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## CLINAL VARIATION IN VOCALIZATIONS OF AN ANTBIRD (THAMNOPHILIDAE) AND IMPLICATIONS FOR DEFINING SPECIES LIMITS

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**ABSTRACT.**—In avian taxa in which vocalizations are considered innate, such as suboscine passerines, vocal characters are increasingly being used to help determine whether populations have achieved species status. In comparing vocal characteristics of distant populations, however, one must be concerned with the possibility of character gradation through intermediate populations. The first quantitative study of a species in a suboscine family to test for clinal vocal variation, our vocal study found clinal variation in the pace (number of notes per second) of male loudsongs, and revealed that the geographic pattern of the clines was consistent with genetic variation found in the companion molecular study (Brumfield 2005). The result underscores the necessity of searching for intermediacy when analyzing vocalizations of geographically distant populations. Furthermore, given that male loudsong pace was the only vocal character that varied across the intergrading populations, the result also provides support to the guideline that one should expect thamnophilid species to differ in at least three vocal characters (Isler et al. 1998) and indicates that this degree of vocal character differences can be a valuable “yard stick” in determining which thamnophilid populations have achieved biological species status.

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**Key words:** clinal variation, hybrid zone, speciation, systematics, Thamnophilidae, *Thamnophilus caerulescens*, Variable Antshrike, vocalizations.

### Variación Clinal en las Vocalizaciones de un Hormiguero (Thamnophilidae) e Implicaciones para Definir los Límites entre Especies

**RESUMEN.**—En las aves en que las vocalizaciones se consideran innatas, como en los Passeriformes suboscinos, los caracteres vocales han sido empleados cada vez más frecuentemente para ayudar a determinar si distintas poblaciones han alcanzado el estatus de especies diferentes. Sin embargo, al comparar las características vocales de poblaciones distantes, es importante prestar atención a la posibilidad de que exista variación gradual en los caracteres a través de poblaciones intermedias. Este estudio es el primer trabajo cuantitativo que pone a prueba la posibilidad de que exista variación clinal en vocalizaciones en una familia de suboscinos. Nuestro análisis de vocalizaciones indicó que existe variación clinal en la cadencia (número de notas por segundo) de los cantos fuertes de los machos, y que el patrón geográfico de las clinas concuerda con la variación genética documentada en un estudio molecular conjunto (Brumfield 2005). Los resultados recalcan la necesidad de buscar rasgos intermedios al analizar las vocalizaciones de poblaciones geográficamente distantes.

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Más aún, considerando que la cadencia de los cantos fuertes de los machos fue el único carácter que varió a través de las poblaciones, el resultado también apoya el lineamiento de que debería esperarse que las especies de Thamnophilidae difieran en por lo menos tres caracteres vocales (Isler et al. 1998), e indica que este grado de diferenciación en vocalizaciones puede ser una "vara medidora" valiosa para determinar cuáles poblaciones de Thamnophilidae han alcanzado el estatus de especies biológicas.

INCREASINGLY, VOCAL CHARACTERS are considered important parameters in species-level taxonomic analysis of suboscines (Johnson et al. 1999, Helbig et al. 2002) and are being used as such (e.g. Isler et al. 1999, 2001, 2002; Whitney et al. 2000; Alvarez and Whitney 2001; Zimmer 2002). Vocalizations of suboscines are considered innate and reflective of genetic structure, though at least one species provides an exception (Baptista and Kroodsma 2001). An earlier paper developed guidelines for employing vocal characters in taxonomic studies of thamnophilid antbirds (Isler et al. 1998). However, when analyzing vocal data in that context, one must ask whether the data were obtained from populations known to be geographically isolated and thus currently incapable of interbreeding and, if not, whether vocal characters vary clinally through intermediate populations. Clinal variation has been found in thamnophilid plumage coloration (e.g. *Thamnophilus atrinucha*; Isler et al. 1997). Contemporary species concepts usually interpret broad geographic character clines as providing evidence of genetic integration and regard populations along a cline unworthy of taxonomic recognition as separately evolving lineages (Haffer 1998). In contrast, clines that are narrow as compared with the distributions of the interconnected populations may reflect selection against hybridization (i.e. reproductive isolation), and the populations on either side of the steep cline are often treated as biological species (Mayr 1996).

The geographic range of the Variable Antshrike (*T. caerulescens*) extends in a wide arc from the Andes of north-central Peru southward through the Andes to central Bolivia and northern Argentina, where it spreads eastward through the lowlands of Bolivia, Paraguay, and Argentina to Uruguay and southeastern Brazil and thence north to northeastern Brazil (Zimmer and Isler 2003). The English common name reflects the substantial differences in plumage coloration among many of the twelve

subspecies recognized by Peters (1951). A comprehensive review of the status of those taxa using modern analytic tools has yet to be done, but some taxa appear to intergrade morphologically. The plumage of the form found on the southern slope of the arm of the Andes in central Bolivia that extends east to the city of Santa Cruz, known as *T. c. connectens*, is intermediate between those of *T. c. aspersiventer* of the north slope of that arm of the Andes and *T. c. dinellii* of southern Bolivia and northwestern Argentina (Ridgely and Tudor 1994). To the east of the Andes in the Bolivian and Paraguayan chaco, a pale population is found that has been described as *T. c. paraguayensis*.

Those populations have been the subject of a study aimed at understanding patterns of mitochondrial variation (Brumfield 2005). Concurrently, a parallel analysis was undertaken of Variable Antshrike vocalizations in the same region, the results of which are reported here. In conjunction with the results of the molecular study, the vocal study has sought answers to the following questions. To what extent do vocalizations of populations described as *T. c. aspersiventer*, *T. c. connectens*, *T. c. dinellii*, and *T. c. paraguayensis* differ? Is there evidence of broad or narrow clines in vocal characters? How well do differences in vocal characters coincide with genetic differences? What implications do those findings have for the use of vocal characters in species-level systematic studies?

#### METHODS

We compared the vocalizations of five populations in central Bolivia and extreme northwestern Argentina (Fig. 1) defined geographically on the basis of current subspecies ranges, distribution of available vocal recordings, and preliminary results of the molecular study (Brumfield 2005). Those populations were designated as *T. c. aspersiventer* (A), corresponding to population sample A1 in the genetic analysis; *T. c. connectens*

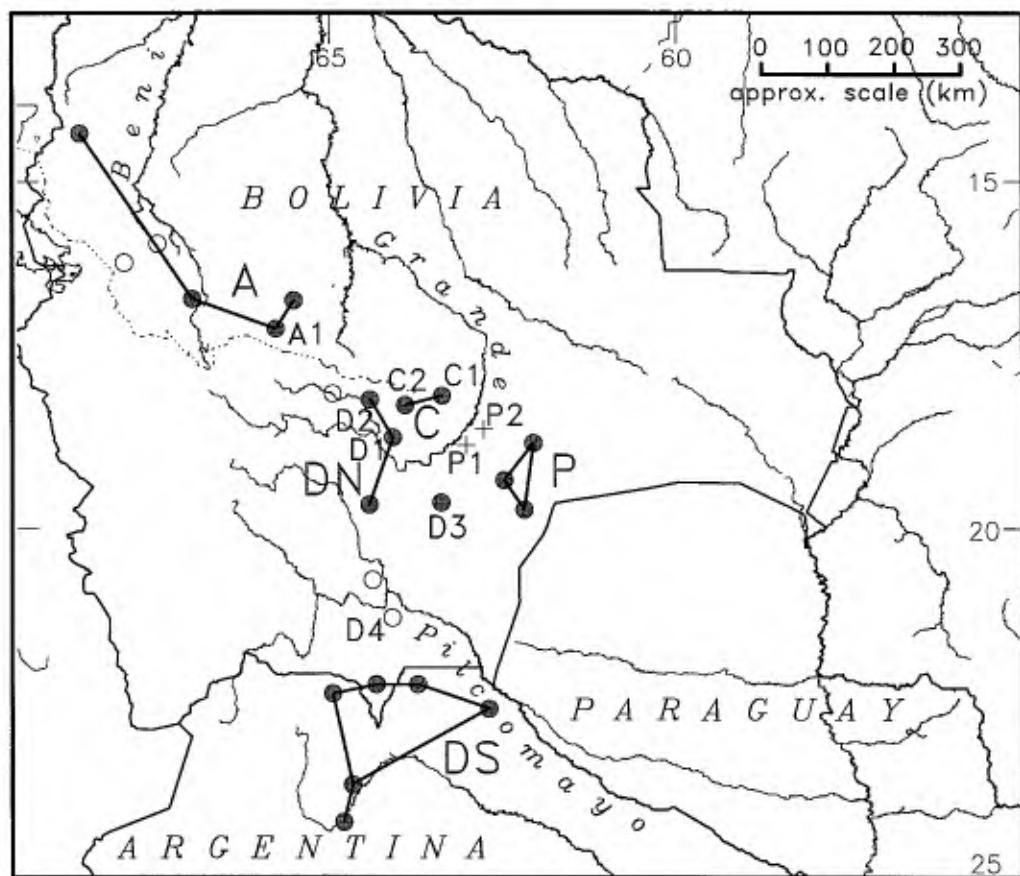


FIG. 1. Identification of populations, locations of vocal recordings, and references to locations employed in a parallel genetic study (Brumfield 2005). Location symbols represent small sectors (Isler 1997) to which individual sites are assigned. Solid circles = locations of recorded male loudsongs. Heavy lines connect locations included in the same population. Large letters identify populations as follows: *T. c. aspersiventer* (A), *T. c. connectens* (C), *T. c. dinellii* North (DN), *T. c. dinellii* South (DS), and *T. c. paraguayensis* (P). Open circles = locations of recordings of calls, but no loudsongs. Crosses = locations from which specimens and tissue were collected and analyzed but from which no vocal recordings were available. Smaller letter and number combinations identify locations of specimens examined in the genetic study. Locations between defined populations mentioned in text: Gutiérrez, Santa Cruz, Bolivia (D3), and Chuquisaca and northern Tarija, Bolivia (D4).

(C), corresponding to population samples C1 and C2 in the genetic analysis; *T. c. dinellii* North (DN), corresponding to population samples D1 and D2 in the genetic analysis; *T. c. dinellii* South (DS) (no genetic samples were available from those populations); and *T. c. paraguayensis* (P), corresponding to population samples P1 and P2 in the genetic analysis. Additional sites, described as "between defined populations," had an insufficient number of recordings for analysis but will be referred to in the Discussion.

Two of those are included in the genetic analysis as population samples D3 and D4.

The northernmost population, *T. c. aspersiventer*, occurs in La Paz and Cochabamba, Bolivia, on the northern flank of the Andean spur that extends eastward to the city of Santa Cruz (Santa Cruz de la Sierra); males are mostly black with white scalloped underparts; and its habitat is the most humid of the populations studied. On the opposite, southern, side of this Andean spur, in the department of Santa Cruz, Bolivia,

recording locations for *T. c. connectens* are centered on Samaipata, Bolivia, the type locality, and extend eastward toward the city of Santa Cruz; plumages are most often intermediate between populations to the north and south, with males showing patches of black feathers and whitish underparts; the habitat is drier than on the north slope, but not as dry as to the west and south.

Within the range of *T. c. dinellii*, two populations were defined because available recordings fell geographically into two clusters, with an inadequate number of recordings from the region between them: *T. c. dinellii* North and *T. c. dinellii* South. Both populations have been identified previously as *T. c. dinellii* on the basis of plumage; males are gray with ochraceous posterior underparts. Note that the range of *T. c. dinellii* North extends to the south slope of the Andean spur west of the range of *T. c. connectens* in the vicinity of El Tambo (Tambo), Bolivia (location D2 on Fig. 1; habitat described in Schmitt et al. 1997). To the east, *T. c. paraguayensis* occupies dry scrub (chaco; habitat described in Kratter et al. 1993); its plumage is the whitest of any of the populations studied. In most cases, tape recordings of vocalizations were obtained at the sites of tissue collection. Recordings were inadequate for vocal analysis at two tissue collection sites (D3 and D4), and the precise locations of two other sites (P1 and P2) were slightly west of the recording locations. As noted above, tissue was unavailable for *T. c. dinellii* South.

We obtained vocalization recordings for each of the populations from our own inventory, from contributions from other individuals, and from archives of the Macaulay Library of Natural Sounds (Cornell University, Ithaca, New York) and the National Sound Archive (The British Library, London). The assemblage included a number of recordings made by R.T.B. of individuals that were collected subsequently to obtain tissue employed in the molecular analysis. We examined 154 recordings. The Appendix provides sample sizes and a list of recordings employed in the study with locality names, recordists, and archival identification.

We reviewed each recording to identify the number and sex of individuals vocalizing and to label every vocalization as to type. We identified six types of vocalizations: "loudsong," "softsong," "caw," "whine," "growl," and "chuck call" (Figs. 2 and 3). Loundsongs (following Willis 1967) refer to the ringing series

of notes delivered in a consistent pattern and were identified as to sex of the vocalizing individual whenever possible. We used CANARY 1.2.4 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, New York) to make a spectrogram of every vocalization type delivered by each individual of either sex on every recording. All spectrograms were used initially in visual analyses. In a visual analysis, we considered a character to be diagnostic only when examination of the character could distinguish every spectrogram of one population from those of another with certainty.

After examining spectrograms of all vocalization types and recordings for diagnostic visual differences, we selected a sample of male loundsongs to obtain quantitative measures of other vocal characters (e.g. pace) that appeared, from spectrograms, to vary geographically. The analysis was limited to loundsongs of males to eliminate possible sexual differences. A parallel analysis of female loundsongs was impractical, because too few loundsong recordings identifiable as representing females were available from most populations. Spectrograms were projected on the screen of a Macintosh G4 computer using the default settings in CANARY, except that the display was set to smooth, overlap was adjusted from 50% to 93.75% depending on recording quality, and contrast was adjusted according to recording intensity, with care taken to retain all elements of the vocalization. Cursor measurements were made by P.R.I., typically at scales of 0.3 s per inch and 4.0 kHz per inch. We measured three loundsongs on each recording for five to seven individuals of each population. Frequency measurements refer to the highest frequencies of notes.

As described in more detail in earlier papers (Isler et al. 1998, 1999), to be considered diagnostic, differences must be unambiguously distinct character states or, in the case of continuous variables, ranges could not overlap and the means ( $\bar{x}$ ) and standard deviations (SD) of the population with the smaller set of measurements (*a*) and the population with the larger set of measurements (*b*) had to meet the requirement:

$$\bar{x}_a + t_a \text{SD}_a \leq \bar{x}_b - t_b \text{SD}_b$$

where  $t_i$  = the  $t$ -score at the 97.5 percentile of the  $t$ -distribution for  $n - 1$  degrees of freedom (except for ratios where that statistical test is inappropriate).

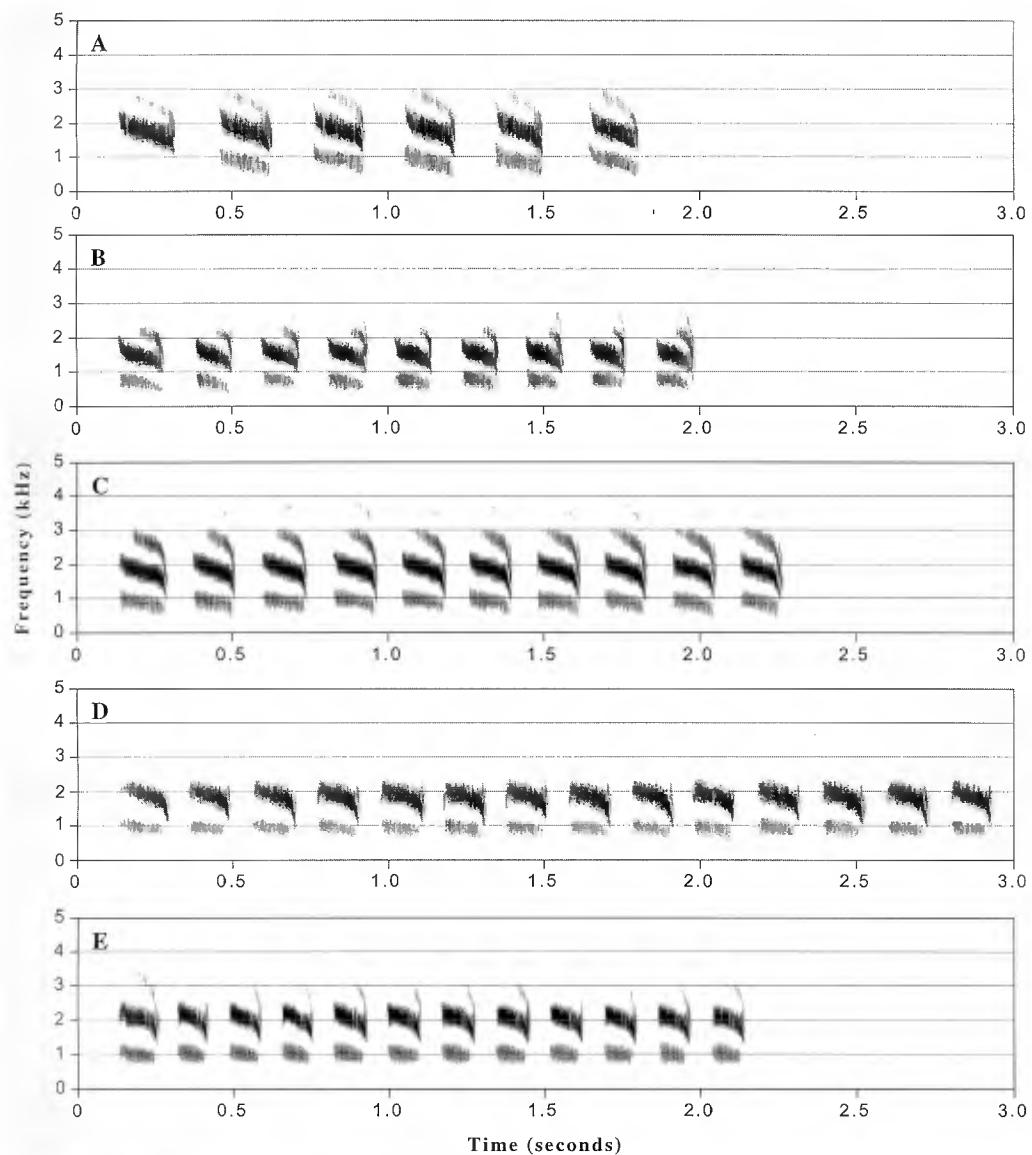


FIG. 2. Loudsongs of male *T. caerulescens* in Bolivia and northwestern Argentina. Spectrograms were selected to reflect means of diagnostic measures (Table 1) for populations. The following annotations include population, location, recordist, and archive reference. See Appendix for explanations of archive references. (A) *T. c. aspersiventer*; San Onofre, Cochabamba, Bolivia (Brumfield ISL-RTB.2:21). (B) *T. c. connectens*; Bermejo, Santa Cruz, Bolivia (Brumfield ISL-RTB.2:08). (C) *T. c. dinellii* North; El Tambo, Santa Cruz, Bolivia (Parker MLNS 33662). (D) *T. c. dinellii* South; Palomitas, Salta, Argentina (Pearman ISL-MISC.7:31). (E) *T. c. paraguayensis*; Perforación, Santa Cruz, Bolivia (Parker MLNS 82692).

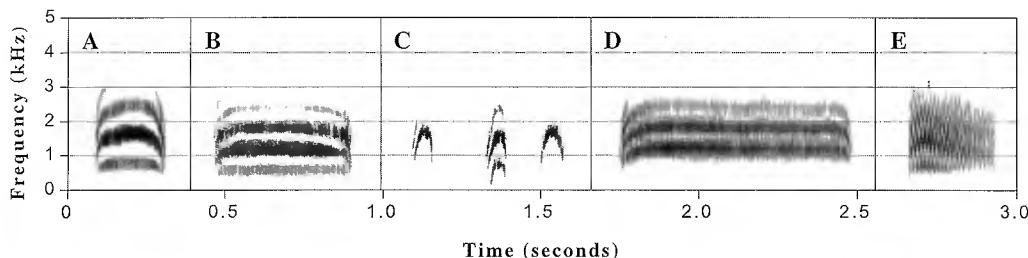


FIG. 3. Calls of *T. caerulescens* in Bolivia and northwestern Argentina. The following annotations include name of call, location, recordist, and archive reference. See Appendix for explanations of archive references. (A) Short caw; San Lorenzo, Santa Cruz, Bolivia (Brumfield ISL-RTB.4:03). (B) Long caw; Bermejo, Santa Cruz, Bolivia (Brumfield ISL-RTB.2:02). (C) Chuck calls of two males counter-calling; San Lorenzo, Santa Cruz, Bolivia (Brumfield ISL-RTB.3:09). (D) Whine; 10 km east Gutierrez, Santa Cruz, Bolivia (Brumfield ISL-RTB.5:02). (E) Growl; San Lorenzo, Santa Cruz, Bolivia (Brumfield ISL-RTB.3:21).

Finally, we assessed the taxonomic status of each consolidated population, employing guidelines developed in previous papers. In summary, based on a study of vocalizations of closely related, syntopic antbirds and subsequent applications (Isler et al. 1998, 1999, etc.), we would recommend species status for allopatric populations under the biological species concept (BSC) if they differ diagnostically in both vocal and nonvocal (morphological or molecular) characters. Three independent vocal characters are employed as a point of reference for using vocalizations in this context, though not as a requirement; and for populations that are highly differentiated in nonvocal characters, fewer vocal characters may be acceptable. Morphological analysis was not undertaken in the present study, and current subspecies definitions were accepted as representing plumage differences. Results of molecular analysis are described in a companion paper (Brumfield 2005). We recommend that taxa that do not meet those requirements but that are distinguished by at least one diagnostic vocal character be maintained as subspecies under the BSC, though such taxa may be recognized as species under alternate approaches, such as the phylogenetic species concept.

#### RESULTS

**Loudsongs.**—Male loudsongs of all five populations were extremely similar in all visual aspects, except in length and pace (Fig. 2). Note shapes did not differ diagnostically

among populations. Individual notes typically descended slightly in frequency before dropping more sharply at the end of the note; and though there was variability in slope and in details of note shape at the beginning of the note, those differences varied individually and not geographically. When compared with one another within a loudsong, note shapes remained constant, frequencies of note peaks essentially remained at the same level, and a slight acceleration of pace was apparent.

Comparisons of 43 quantitative measurements of male loudsong samples supported the paucity of differences found in the visual analysis. Across five populations, ranges of values overlapped in all but 39 of the 430 pairwise comparisons. The 39 comparisons in which ranges did not overlap were confined to 12 measures: number of notes (1), duration (2), overall pace (3), length of five selected notes (4–8), and length of four selected intervals between notes (9–12). When statistical tests of significance were applied subsequently, the number of pairwise comparisons found to be diagnostic was reduced from 39 to 9 (Table 1), and the number of measures that expressed diagnosable differences was reduced from 12 to 6: overall pace (1), four interval measures (2–5), and length of initial note (6). Although note length has been considered an aspect of note shape in situations where note shapes differed visually, we considered all those significant measures reflective of a single vocal character—overall pace of the loudsong.

All diagnostic differences in measurements

TABLE 1. Vocal measurements of male loudsongs of five populations of *Thamnophilus caerulescens* in central Bolivia and northwest Argentina. Pace is given in notes per second. Remaining length measurements are given in microseconds. Interval 1 lies between first and second notes, interval 2 lies between second and third notes, interval 3 lies between middle and next notes, and interval 4 lies between next-to-last and last notes. Note 1 = first note. Means  $\pm$  SD (range; *n*).

Population	Pace <sup>a</sup>	Interval 1 <sup>b</sup>	Interval 2 <sup>b</sup>	Interval 3 <sup>b</sup>	Interval 4 <sup>a</sup>	Note 1 <sup>a</sup>
<i>T. c. aspersiventer</i>	3.35 $\pm$ 0.19 (3.06–3.63; 6)	141 $\pm$ 15 (116–160; 6)	137 $\pm$ 14 (115–156; 6)	139 $\pm$ 15 (115–160; 6)	152 $\pm$ 20 (119–179; 6)	175 $\pm$ 13 (162–190; 6)
<i>T. c. connectens</i>	4.30 $\pm$ 0.28 (3.98–4.61; 5)	100 $\pm$ 10 (89–111; 5)	98 $\pm$ 8 (91–107; 5)	97 $\pm$ 8 (90–108; 5)	111 $\pm$ 18 (95–140; 5)	147 $\pm$ 20 (119–175; 5)
<i>T. c. dinellii</i>	4.56 $\pm$ 0.65 (3.65–5.39; 7)	87 $\pm$ 17 (67–110; 7)	87 $\pm$ 16 (72–110; 7)	87 $\pm$ 19 (67–114; 7)	101 $\pm$ 30 (72–158; 7)	145 $\pm$ 9 (135–163; 7)
North	4.82 $\pm$ 0.53 (4.05–5.44; 7)	74 $\pm$ 11 (59–93; 7)	78 $\pm$ 7 (69–92; 7)	75 $\pm$ 8 (64–85; 7)	85 $\pm$ 8 (74–96; 7)	149 $\pm$ 22 (125–183; 7)
South	5.60 $\pm$ 0.36 (4.98–5.94; 5)	68 $\pm$ 4 (65–74; 5)	70 $\pm$ 6 (63–79; 5)	69 $\pm$ 5 (64–78; 5)	78 $\pm$ 7 (71–89; 5)	127 $\pm$ 3 (122–129; 5)

<sup>a</sup> Difference between *T. c. aspersiventer* and *T. c. paraguayensis* diagnostic.

<sup>b</sup> Differences between *T. c. aspersiventer* and both *T. c. dinellii* South and *T. c. paraguayensis* diagnostic.

were between the most remote populations: between *T. c. aspersiventer* and the southernmost and easternmost populations, *T. c. dinellii* South and *T. c. paraguayensis* (Fig. 1). Ranges of measurements for geographically intermediate populations fell between the extremes and did not differ significantly from those of neighboring populations. Measurements of the interval between the second and third notes (interval 2; Table 1) exemplify those relationships. Measurements of interval 2 differed significantly between *T. c. aspersiventer* and both *T. c. dinellii* South and *T. c. paraguayensis*. However, although values in our sample for *T. c. aspersiventer* did not overlap those of either *T. c. connectens* or *T. c. dinellii* North, and values of *T. c. paraguayensis* did not overlap those of *T. c. connectens*, pairwise comparisons did not pass our statistical test. The value of the test was borne out when additional vocal recordings, not falling into our measurement sample, were measured for that variable; extreme examples were found whose pace overlapped slightly in both pairwise comparisons. Ranges of measurements for interval 2 from *T. c. connectens* and *T. c. dinellii* North overlap completely, and ranges for both overlap slightly with *T. c. dinellii* South.

As exemplified by interval 2, a cline in pace of male loudsongs was found in the region between populations identified as *T. c. aspersiventer* and *T. c. dinellii* South (Fig. 4A). Most (66%) of that transition occurs between populations A and C. The lack of samples between populations A and C prevented us from assessing whether the transition represents a gradual change from one vocal type to the other, or a narrow cline that is centered somewhere in the unsampled region (Fig. 1). The remaining transition occurs gradually; 19% between C and DN, and 15% between DN and DS. A similar cline in pace was found on a west-east axis (Fig. 4B), between *T. c. dinellii* North and *T. c. paraguayensis*; one should bear in mind that only a single example of a male song was available from Gutiérrez (locality D3; Fig. 1). Considering all populations, the pace of male loudsongs is slowest in *aspersiventer* on the north slope of the Andean spur, with a sharp transition to faster-paced songs in southern-slope populations, which become even faster-paced, but at a lessened rate of change, as one moves south through the distribution of *connectens* and *dinellii*, and thence into the distribution of *paraguayensis* (Table 1).

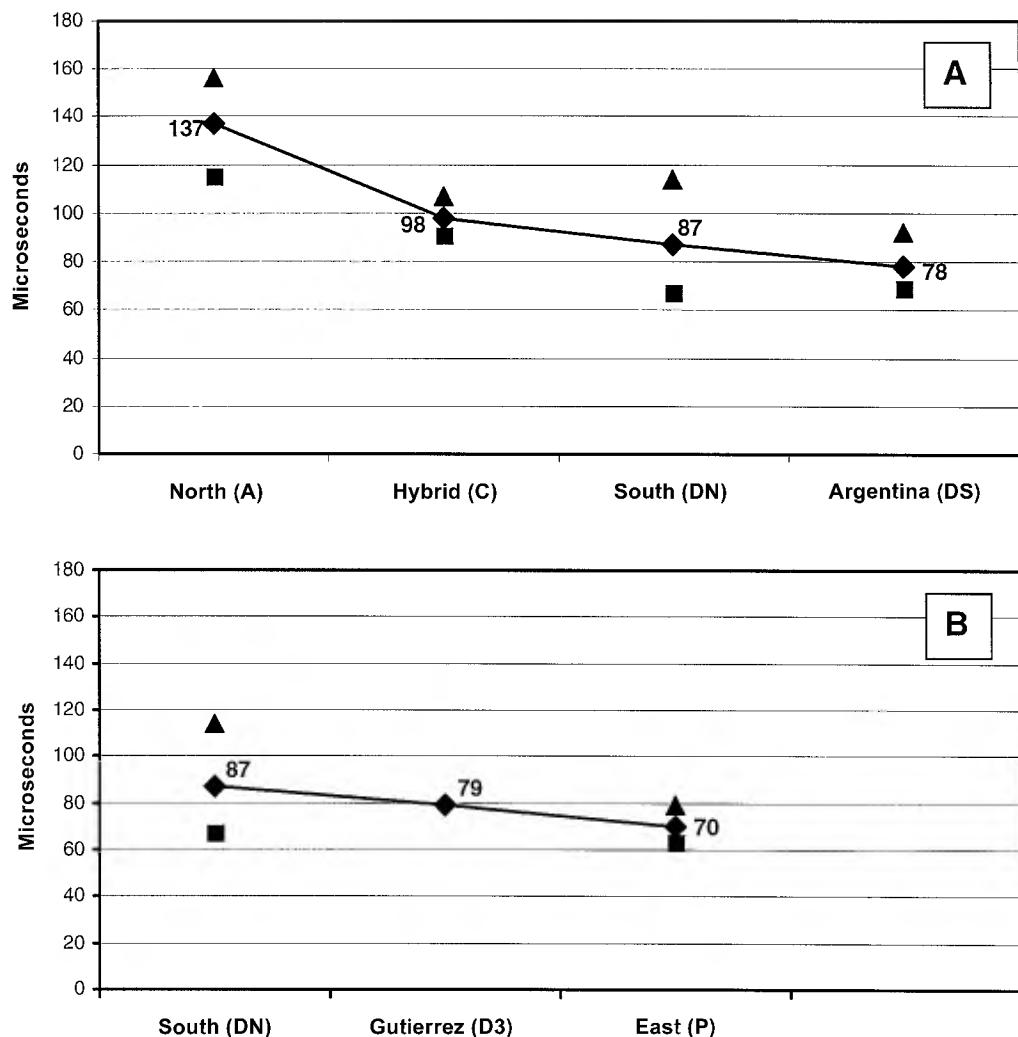


FIG. 4. Intervals (microseconds) between second and third notes of *T. caerulescens* male loudsongs for six populations. (A) Populations arranged on a north-south transect. (B) Populations arranged on a west-east transect. Line connects means (diamonds) of values for populations. Symbols above and below line reflect maximum (triangle) and minimum (square) values for each population. North (A) = *T. c. aspersiventer* ( $n = 6$ ); hybrid (C) = *T. c. connectens* ( $n = 5$ ); south (DN) = *T. c. dinellii* North ( $n = 7$ ); Argentina (DS) = *T. c. dinellii* South ( $n = 7$ ); Gutierrez (D3) = Gutiérrez, Bolivia ( $n = 1$ ); east (P) = *T. c. paraguayensis* ( $n = 5$ ).

**Calls.**—The commonly delivered call was a deep (mostly 1.5–2.0 kHz with overtones), moderately long (typically 0.2–0.3 s), mostly flat note, typically described as a *caw*. The length of this call varies within populations (Fig. 3). Samples of the call were plentiful from all populations, except for *T. c. dinellii* South where it was found in only one recording. Visual comparison of

spectrograms produced no diagnostic differences in the *caw* call among populations.

Other calls included a longer (0.5–0.6 s) “whine,” similar but usually flatter than the *caw*; a frequency-modulated but more downslurred version of the *caw* that might be described as a “growl”; and a short (0.05–0.10 s) “chuck call” that took the shape of an inverted U. A

"softsong," recorded only for *T. c. dinellii* North and in Gutiérrez, consisted of a short downslurred note, shaped like the note of the loudsong, repeated a variable number of times at the rate of 2.0–2.5 notes per second (not illustrated). All those secondary calls were recorded rarely, and sample sizes were too small to provide a basis, if any exists, for identifying diagnostic differences among populations.

#### DISCUSSION

This is the first analysis to demonstrate clinality of a vocal character in contiguous populations of a thamnophilid antbird. Lindell (1998) studied vocalizations of a species in another suboscine family (Furnariidae) at three locations in northern Venezuela; and though she found differences between populations (which do not appear to meet our more severe tests for accepting vocal differences as diagnostic), the geographic pattern did not suggest clinality. In the present study, six quantitative vocal measures differed diagnostically between the population on the northeastern slope of the Bolivian Andes (*T. c. aspersiventer*) and noncontiguous populations in northwestern Argentina (*T. c. dinellii*) and the Bolivian–Paraguayan chaco (*T. c. paraguayensis*), but those measures reflected a single vocal character—loudsong pace. Furthermore, intermediate values were exhibited by intervening populations. Thus, the pace of notes, though an important character in distinguishing loudsongs of syntopic species-pairs (Isler et al. 1998), can vary clinally, even though antbird loudsongs are almost certainly innate. It remains to be seen whether other antbird vocal characters are subject to a similar pattern of geographic variation.

The clinal variation in vocal characters is strikingly concordant with the patterns of mitochondrial variation. Focusing on measures reflecting the change of pace from *aspersiventer* (A) through *connectens* (C) to south-slope *dinellii* foothill (DN) populations, most (76–93%) of the transition in the six loudsong pace vocal characters (Table 1) occurs between populations A and C. That is remarkably similar to the 83% transition in mitochondrial haplotype frequency between those same populations. The gradual increase in loudsong pace that explains the remaining clinal transition within the distribution of *dinellii* may be explained by a gradual increase in the amount of introgression of *paraguayensis* into *dinellii*

populations. Eight percent of individuals from population D3 and 23% from population D4 (Fig. 1) had *paraguayensis* mitochondrial haplotypes. Although the nature of the contact between *dinellii* and *paraguayensis* is still unknown, both the vocal and genetic data suggest that introgression is influencing the patterns of variation in those characters. Unfortunately, genetic data from the type locality of *dinellii* in northern Argentina are lacking but, extrapolating from the vocal and genetic patterns, it seems possible that the population found at the type locality of *dinellii* is an intergrade between populations of *dinellii* to the north and *paraguayensis*.

Turning to questions of current taxonomic status of the populations of *T. caeruleascens* in Bolivia and northwestern Argentina, even if there were no geographically intermediate populations, the extent of vocal differences between the outliers would fall well short of vocal distinctions found between closely related, syntopic antbird species. Reproductively isolated thamnophilid species are typically distinguished by three or more independent vocal characters (Isler et al. 1998, 1999). Because significant variation between outlying populations was found in only one character, vocal differences would be insufficient to support the hypothesis that the outlying populations are specifically distinct even without a finding of clinality.

Clinality was found, however, and no vocal characters distinguished adjacent populations. Thus, from a purely vocal perspective, the named taxa that occur in Bolivia and northwest Argentina (*T. c. aspersiventer*, *T. c. connectens*, *T. c. paraguayensis*, and *T. c. dinellii*) could be merged into a single taxon. The name *T. c. connectens* should be eliminated, because individuals of this population are clearly intergrades (based on genetic data and morphological features as well as vocal analysis), but to merge the remaining three populations into a single taxon obscures the fact that they may represent distinct evolutionarily significant units, as suggested by morphology. The decision rests largely on the nature of the hybrid zones between them, and further analysis of morphological and other characters is required. In the north, additional sampling is needed to characterize the transition from *aspersiventer* to *dinellii*, but the available genetic and vocal evidence suggests that the transition may be restricted to a relatively narrow contact zone, with *connectens* representing one tail of

the zone. *Thamnophilus c. aspersiventer* should continue to be maintained as a subspecies pending further study of the contact zone when its status can be reassessed. To the south and east, intergradation between *dinellii* and *paraguayensis* is also reflected in genetic as well as in vocal data. Compared with that of *aspersiventer-dinellii*, however, the transition zone of *dinellii* and *paraguayensis* appears to be broader geographically, though its extent and configuration are poorly known. Given the absence of genetic data from extreme southern Bolivia, northwest Argentina, and southwestern Paraguay, and the paucity of geographically fine-grained vocal and morphological information in that region, as well as the contrasts in plumage between populations of *dinellii* and *paraguayensis* found in central Bolivia, the best treatment at this time is to maintain *dinellii* as a subspecies.

The companion analysis of genetic variation (Brumfield 2005) found geographic patterns of interrelationships among those populations similar to the patterns reported here and demonstrated that introgression is occurring or has occurred recently among them despite substantial differences in plumage coloration. The concordance of results between the two studies reinforces their individual conclusions. Consistency of results also provides further evidence of the value of vocal characters as an expression of genetic similarities and differences among thamnophilid antbird populations. More specifically, the earlier finding (Isler et al. 1998) that at least three independent vocal differences distinguished closely related, syntopic thamnophilid species is supported. We note that the critical assumption that those vocal differences evolved independently remains untested. Although it is likely that vocal differences serve as a mechanism for effecting reproductive isolation of thamnophilid species (Seddon 2005), it remains to be seen whether the observed gene flow between populations is a result of the lack of significant vocal differences between them or, conversely, whether the paucity of vocal differences is a reflection of a low level of genetic differentiation in the absence of other isolating mechanisms.

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#### APPENDIX: INVENTORY OF VOCALIZATION RECORDINGS EXAMINED

For each population, recordings are listed by country, state or department, and recording location. Recordist names aid in identifying the recording as well as providing acknowledgments. Numbers following the recordist's name identify the number of cuts per recordist per location. Acronym for recording archive: MLNS = Macaulay Library of Natural Sounds,

Cornell Laboratory of Ornithology, Ithaca; NSA = National Sound Archive, The British Library, London; ISL = recordings not yet archived in an institutional collection but that have been copied into the inventory maintained by M. L. and P. R. Isler.

*Thamnophilus caerulescens aspersiventer*. 19 recordings. BOLIVIA: La Paz: Caranavi 33–48 km north of (Parker 3 NSA; Sacramento Alto (Pearman 3 NSA; Whitney 1 ISL); Río Tuichi (Whitney 2 ISL). Cochabamba: P. N.

Carrasco (Whitney 1 ISL); Saila Pata (Krabbe 2 ISL); San Onofre (Brumfield 5 ISL); Villa Tunari (D. Willis 1 NSA); Villa Tunari-Cochabamba Road (Whitney 1 ISL).

*Thamnophilus caerulescens connectens*. 30 recordings. BOLIVIA: Santa Cruz: Bella Vista (Brumfield 5 ISL); Bermejo (Brumfield 13 ISL; Whitney 1 ISL); La Pajcha (Lane 4 ISL); Samaipata (Lane 1 ISL, Mayer 5 ISL); Yungas de Mairana (Parker 1 ISL).

*Thamnophilus caerulescens dinellii* North. 48 recordings. BOLIVIA: Santa Cruz: Comarapa, 35 km west (Lane 2 ISL); El Tambo (Parker 1 MLNS; Whitney 1 ISL); Masicuri (Mayer 3 ISL); San Lorenzo (Brumfield 32 ISL); Vallegrande (Mayer 4 ISL). Chuquisaca: Sopachuy (Krabbe 5 ISL).

Between defined populations. 25 recordings. BOLIVIA: Santa Cruz: Gutiérrez, 10 km east of: (Brumfield 11 ISL); Itai (Brumfield

7 ISL); Pirirenda, Laguna (Brumfield 1 ISL). Chuquisaca: Bufete, Cerro (Schulenberg 2 ISL); El Palmar (Mayer 1 ISL). Tarija: Entre Ríos (Brumfield 2 ISL; Parker 1 MLNS).

*Thamnophilus caerulescens dinellii* South. 17 recordings. ARGENTINA: Chaco: Chaco, P. N. (Mazar Barnett 1 ISL). Jujuy: Aguas Negras (Krabbe 1 ISL); Calilegua, P. N. (Mazar Barnett 5 ISL; D. Willis 1 NSA). Precise location unknown: (Pearman 1 ISL); Salta: Joaquin V. Gonzales (Pearman 1 ISL); Lipeo-Papachacra Trail (Pearman 1 ISL); Palomitas (Pearman 1 ISL); Piquirenda (Pearman 1 ISL); Tartagal (Pearman 1 ISL). BOLIVIA: Tarija: Cambari (Mayer 1 ISL); Pampa Grande (Mayer 2 ISL).

*Thamnophilus caerulescens paraguayensis*. 15 recordings. BOLIVIA: Santa Cruz: Curuyuqui (Parker 2 ISL, 2 MLNS); Ibasiriri (Mayer 6 ISL); Perforación (Parker 5 ISL).