphology controlling for body mass.

FIG. 3. Scatterplot of phylogenetically independent linear contrasts illustrating the relationship between bill width residuals (controlling for body mass) and the temporal characteristics of loudsongs ($PC2_a$; see Table 1 for factor loadings).

correlations arise if natural selection favors modifications in traits functionally related to the production of mating signals. In birds, body size and bill morphology are under strong selective pressures relating to diet (Gibbs and Grant 1987) and this study showed that in antbirds both these traits influence loudsong structure: body mass had a strong negative relationship with loudsong frequency and bill width was negatively correlated with temporal patterning. Notwithstanding the limitations of independent contrasts (see Price 1997), the finding that these relationships were retained after controlling for phylogeny suggests that the evolution of loudsong structure in antbirds is correlated with morphology. Negative correlations between body mass and song frequency have been reported in numerous bird species and the functional relationship underlying them is largely understood. Tight linkage between bill width and temporal patterning is less easy to explain but probably relates to the trade-off between the force a bill can generate and the speed with which it opens and closes (Podos 1997). Selection for big, broad bills—such as those used by large *Frederickena* and *Mackenziaena* antshrikes to feed on small vertebrates—should therefore lead to a decrease in the ability to produce notes rapidly.

The lack of correlation between bill morphology and acoustic frequency suggests that body mass (and hence the size of the syringeal membrane) is more important in determining the frequency of antbird loudsongs. Interestingly, a correlation between bill size and song frequency has been reported in the woodcreepers (family: Dendrocolaptidae), a suboscine family with great interspecific variation in bill length (12–74 mm, Palacios and Tubaro 2000). In antbirds, bill length shows relatively little variation, ranging from about 5 mm in *Myrmotherula* spp. to about 30 mm in *Batara cinerea*, probably making it difficult to detect a relationship between bill length and acoustic frequency. However, it is worth noting that in Palacios and Tubaro's (2000) study the negative relationship between residuals of bill length and emphasised frequency was weak and only significant ($P = 0.05$) when two species were excluded from the analysis; further studies exploring this relationship are therefore required.

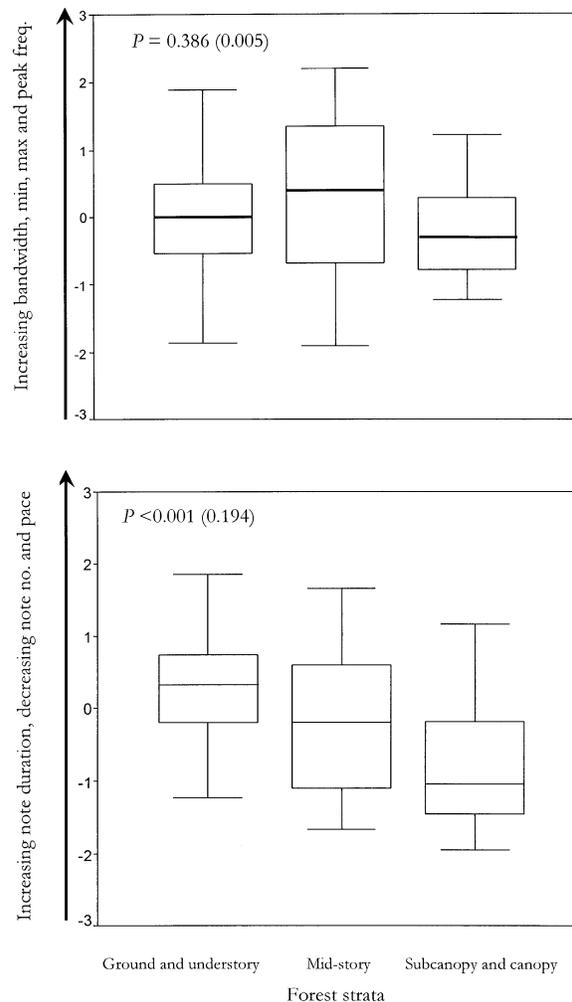Overall, the present study suggests that in the antbirds, morphology and the acoustic features of loudsongs have

FIG. 4. Box plots illustrating the relationship between strata and antbird loudsong structure as described by (a) $PC1_b$ and (b) $PC2_b$. The lower and upper boundaries of the boxes represent first and second interquartile ranges, respectively, the median is shown by the horizontal bar, and the lower and upper bars relate to the 10th and 90th percentiles, respectively. $PC1_b$ reflects increasing loudsong frequency and $PC2_b$ reflects increasing note duration and decreasing pace (see Table 1 for factor loadings). Initial P-values are derived from one-way ANOVAs, whereas values in parentheses are derived from GLMs controlling for effects of morphology; $n = 97$ species restricted to intact lowland forest.

evolved in a correlated fashion. Therefore, song diversification may have occurred, at least in part, as a by-product of adaptation to different foraging niches (see also Podos 2001). Future studies could investigate the extent to which antbirds match bill configuration (e.g., gape) to the acoustic properties of their songs (e.g., peak frequency) as shown in Darwin's finches (Podos et al. 2004). If performance-related loudsong properties are also used in species recognition and mate choice, correlated evolution of bill morphology and loudsong structure might facilitate reproductive isolation and speciation in this diverse assemblage.

Evidence for Acoustic Adaptation

Correlations between habitat structure and the acoustic properties of birdsongs have been found in numerous species

TABLE 3. GLM and REML analyses of the effect of forest strata on antbird loudsong structure as defined by the first two principal components ($PC1_b$ and $PC2_b$). The GLM analysis uses species values, whereas the REML analysis takes account of greater similarity of the loudsongs of species within genera than between genera (see text). Significant probability values are given in bold. They derive from having all significant terms ($P < 0.1$) fitted in the final model together; those of nonsignificant terms were derived from having all significant terms in the model and each nonsignificant term ($P > 0.1$) fitted individually; $n = 97$ species, of which there were bill data for 70.

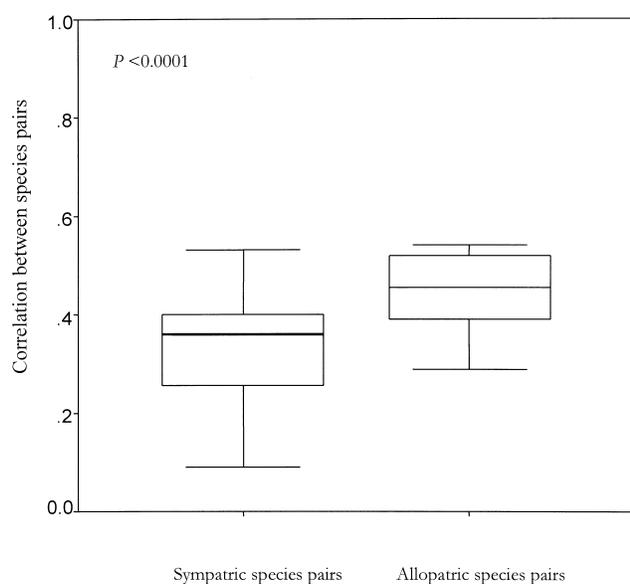| Response variable	Model terms	GLM			REML		
		F	df	P	Wald (χ^2)	df	P
$PC1_b$	strata	5.89	2	**0.005**	7.46	2	**0.024**
	mass	1.28	1	0.262	0.00	1	0.991
	bill size ($PC1_{bill}$)	0.04	1	0.841	0.02	1	0.880
	mass $\times$ $PC1_{bill}$	0.41	1	0.525	0.13	1	0.717
	mass $\times$ strata	4.06	2	**0.011**	3.83	3	0.281
	$PC1_{bill}$ $\times$ strata	3.51	2	**0.020**	9.43	2	**0.009**
	mass $\times$ $PC1_{bill}$ $\times$ strata	0.75	3	0.528	0.20	3	0.896
$PC2_b$	strata	1.68	2	0.194	0.92	2	0.398
	mass	10.64	1	**0.002**	15.02	1	**<0.001**
	$PC1_{bill}$	1.73	1	0.193	1.70	1	0.192
	mass $\times$ strata	1.08	2	0.346	0.15	2	0.861
	mass $\times$ $PC1_{bill}$	2.40	1	0.126	1.72	1	0.190
	strata $\times$ $PC1_{bill}$	1.75	2	0.165	0.74	2	0.475
	mass $\times$ strata $\times$ $PC1_{bill}$	1.75	3	0.166	0.30	3	0.744
	Minimal models for REML analyses	Average effect			SE		
$PC1_b$	Constant	−0.10			0.21		
	Strata 1	0			0.34		
	2	0.43			0.45		
	3	−0.77			0.29		
	Strata $\times$ $PC1_{bill}$ 1	−0.26			0.35		
	2	0.34			0.42		
	3	−0.69			0.24		
$PC2_b$	Constant	−0.13			0.14		
	Mass	1.58			0.41		

FIG. 5. Boxplots showing allopatric species pairs to have more similar loudsongs (i.e., a higher average correlation coefficient) than sympatric species pairs, as determined by cross-correlation analysis. A correlation close to 0 indicates an orthogonal relationship between two loudsongs, and a correlation close to 1 indicates that they are very similar. P-value are derived from a paired t-test (see text); $n = 21$ trios.

but the functional relationships underscoring them are only partially understood (Morton 1975; Wiley and Richards 1982; Ryan and Brenowitz 1985; Wiley 1991). In forest, sound attenuation and reverberation is greatest in the densely vegetated understory and canopy (Marten and Marler 1977; Marten et al. 1977), and reverberation is reduced in sounds of 2–5 kHz (Ellinger and Hodl 2003). It follows that if loudsongs are adapted to maximize transmission, forest-dwelling antbirds habitually vocalizing in the understory and canopy should produce loudsongs of low frequency to minimize attenuation, whereas to reduce the effects of reverberation their loudsongs should lie in a ''sound window'' between 2–5 kHz and be uttered at a slow pace. Analyzing the loudsongs of only lowland rainforest species, my results generally agree with these predictions. First, I found that controlling for the effects of morphology, antbird loudsong frequency was lowest in the understory and canopy and highest in the more open midstory. Second, the mean peak frequency of forest-dwelling antbird loudsongs (3.71 ± 1.40 kHz; range 1.38–7.41) fell well within the 2–5 kHz range. Moreover, five of the ten species with loudsongs less than 2 kHz and seven of the nine species with loudsongs more than 5 kHz vocalized in the midstory. However, contrary to predictions regarding song pace, although species of the open midstory had faster loudsongs than those of the closed understory, this effect was removed when controlling for morphology and phylogenetic effects. Further, I found that canopy loudsongs were of higher pace than those of the mid- and understory, an unexpected finding that was also reported in a study across five antbird

TABLE 4. Mean ($\pm$ SD) differences between species in sympatric and allopatric antbird pairs for 16 temporal and frequency loudsong variables. Statistics are derived from Wilcoxon signed-ranks tests; n = 21 trios except for those marked with an asterisk, for which n = 18. Bold denotes P-values that were significant ($P < 0.05$) when trios containing allopatric superspecies were excluded.

			Test Statistics	
Acoustic variable	Sympatric pairs	Allopatric pairs	z	P^1
Max. frequency of loudsong	0.72 $\pm$ 0.51	0.62 $\pm$ 0.49	-0.68	0.498
Min. frequency of loudsong	0.59 $\pm$ 0.53	0.32 $\pm$ 0.36	-2.47	**0.014**
Bandwidth of loudsong	0.58 $\pm$ 0.53	0.55 $\pm$ 0.60	-0.64	0.520
Max. frequency of 1st note	1.01 $\pm$ 1.01	0.65 $\pm$ 0.53	-1.56	0.118
Max. frequency of middle note*	1.05 $\pm$ 0.71	0.64 $\pm$ 0.45	-2.16	**0.031**
Max. frequency of final note	1.01 $\pm$ 0.92	0.64 $\pm$ 0.48	-1.58	**0.114**
Peak frequency	0.71 $\pm$ 0.58	0.49 $\pm$ 0.43	-2.59	**0.010**
1st frequency change*	0.28 $\pm$ 0.30	0.18 $\pm$ 0.30	-1.70	0.089
2nd frequency change*	0.21 $\pm$ 0.32	0.08 $\pm$ 0.12	-2.18	0.029
Final note frequency change*	0.32 $\pm$ 0.36	0.12 $\pm$ 0.12	-2.75	**0.006**
Duration of loudsong	1.15 $\pm$ 0.87	0.88 $\pm$ 0.75	-0.24	0.808
Pace	4.41 $\pm$ 3.99	2.16 $\pm$ 3.00	-1.96	0.050
Number of notes	13.3 $\pm$ 11.6	6.48 $\pm$ 8.94	-1.99	0.046
Duration of 1st note	0.11 $\pm$ 0.11	0.09 $\pm$ 0.11	-0.07	0.945
Duration of middle note*	0.10 $\pm$ 0.08	0.08 $\pm$ 0.09	-1.59	0.113
Duration of final note	0.10 $\pm$ 0.10	0.12 $\pm$ 0.13	0.23	0.821

[1] A Fisher's combined probability test showed the overall significance to be $P < 0.001$ (i.e., $-2\Sigma \ln P > \chi^2_{0.01[2k]}$, where k = number of separate tests; (Sokal and Rohlf 1995).

species (Nemeth et al. 2001). Fast canopy loudsongs may be an adaptation to irregular amplitude fluctuations caused by wind or atmospheric turbulence (Wiley and Richards 1982), but this is unlikely to apply to antbirds which rarely venture into the upper canopy. Moreover, the modulation frequency of rainforest canopy amplitude fluctuations is only about 0.2 kHz, that is, well below that which would degrade antbird loudsongs (Nemeth et al. 2001). Instead the relationship probably reflects decreasing body mass with increasing height. Indeed, over a much larger sample I found strong correlations between mass and bill morphology and between bill morphology and the temporal patterning of loudsongs. As such we might expect morphology to constrain the ability of loudsongs to respond to habitat-dependent selection.

Although my findings are consistent with the idea that antbird loudsongs are generally well adapted to minimize attenuation and reverberations in their respective strata, close
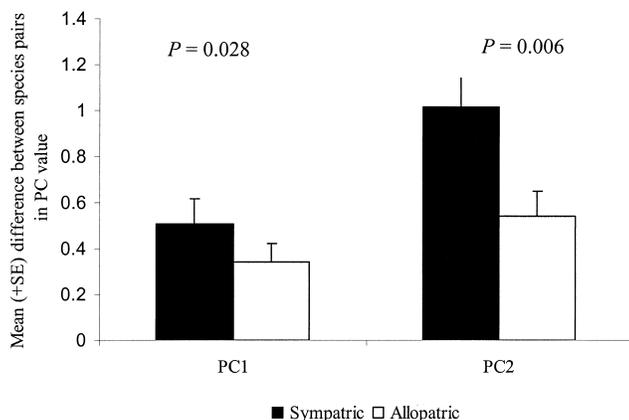

FIG. 6. Relationship between pattern of sympatry and divergence in loudsong structure as described by two principal components (see Table 1 for factor loadings). P-values are derived from paired t-tests (see text); n = 18 trios (i.e., only those including species with loudsongs of three or more notes).

inspection of the data reveals much variation. For example, many midstory species produce low-pitched, fairly slow-paced loudsongs (e.g., *Thamnophilus amazonicus*: mean peak frequency = 1.84 kHz, pace = 8.6 notes min^{-1}), whereas several understory (*Myrmotherula*) and canopy (*Terenura*) antwrens have very high-pitched loudsongs of fast pace (e.g., *M. fjeldsaai*: mean peak frequency = 6.48 kHz, pace = 13.6 notes min^{-1}; *T. humeralis*: mean peak frequency = 7.39 kHz, pace = 9.8 notes min^{-1}). This suggests that the rather coarse classification of the habitat into three strata does not take into account the potential effects of microhabitat on loudsong structure. It may also indicate that the acoustic properties of birdsongs are not as tightly linked to the acoustic properties of habitat as previous studies imply. This may be because songs are selected to optimize rather than maximize transmission (Lemon et al. 1981); what is optimal depends on the type and location of intended receivers. Song degradation might be advantageous if intended receivers are close-by mates or neighbors because it facilitates localization (McGregor and Krebs 1984) and may reduce eavesdropping by more distant receivers that could use the information to exploit the signaler or intended receiver (e.g., Tobias and Seddon 2002). Thus the anomalously high-pitched, fast-paced loudsongs of *Terenura* antwrens may reflect that, as flocking species, their receivers are located close-by. Future studies evaluating the importance of habitat-dependent selection on birdsong should therefore control for differences in the locations of intended receivers, which depends on detailed knowledge of ecological factors such as territory size, population density, and diversity.

Given that song development in suboscines is not thought to depend on learning, the findings of this study are interesting because they suggest that song divergence between habitats does not necessarily require an ability to tune songs through vocal learning (Slabbekoorn and Smith 2002). They indicate that linkage between song structure and habitat could arise through processes such as sensory drive, that is, by

direct selection imposed by the local signaling environment on the design of acoustic sexual signaling systems (for an overview, see Boughman 2002).

Evidence for Species Recognition

Even if there is strong selection on antbird vocalizations for optimal transmission, other selective forces may drive songs away from predicted optima. Among these the need for accurate species identification is likely to be important in Amazonia where antbirds form diverse assemblages, where dim light conditions render visual signals less effective, and where many species are poorly differentiated by plumage. Traditional theory predicts that mating signals will diverge in sympatry because selection against the production of unfit hybrids favors traits that that reliably signal species identity and increase assortative mating (Dobzhansky 1951; Lewontin 1974; Liou and Price 1994). Birdsongs have long been predicted to diverge by this process but evidence for this has hitherto been scarce (Irwin and Price 1999). Therefore, the present study is important because by demonstrating greater loudsong divergence in sympatry than in allopatry, it supports the idea that the need for species recognition may drive vocal evolution in birds. The findings are also important insofar as they make specific predictions about the vocal cues used by antbirds in species recognition and can be used to design future experimental work.

In contrast to the findings reported here, recent studies have demonstrated birdsong convergence in sympatry (de Kort et al. 2002a; Haavie et al. 2004). This may arise when high levels of interspecific territoriality favor recognition of heterospecific competitors to facilitate their spatial separation (Cody 1969). Unlike the *Streptopelia* doves (de Kort et al. 2002a,b) and *Ficedula* flycatchers (Haavie et al. 2004), most antbird species have year-round territories, occur at low population densities, and probably experience low levels of interspecific territoriality (Zimmer and Isler 2003). They are therefore unlikely to evolve similar loudsongs for territorial purposes, and instead it may be more important to remain vocally distinct to avoid costly mating errors.

Demonstration of more pronounced song differences in sympatry than allopatry is consistent with the idea that the need for species identification selects for vocal divergence. As far as could be determined, loudsong divergence was not accompanied by morphological divergence. Moreover, all sympatric species occupied similar ecological niches. This suggests that loudsong divergence is not a by-product of ecological character displacement, but may have been driven either by reinforcement or by disruptive sexual selection (Panhuis et al. 2001). A simple test is unlikely to distinguish these alternatives. However, by gathering data on the fitness of hybrids between taxa that are in the early stages of speciation, the extent of female preference for divergent loudsongs in sympatry, and whether any loudsong divergence reduces the frequency of hybridization, future empirical studies could make a case for vocal divergence and hence speciation by one or other of these processes.

In conclusion, this study suggests that in the Thamnophilidae there has been an evolutionary response to selection for species recognition; this underscores the potential of in-teractions among closely related sympatric species to drive song divergence. Furthermore, the strong relationships between song structure, morphology, and habitat suggest that, as discussed in traditional theories of speciation, ecological adaptation may indeed drive signal evolution.

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APPENDIX 1

The composite phylogeny of Thamnophilidae used to correct for the statistical nonindependence among species samples when assessing the relationships between loudsong structure and morphology. The phylogeny represents 95 antbird species with data on loudsong structure, morphology, and inter- and intrageneric relatedness. The phylogenetic relationships shown are based on a combination of molecular, behavioral, and morphological traits and are given in parenthetical notation, in which hierarchical groupings are indicated within a nested series of parentheses. Body mass data were available for all species listed below; those with data on bill morphology are denoted by a superscript ''b.'' Subspecies' names are given where there are pronounced geographical differences in loudsong structure.

(((((((((((*Batara cinerea*[b], ((*Frederickena unduligera fulva*[b], *Frederickena viridis*[b]), (*Mackenziaena leachii*[b], *Mackenziaena severa*[b]))), ((*Cymbilaimus lineatus*[b], *Cymbilaimus sanctaemariae*[b]), *Taraba major*[b])), ((((*Dysithamnus striaticeps*[b], *Dysithamnus puncticeps*[b], *Dysithamnus mentalis*[b]), *Herpsilochmus rufimarginatus*), (*Sakesphorus luctuosus*[b], *Sakesphorus canadensis*[b])), ((*Thamnophilus aethiops*[b], *Thamnophilus unicolor*[b]), (*Thamnophilus ruficapillus*[b], *Thamnophilus tenuepunctatus*[b], *Thamnophilus torquatus*[b], *Thamnophilus palliatus*[b], *Thamnophilus doliatus doliatus*[b])))), (*Dichrozona cincta*[b], *Megastictus margaritatus*[b])), ((*Thamnomanes saturninus*, *Thamnomanes ardesiacus*), (*Thamnomanes schistogynus*, *Thamnomanes caesius*[b]))), (((((*Cercomacra nigrescens*[b], *Cercomacra serva*[b], *Cercomacra laeta*[b], *Cercomacra tyrannina*[b]), (*Myrmeciza hemimelaena*[b], *Myrmeciza castanea*[b])), (((*Drymophila devillei*, *Drymophila caudata*[b]), *Drymophila squamata*[b], *Drymophila malura*[b], *Drymophila rubricollis*, *Drymophila genei*[b]), *Hypocnemis cantator saturata*[b])), ((((((*Gymnopithys lunulatus*, *Gymnopithys salvini*), (*Rhegmatorhina hoffmannsi*, *Rhegmatorhina gymnops*[b], *Rhegmatorhina melanosticta*)), (*Phlegopsis nigromaculata*[b], *Phlegopsis erythroptera*)), *Phaenostictus mcleannani*[b]), *Hylophylax poecilinotus duidae*[b])), (((((*Gymnocichla nudiceps*[b], (((*Myrmeciza immaculata*[b], *Myrmeciza fortis*[b], *Myrmeciza hyperythra*[b], *Myrmeciza goeldii*, *Myrmeciza melanoceps*[b]), (*Myrmeciza loricata*[b], *Myrmeciza squamosa*[b])), *Percnostola rufifrons*[b])), (*Pyriglena leuconota*[b], *Pyriglena atra*, *Pyriglena leucoptera castanoptera*[b])), (*Percnostola lophotes*, *Myrmoborus myotherinus myotherinus*[b])), ((*Hylophylax naevioides*[b], (*Hypocnemoides melanopogon*[b], *Hypocnemoides maculicauda*[b])), *Sclateria naevia*[b])))), (((*Formicivora serrana*, *Formicivora littoralis*, (*Formicivora rufa*[b], *Formicivora grisea rufiventris*)), *Myrmotherula axillaries melaena*[b]), (*Myrmochanes hemileucus*, ((*Myrmotherula longicauda*[b], *Myrmotherula klagesi*), (*Myrmotherula surinamensis*[b], *Myrmotherula multostriata*[b]), (*Myrmotherula pacifica*[b], *Myrmotherula cherriei*[b]), (*Myrmotherula sclateri*[b], *Myrmotherula ambigua*), (*Myrmotherula brachyura*[b], *Myrmotherula ignota*))))), (*Microrhopias quixensis consobrinus*[b], ((*Myrmeciza pelzelni*, *Myrmeciza atrothorax*[b]), *Myrmochilus strigilatus*[b]))), (*Myrmornis torquata torquata*[b], (*Pygiptila stellaris*[b], (*Thamnistes anabatinus aequatorialis*[b])))), ((*Terenura sharpei*, *Terenura humeralis*[b], *Terenura callinota*[b]), (*Terenura maculata*[b], *Terenura sicki*)))

APPENDIX 2

Pairs of sympatric and allopatric antbird taxa used to test the species recognition hypothesis, with current taxonomic relationship between the pairs,[1] total number of species in a monophyletic species group,[2] and the location and source of recordings.[3]

	Sympatric pairs of species		Allopatric pairs of species	Taxonomic relationship of species[1]	Total no. of spp.[2]
	A	B	C		
1	Thamnophilus palliatus puncticeps	T. doliatus radiatus	T. z. zarumae	A (BC)	7
2	Thamnomanes ardesiacus obidensis	T. caesius glaucus	T. s. schistogynus	(AX) (BC)	4
3	Thamnomanes schistogynus intermedius	T. saturninus huallagae	T. ardesiacus obidensis	(AX) (BC)	4
4	Myrmotherula cherriei	M. surinamensis	M. pacifica	(ABX) C	10
5	Myrmotherula brachyura	M. sclateri	M. ambigua	(AX) (BC)	10
6	Myrmotherula menetriesii pallida	M. s. sunensis	M. minor	A (BCX)	(12)
7	Myrmotherula m. menetriesii	M. longipennis garbei	M. urosticta	(ABC)	(12)
8	Myrmotherula l. leucophthalma	M. haematonota amazonica	M. spodionota	A (BCX)	8
9	Herpsilochmus gentryi	H. dugandi	H. sticturus	(AX) (BC)	–
10	Herpsilochmus sticturus	H. stictocephalus	H. gentryi	(AX) (BC)	–
11	Herpsilochmus sellowi	H. atricapillus	H. pileatus	(ABC)	5
12	Formicivora g. grisea	F. serrana interposita	F. littoralis	(AX) (BCX)	6
13	Drymophila ferruginea	D. rubricollis	D. d. devillei	(AB) (CX)	8
14	Drymophila genei	D. ochropyga	D. caudata klagesi	(AB) (CX)	8
15	Cercomacra laeta waimiri	C. tyrannina saturatior	C. nigrescens fuscicauda	(AB) C	5
16	Cercomacra serva	C. nigrescens fuscicauda	C. tyrannina saturatior	(AB) (CX)	5
17	Cercomacra t. tyrannina	C. nigricans	C. carbonaria	A (BCXX)	–
18	Myrmeciza berlepschi	M. nigricauda	M. l. laemosticta	A (BC)	–
19	Myrmeciza hyperythra	M. f. fortis	M. immaculata macrorhyncha	A (BC)	(5)
20	Myrmeciza hyperythra	M. goeldii	M. melanoceps	A (BC)	(5)
21	Hylophylax punctulatus	H. naevius theresae	H. n. naevioides	(ABC)	3

[1] A and C denote the focal species' (B) closest sympatric and allopatric relative, respectively; all three have similar habitat requirements and altitudinal ranges; X denotes species that are more closely related to A, B, and C than others within the monophyletic species group of which A, B, C, and X are members. The latter were excluded from the analysis because of morphological and/or ecological divergence from the focal species (see text). Parentheses denote membership of a superspecies. All species pairs assignations are based on Zimmer and Isler (2003).

[2] Total number of closely related species in the monophyletic group from which trio members were selected; a paraphyletic group is denoted by an en dash (–); parentheses denote cases where the monophyly of a group is uncertain.

[3] Recordings are identified in the following order: (**Trio #**) country (region): number of loudsongs (recordists); within each trio, taxa are ordered as above (left to right) and separated by periods. Country codes: BOL, Bolivia; BRA, Brazil; COL, Colombia; COS, Costa Rica; ECU, Ecuador; GUY, Guyana; PAN, Panama; PER, Peru; SUR, Suriname; VEN, Venezuela. Recordists: GB, G. Budney; BC, B. Coffey; MI, M. Isler; PI, P. Isler; TP, T. Parker; PS, P. Schwartz; NS, N. Seddon, JT, J. Tobias; BW, B. Whitney, and KZ, K. Zimmer. All recordings are archived, or will soon be archived, at the Library of Natural Sounds, Cornell Laboratory of Ornithology, Ithaca, NY or the British Library of Sounds, London, U.K. Those marked with an asterisk (*) are taken from an audio CD (Isler and Whitney 2002). (1) PER (Madre de Dios): 3 (NS, JT). PER (Piura): 3 (JT). PER (Tumbes): 1 (BW). (2) COL (Amazonas): 1 (MI); GUY: 1 (BW); VEN (Bolivar): 2 (BC). BRA (Acre): 4 (BW). COL (Amazonas): 1 (MI); GUY: 1 (BW); VEN (Bolivar): 2 (BW). (4) VEN (Amazonas): 3 (KZ). GUY: 3 (BW); BRA (Amapa): 1 (KZ), ECU (Pinchincha): 3 (KZ)*. (5) PER (Madre de Dios): 5 (NS, JT). PER (Madre de Dios): 3 (JT). BRA (Amazonas): 3 (BW, KZ). (6) ECU (Napo): 3 (NS, JT). ECU (Napo): 3 (NS, JT). BRA (Bahia): 1 (BW); BRA (Rio de Janeiro): 2 (BW). (7) PER (Madre de Dios): 3 (BW, KZ). PER (Madre de Dios): 3 (JT). BRA (Bahia): 3 (JT). BRA (Bahia): 3 (BW, KZ). (8) BOL (Santa Cruz): 3 (NS, JT). BOL (Pando): 3 (NS, JT). ECU (Napo): 3 (BW)*. (9) PER (Loreto): 3 (BW). ECU (Napo): 4 (BW, KZ). VEN (Bolivar): 3 (BW). (10) GUY: 3 (BW); VEN (Bolivar): 1 (BW). GUY: 3 (BW). PER (Loreto): 3 (BW). (11) BRA (Bahia): 3 (BW, KZ). BRA (Rio de Janeiro): 4 (BW, KZ). BRA (Bahia): 3 (BW, KZ). (12) BRA (Bahia): 3 (BW, MI, KZ). PER (Madre de Dios): 4 (NS, JT). (14) BRA (Rio de Janeiro): 3 (BW, KZ). (13) BRA (Rio de Janeiro): 2 (KZ); BRA (São Paulo): 2 (BW). VEN (Aragua): 3 (PS)*. (15) BRA (Amazonas): 4 (BW, KZ), PAN (Colón): 2 (BW); PAN (Darién): 1 (BW); PAN (San Blas): 2 (BW); BRA (Roraima): 4 (KZ). (18) COL (Valle): 3 (BW)*. Col (Valle): 3 (BW)* PAN (17) PAN (Canal Zone): 4 (BW, KZ), PAN (Colón): 2 (BW); COS (San José): 1 (KZ), (19) PER (Madre de Dios): 5 (NS, JT). ECU (Cañar): 3 (TP)*. (20) PER (Madre de Dios): (Panamá): 1 (BW); PAN (Darién): 2 (BW). (20) PER (Loreto): 3 (BW)*. PER (Loreto): 3 (TP)*. PAN (Darién): 2 (BW); PAN (Colón): 3 (KZ). 5 (NS, JT). PER (Madre de Dios): 4 (NS, JT). ECU (Pastaza): 2 (BW), Napo: 2 (KZ). (21) PER (Loreto): 3 (BW)*. PER (Loreto): 3 (TP)*. PAN (Darién): 2 (BW); PAN (Colón): 3 (KZ).