Genetic evidence supports song learning in the three-wattled bellbird *Procnias tricarunculata* (Cotingidae)

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Abstract

Vocal learning is thought to have evolved in three clades of birds (parrots, hummingbirds, and oscine passerines), and three clades of mammals (whales, bats, and primates). Behavioural data indicate that, unlike other suboscine passerines, the three-wattled bellbird *Procnias tricarunculata* (Cotingidae) is capable of vocal learning. *Procnias tricarunculata* shows conspicuous vocal ontogeny, striking geographical variation in song, and rapid temporal change in song within a population. Deprivation studies of vocal development in *P. tricarunculata* are impractical. Here, we report evidence from mitochondrial DNA sequences and nuclear microsatellite loci that genetic variation within and among the four allopatric breeding populations of *P. tricarunculata* is not congruent with variation in vocal behaviour. Sequences of the mitochondrial DNA control region document extensive haplotype sharing among localities and song types, and no phylogenetic resolution of geographical populations or behavioural groups. The vocally differentiated, allopatric breeding populations of *P. tricarunculata* are only weakly genetically differentiated populations, and are not distinct taxa. Mitochondrial DNA and microsatellite variation show small (2.9% and 13.5%, respectively) but significant correlation with geographical distance, but no significant residual variation by song type. Estimates of the strength of selection that would be needed to maintain the observed geographical pattern in vocal differentiation if songs were genetically based are unreasonably high, further discrediting the hypothesis of a genetic origin of vocal variation. These data support a fourth, phylogenetically independent origin of avian vocal learning in *Procnias*. Geographical variations in *P. tricarunculata* vocal behaviour are likely culturally evolved dialects.

Keywords: conservation suboscine, cotinga, cultural evolution, gene flow, population genetics, song learning

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Introduction

Vocal learning has been definitively demonstrated in humans and three avian clades: parrots, hummingbirds, and oscine passerines (Nottebohm 1972; Baptista & Schuchmann 1990; Jarvis *et al.* 2000; Wilbrecht & Nottebohm 2003). Geographical dialects and rapid cultural evolution provide strong additional evidence of vocal learning in

whales (Noad *et al.* 2000) and bats (Boughman 1998). In chimpanzees, genetic analysis of populations with different vocal dialects provides evidence of cultural transmission of vocal behaviour (Crockford *et al.* 2004).

The best-studied nonhuman vocal learning system is in the oscine passerines, or song birds (Wilbrecht & Nottebohm 2003) which include over 3000 avian species. Song learning in oscines produces rapid cultural evolution within populations (Payne 1982; Payne *et al.* 1988), and extensive geographical variation, or culturally evolved vocal dialects, among populations (Kroodsma 2005).

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The sister group to the oscines is the suboscine passerine clade (Tyranni) (Barker *et al.* 2004). The ~1000 species of suboscine perching birds have a pantropical distribution with a few species in temperate North America. They are ecologically highly diverse, and comprise a substantial portion of the avifauna of the Neotropics (del Hoyo *et al.* 2003, 2004). Because of overwhelming evidence of their lack of vocal dialects (i.e. vocal variations across geographical distributions of a species), suboscines have been hypothesized to lack vocal learning generally. Acoustic deprivation experiments on three species of suboscine tyrant flycatchers (*Empidonax traillii, Empidonax alnorum, Sayornis phoebe,* Tyrannidae) demonstrate that their songs are largely innate (Kroodsma 1984, 1985; Kroodsma & Konishi 1991). Neuroanatomical investigations on a few species have also demonstrated that suboscines lack the forebrain song nuclei used in song learning by oscine passerines (Gahr *et al.* 1993). These results have corroborated the conclusion that suboscine passerines do not have the ability to learn their songs (but see Raposo & Höfling 2003 for an alternative view).
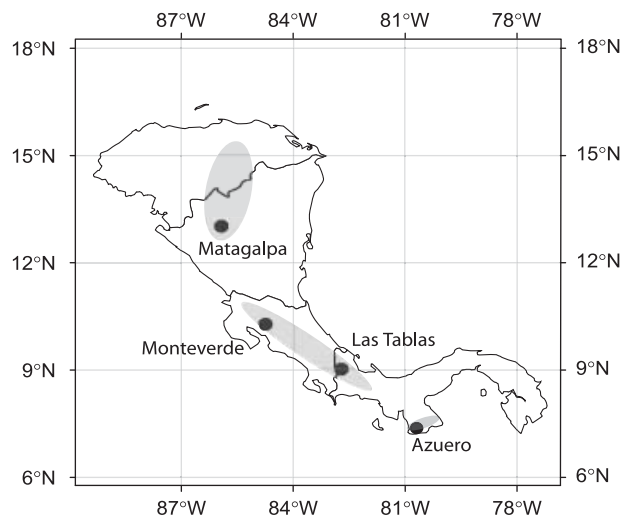
The three-wattled bellbird *Procnias tricarunculata* is a threatened species distributed in Central America from southern Honduras and northern Nicaragua to western Panama (Figs 1 and 2) (Ridgely & Gwynne 1989; Stiles & Skutch 1989; Snow 2004). The genus *Procnias* also includes three additional South American species, and is placed in the suboscine passerine family of cotingas (Cotingidae) (Snow 1973b, 1982; Prum *et al.* 2000; Ohlson *et al.* 2007). Male *Procnias* produce some of the loudest of all animal vocalizations, which include thunderous, electronic, bell-, or gong-like notes (Snow 1970, 1973a, 1977; Snow 1973b, 1982, 2004). Unlike other suboscine species, *P. tricarunculata* shows extensive qualitative and quantitative vocal variation among breeding populations (Kroodsma 2005). Throughout this study, we refer to these discreet classes of vocal variation as song types; we use the term dialect to refer to learned geographic variations in vocal behaviour.

Kroodsma (2005) has described three song types in *P. tricarunculata*: the Nicaraguan song type found in northern Nicaragua and southern Honduras; the Monteverde song type found in the Tilarán Mountains of north-central Costa Rica; and the Talamancan (or Panamanian of Kroodsma) song type found in the Cordillera de Talamanca of southeastern Costa Rica and western Panamá. Here, we also document an additional, Azuero song type found in the allopatric breeding population in the highlands at the southern tip of the Azuero Peninsula of Panamá (Fig. 2). The regional distribution of these song types (i.e. vocal variants with fixed quantitative and qualitative differences) indicates that this species may learn a major component of its vocal behaviour (Snow 1977; Kroodsma 2005).

Additional behavioural evidence also supports vocal learning in *P. tricarunculata*. First, young males of *P.*

Fig. 1 An adult, male three-wattled bellbird, *Procnias tricarunculata* (Cotingidae), singing from its display perch — a dead branch emerging above from the forest canopy in Monteverde, Costa Rica. Photo taken by Clayton Taylor of Swarovski Optik N.A. with Swarovski AT80 HD spotting scope with 20–60× eyepiece and a Pentax EI200 camera on 16 June 2001.

Fig. 2 Breeding distribution (grey) and the four study sampling localities of the three-wattled bellbird, *Procnias tricarunculata.*

tricarunculata, and other *Procnias* species from South America, exhibit conspicuous vocal ontogeny — that is inept repetition of incomplete and inappropriately formed vocalizations (Snow 1970, 1973a, 1977; Kroodsma 2005). Vocal development requires many months in wild male *Procnias* (Snow 1970), but is conspicuously absent in other suboscines, including closely related, highly vocal, lekking cotinga species (e.g. screaming piha, *Lipaugus vociferans;* and capuchinbird, *Perissocephalus tricolor*). Second, Kroodsma (2005) and Snow (1977) documented the existence of a few 'bilingual' or dual-song type male *P. tricarunculata* at Monteverde, in the Tilarán Mountains, Costa Rica. Third,

Kroodsma (2005) has documented rapid temporal change in the dominant frequencies of whistled notes of the primary call of *P. tricarunculata* at Monteverde. There has been a linear drop in the fundamental frequency of these notes from 5.58 kHz in 1974 to 3.72 kHz in 2000 (Kroodsma 2005). This rapid rate vocal change in a single population indicates that the mode of transmission is cultural rather than genetic (Payne *et al*. 1988; Noad *et al*. 2000; Kroodsma 2005). The frequency of the whistle notes of one individual male *P. tricarunculata* actually dropped 75 Hz in 1 year, which is very close to the population's average rate of change over 20 years (Kroodsma 2005).

As with whales, experimentally testing the hypothesis of song learning in *P. tricarunculata* with developmental deprivation studies would be extremely difficult. The nest of *P. tricarunculata* is undescribed (Stiles & Skutch 1989; Snow 2004), and the clutch size of other *Procnias* species is one (Snow 1970, 2004). Male *Procnias* take 5–7 years to fully mature (Snow 1973b; Powell & Bjork 2004), and *P. tricarunculata* is considered threatened throughout its range and is locally endangered in some areas (Powell & Bjork 2004). However, one compelling anecdotal observation of developmental deprivation comes from a young male bare-throated bellbird, *Procnias nudicollis* that was raised in captivity with a chopi blackbird (*Gnorimopsar chopi*) by an avicultural hobbyist in Brazil (Kroodsma 2005). This male *nudicollis* incorporated trills and whistles with acoustic structure and timing that were extremely similar to *Gnorimopsar* and completely unlike anything in the normal repertoire of this species (Kroodsma 2005).

In absence of deprivation experiments, we present an alternative test of whether vocal variation in *P. tricarunculata* is learned through cultural transmission, or genetically inherited within differentiated populations. Here, we examine whether patterns of behavioural variation are congruent with patterns of genetic variation among the four allopatric breeding populations and song type groups within *P. tricarunculata*. In other suboscine species, the striking vocal differences among *P. tricarunculata* song types would indicate the presence of distinct species (Isler *et al*. 1998; Whitney *et al*. 2000, but see contrary opinion in Raposo & Höfling 2003). Differentiated species should also be diagnosable by their genetic differentiation. In contrast, studies of oscines and parrots, which have vocal learning, document very limited correlation between genetic structure of populations and learned vocal dialects (Lougheed & Handford 1992; Zink & Barrowclough 1984; Wright & Wilkinson 2001; Soha *et al*. 2004; Wright *et al*. 2005; Nicholls *et al*. 2006). A correlation between genetic and vocal variation would not falsify vocal learning because mating preferences for learned vocal variations could evolutionarily reinforce genetic differentiation, but incongruence between genetic and vocal variation will generally contradict the hypothesis that song types are genetically

determined (unless song type is under strong selection; see below).

We examined genetic variation in 44 individuals from all four allopatric breeding populations of the threatened *P. tricarunculata*. We analysed a 500-bp fragment of the mitochondrial (mt)DNA control region and 11 nuclear microsatellite loci to investigate congruence between vocal and genetic variation. We performed phylogenetic and network analyses of the mtDNA variation, calculated F_{ST} values from both mtDNA and microsatellite data, examined the correlations between geographical distance, song type, and genetic variation, and performed Bayesian modelling of cryptic population structure in the microsatellite data set. To further examine the hypothesis that vocal variation is genetically determined, we also estimated the strength of selection necessary to maintain geographical variation in hypothetical song type alleles using Wright's (1931) migration–selection balance model. These genetic analyses provide independent support for the hypothesis that vocal variation in *P. tricarunculata* is a consequence of cultural evolution through vocal learning.

Materials and methods

Vocal sampling

Vocal recordings of male *Procnias tricarunculata* were collected during fieldwork by D.E.K. and colleagues in Costa Rica between 1998 and 2001, and near Matagalpa, Nicaragua in 2001. These samples were augmented by recordings from the Azuero Peninsula, Panamá by Robert Ridgely, and additional recordings from the Macaulay Library of Natural Sounds, Cornell University, and the Borror Laboratory of Natural Sounds, Ohio State University. *Procnias tricarunculata* has a repertoire of three main songs (Snow 1977; Kroodsma 2005). Sonograms of the vocalizations were made using RAVEN 1.2.1. Full analysis of the vocal variation of *P. tricarunculata* will appear elsewhere (Kroodsma *et al*. in preparation).

Genetic sampling

Specimens of contour feathers or blood of *P. tricarunculata* were collected by G.N.P., D.H., and collaborators during the breeding seasons of 1991–1995 from 1 female and 25 male individuals in Monteverde National Park in the Cordillera de Tilarán, Costa Rica (10°15′N, 84°46′W), and from 11 male individuals in 2000 in Las Tablas National Park in the Cordillera de Talamanca (~8°58′N, 82°49′W). *Procnias tricarunculata* were captured using canopy mist nets as part of a radio-tracking research program examining the breeding biology and migratory behaviour of the species (Powell & Bjork 2004). The song types of 20 of the 26-banded individuals in the vocally mixed Monteverde

population were observed and recorded during later observations. The Las Tablas population includes exclusively Talamacan song types (D.H.).

In addition, samples from Costa Rican populations were augmented by five frozen tissue specimens from males collected 10 km north of Matagalpa, Nicaragua (13°00.9′N, 85°55.4′W; Burke Museum Natural History, University of Washington), and two specimens from the southwestern Azuero Peninsula, Veraguas, Panamá (7°17′N, 80°43′W; Academy of Natural Sciences of Philadelphia). The Matagalpa, Nicaragua population is vocally uniform, and sings a distinct song type (Kroodsma 2005). Vocal recordings of males from the Azuero Peninsula, Panamá were made by R. S. Ridgely in 1996, and are presented here for the first time.

Molecular data

We extracted DNA from feathers using the DNeasy kit (QIAGEN) based on a protocol modified from Mundy *et al.* (1997), and from blood samples using EasyDNA kit (Invitrogen). We sequenced 500 bp of the rapidly evolving domain I (Baker & Marshall 1997) of the mitochondrial control region using primers Prca537R (5′-CTGACCGAG-GAACCAGAGG-3′) and RupiF (5′-GGTCTTGTAAACC-AAAGACTGAAG-3′) (modified from L547 and H16064 tRNA-Thr of Sorenson *et al.* 1999). Products were polymerase chain reaction (PCR) amplified in 50-µL volumes containing ~20–50 ng of DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3.5 mM MgCl$_2$, 200 µM dNTPs, 20 µg bovine serum albumin (BSA, New England BioLabs), 0.8 µM of each primer and 2 U of Ampli*Taq* Gold DNA polymerase (PE Applied Biosystems) and ultrapure water for volume. PCRs were performed on a PE 9700 thermocycler (PE Biosystems) with a profile of 94 °C for 10 min followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, followed by 10 min of extension at 72 °C. We sequenced both strands of the product using BigDye Terminator version 3.1 (PE Applied Biosystems) on an ABI PRISM 3700 DNA Sequencer running SEQUENCING ANALYSIS 3.7 (PE Applied Biosystems). Sequences were edited and aligned with the REALIGNER option using SEQUENCHER 4.6 (Gene Codes) and collapsed into haplotypes using MACCLADE 4.06 (Maddison & Maddison 2000).

We also amplified 11 polymorphic microsatellite loci using primers originally designed by Francisco *et al.* (2004) for *Chiroxiphia caudata* (Pipridae), which is in the sister family to Cotingidae (Prum 1990; Ericson *et al.* 2006). PCRs were performed on a PE 9700 thermocycler (PE Biosystems) and carried out in a 12.5-µL volume containing ~20–50 ng of DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3.5 mM MgCl$_2$, 200 µM dNTPs, 7.5 µg bovine serum albumin (BSA, New England BioLabs), 0.8 µM of each primer and 0.5 U of Ampli*Taq* Gold DNA polymerase (PE Applied Biosystems).

Reaction conditions for all primers were optimized using a two-step PCR, with the PCR product of the first step used as the template for the subsequent PCR. The reaction profile for both steps consisted of: 95 °C for 10 min, 30 or 35 cycles of 95 °C for 30 s, annealing for 30 s, and 72 °C for 45 s and a final step of 72 °C for 7 min. Annealing temperatures were 50 °C for both steps and no fluorescent dye (M13-FAM or M13-HEX; see below) were added in the first step.

All forward primers were 5′-tailed with the M13 sequence (5′-TCCCAGTCACGACGT-3′) and used in combination with an M13 primer of the same sequence but 5′-labelled with the fluorescent dye 6-FAM or 5HEX, to directly incorporate the fluorescent label into the resulting PCR amplicon and facilitate automated genotyping (Schuelke 2000). Alleles were visualized on 2% EtBr-stained agarose gels and analysed using an ABI PRISM 3700 DNA Sequencer (PE Applied Biosystems) with internal lane standards and GENESCAN 3.1 software (PE Applied Biosystems) for determining allele sizes. GENEPOP version 3.4 (Raymond & Rousset 1995) and ARLEQUIN version 3.01 (Excoffier *et al.* 2005) were used to test for deviation from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium between loci. Type I error rates for both tests were corrected for multiple comparisons using the sequential Bonferroni procedure (Rice 1989).

Genetic analyses

We constructed a control region haplotype network using TCS 1.21 (Clement *et al.* 2000) based on 99% statistical parsimony probability. We also used PAUP* 4.0b10 (Swofford 1993) to perform heuristic searches [TBR (tree-bisection–reconnection) branch swapping and 'as-is' stepwise addition sequence] on the collapsed control region haplotypes with both maximum-parsimony (MP; gaps treated as a new state) and maximum-likelihood (ML) criteria. We specified the ML parameters using the best-fit model of sequence evolution — HKY + G — obtained by performing hierarchical likelihood-ratio tests (LRT) on the 56 models specified in MODELTEST 3.7 (Posada & Crandall 2001), applied to our data set. In addition to these unconstrained searches, we report the likelihood scores and tree lengths, respectively, for the 50% majority-rule consensus topologies obtained from ML and MP searches that were constrained to include monophyletic clades based on geography or song type.

Seven of the 11 microsatellite loci designed for *C. caudata* (Francisco *et al.* 2004) amplified successfully and consistently for *P. tricarunculata* (Table 1). Three poorly amplifying loci — CHIR 1–16, CHIR 1–6, and CHIR 4–21 — were found to violate Hardy–Weinberg equilibrium conditions, probably because of the presence of null alleles, and they were excluded from analyses. One locus (CHIR4-33) exhibited little heterozygosity and so was excluded as well. All seven remaining loci were polymorphic with 2–11

Table 1 Polymorphic nuclear microsatellite markers analysed for 44 specimens from four breeding populations of the three-wattled bellbird *Procnias tricarunculata* (Cotingidae). Loci were designed by Francisco *et al.* (2004) for *Chiroxiphia caudata* (Pipridae)

Locus	Scoring success (%)	No. of alleles	H_O	H_E
CHIR1-18	100	11	0.82	0.84
CHIR2-9	91	11	0.50	0.63
CHIR3-2	98	3	0.14	0.13
CHIR3-15	95	5	0.34	0.36
CHIR3-22	80	7	0.28	0.36
CHIR3-27	100	6	0.30	0.33
CHIR4-34	100	2	0.36	0.33
Average	95	6	0.35	0.38

alleles per locus among the 44 individuals of *P. tricarunculata* analysed (Table 1). These seven loci were each in Hardy–Weinberg equilibrium and not in linkage disequilibrium with one another.

We calculated F_{ST} (Weir & Cockerham 1984) values for mtDNA and microsatellite variation between groups of individuals organized by geographical population and by song type. We also derived a matrix of pairwise microsatellite and mitochondrial genetic distances between all individuals based on D_{ps}, the proportion of shared alleles (Bowcock *et al.* 1994) and pairwise mtDNA differences using MICROSATELLITE ANALYSER (MSA) 4.0 (Dieringer & Schotterer 2003) and ARLEQUIN 3.01 (Excoffier *et al.* 2005), respectively. Correlations between interindividual genetic distances, interpopulation geographical distances, and song type (coded as a binary variable) were tested using the Mantel and partial Mantel tests implemented in ARLEQUIN version 3.01 (Excoffier *et al.* 2005). In the partial Mantel tests, song type was coded as a binary categorical variable: a value of 0 for pairs of individuals with the same song type, and a value of 1 for any pairs of individuals with different song types.

We investigated the presence of cryptic population structure in the microsatellite data among the geographical populations and song type groups using the Bayesian clustering program STRUCTURE 2.1 (Pritchard *et al.* 2000). To elucidate the most probable number of populations supported by our data, we ran 10 replicates with the assumed number of populations, *K*, ranging from 1 to 7, under all possible combination of ancestry (genetic admixture, no admixture, population information) and allele frequency model assumptions (correlated, or independent). The average log-likelihood (–ln L) values for each run were obtained using a Markov chain Monte Carlo (MCMC) chain length of 1 000 000 after an initial burn-in of 100 000 steps. We also computed the posterior probabilities using the mean –ln L values of all replicates for a given *K* and model assumptions, following Pritchard *et al.* (2000).

Estimating selection coefficients

An alternative explanation for a lack of concordance between vocal variation and neutral genetic variation is that the genetic variation for song type is under strong differential selection in different populations. To estimate the strength of natural selection on hypothesized alleles for different song types, we used the migration–selection balance model of Wright (1931 Hoekstra *et al.* 2004) which assumes an equilibrium between interpopulation migration and intrapopulation selection with stable population sizes. We estimated the number of migrants per generation (N_em) in two ways. Following Hoekstra *et al.* (2004), we first estimated effective population size (N_e) from the nucleotide diversity (Π) of the mtDNA data: $N_e = \Pi\mu$, using a neutral mutation rate of $\mu = 1 \times 10^{-6}$ per site per generation for mtDNA (Brown *et al.* 1982), and $\mu = 1 \times 10^{-3}$ for nuclear microsatellite loci (Goldstein & Schlotterer 1999). We then estimated the number of migrants per generation (*Nm*) from the mtDNA F_{ST} values following Hoekstra *et al.* (2004). Note that for mtDNA N_em is really female effective population size, N_fm_f, as mtDNA allows estimation of female demographics only. Alternatively, we used MIGRATE version 2.1.3 (Beerli & Felsenstein 1999) to obtain ML estimates of θ (*N*μ) and *Nm* for mtDNA. We used the Bayesian option to estimate θ and *Nm* for microsatellite loci. We optimized the parameter search with start parameters obtained using default settings. We estimated migration parameters under two different parameter assumptions: constant number of mutations per generation (θ), or constant migration rate (*m*).

Then, using the various estimates of migration, we calculated the selection coefficient, *s*, by setting the change in deleterious allele frequency in the population under consideration, Δ*q*, to 0, in equation 1 of Hoekstra *et al.* (2004):

$$\Delta q = \frac{-spq[q + h(p - q)]}{1 - sq(2hp + q)} + mQ - Mq$$

where *s* is the selection coefficient against the deleterious allele, *q* is the frequency of the deleterious allele in the population, *p* is the frequency of nondeleterious alleles (1 – *q*), *h* is the dominance coefficient, and *m* is the migration rate of individuals into the population, *Q* is the frequency of the deleterious allele outside the population, and *M* is the emigration rate of individuals out of the population. We estimated *s* assuming two different values of the dominance coefficient, $h = 1$ (complete dominance) and $h = 0.5$ (partial dominance). However, because only male individuals sing, only half of all individuals in the population would potentially be exposed to selection on vocalizations. Consequently, we also multiplied *h* by 0.5 in all calculations. We conservatively set the frequency of the nonlocal, 'deleterious' song allele, *q*, to 0.01.
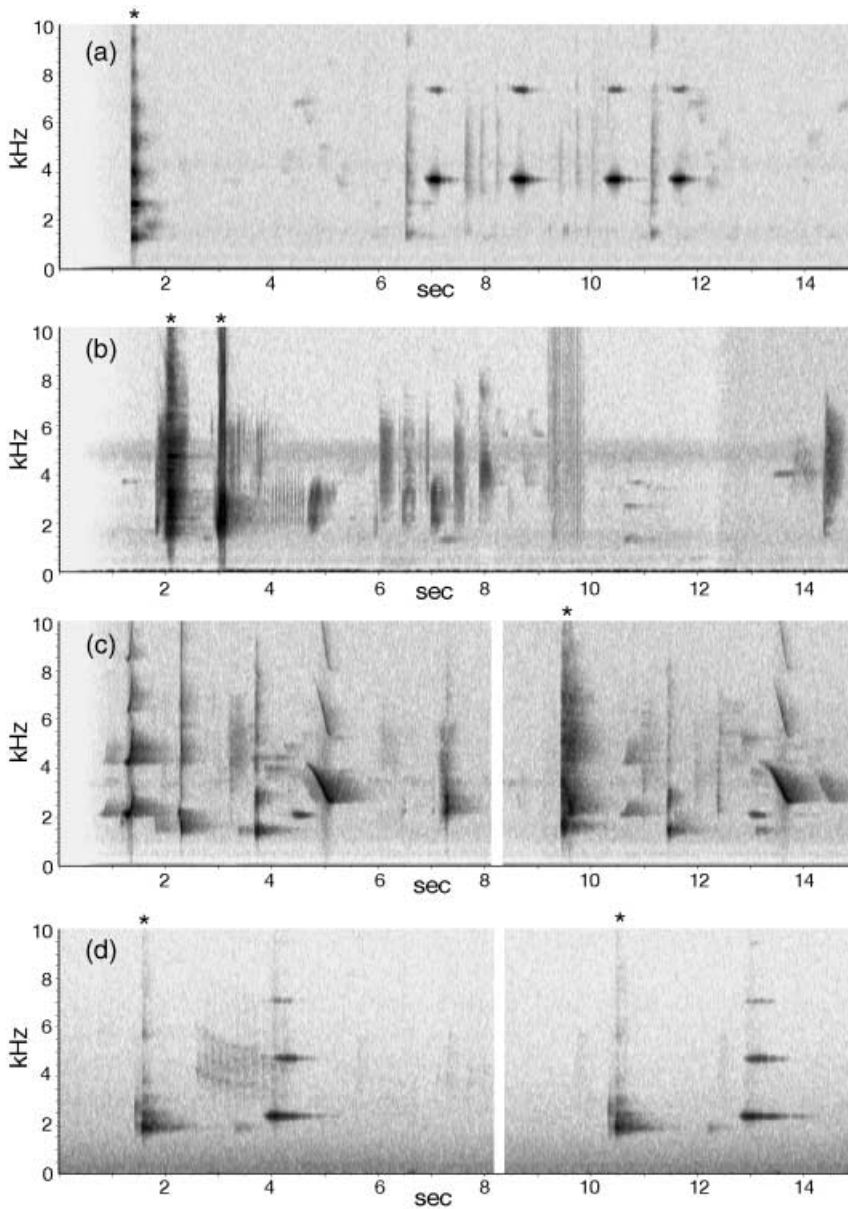
Fig. 3 Sonograms of four documented song types of three-wattled bellbird, *Procnias tricarunculata* (Cotingidae). (a) Monteverde song type (Monteverde, Costa Rica). (b) Talamancan song type (Monteverde, Costa Rica). (c) Nicaraguan song type (Matagalpa, Nicaragua). (d) Azuero song type (Cascajilloso, Azuero Peninsula, Panamá). Asterisks indicate the loud, ringing *Bonk, bock,* and *gbink* notes in the different song types.

Results

Vocalizations

In Monteverde, the vast majority of male *Procnias tricarunculata* sing the Monteverde song type (19 of 20 banded males with known song types) (Snow 1977; Kroodsma 2005). Individual repertoires include several songs, but all Monteverde songs are characterized by a loud, ringing initial *Bonk!* that is followed by a variety of complex swishing notes interspersed with one or more loud, pure tone whistles (Fig. 3a). Interestingly, the Monteverde song type appears to have changed qualitatively as well as quantitatively between 1974 (Snow 1977) and 1999–2001 (Kroodsma 2005).

All individuals in Las Tablas and two individuals in Monteverde (2 of 20 banded males with known song types) sang the 'Talamancan' song type ('Panamanian' of Kroodsma 2005). The Talamancan songs are characterized by an initial pair of squawking or quacking notes, with the second note higher in pitch, followed by a series of variable swishing notes (Fig. 3b). Although these birds do insert an occasional *bock* note, these notes are shorter and lack the loud ringing quality of the Monteverde *Bonk!* note.

The birds in Matagalpa region, Nicaragua and apparently southern Honduras have an entirely different 'Nicaraguan' song type (Fig. 3c). The Nicaraguan song type is dominated by phrases of slurred whistles with only occasional *bonk* notes with a chirpy quality.

The birds in the isolated breeding population at the southern end of the Azuero Peninsula sing the simplest songs (Fig. 3d). The 'Azuero' song type begins with a distinctive, loud, and higher pitched *gbink* with a brief introductory chirp, followed after a pause by a single pure tone at 2.3 kHz. The Azuero song type is most similar to, although simpler than, the Monteverde song type. However, the introductory note is higher in pitch and different in quality and the single unrepeated tone that follows is lower in pitch than the whistled notes of Monteverde. The Azuero song type is most different from the geographically closest Talamancan song type.

A single 'bilingual' male in our genetic sample at Monteverde (1 of 20 males with known song types) sang songs including both the Monteverde and Talamancan motifs (Kroodsma 2005). Interestingly, Snow (1977: Fig. 3i) documented a single bird (Male 5) that occasionally sang pure-whistled notes among its normal Monteverde songs. In acoustic structure, these notes are very similar to the whistled phrases found in the current Nicaraguan song type (Fig. 3c). Snow may have documented a bilingual Monteverde-Nicaraguan bird.

Mitochondrial DNA sequence data

We identified 17 different mtDNA control region haplotypes among the 44 individuals of *P. tricarunculata* examined. Eight haplotypes were shared by multiple individuals. A haplotype network shows the extensive haplotype sharing between individuals belonging to different geographical populations (Fig. 4). Five of the shared haplotypes were shared among individuals from different localities with distinct song types: two shared by Monteverde–Las Tablas, one by Nicaragua–Monteverde, one by Monteverde–Azuero Peninsula, and one by Nicaragua–Monteverde–Las Tablas (Fig. 4). Nicaraguan (dark grey) and Azuero (light grey) haplotypes are slightly segregated towards opposite, peripheral regions of the network, suggesting the preliminary stages of lineage sorting among populations, while the central Monteverde (white) and Las Tablas (black) individuals are widely distributed across the cluster implying continued genetic continuity. A haplotype network based on known song types showed similar structure (not shown).

The outgroup *Procnias alba* averaged 2.6% sequence divergence from all *P. tricarunculata* samples. Sequence divergence among the ingroup haplotypes was typically between 0.2% and 0.6%. The largest sequence divergence within the ingroup was just over 1% between one Nicaraguan haplotype and two of the other Nicaraguan haplotypes (one of which was also shared with Monteverde).

Phylogenetic analyses of the mtDNA control region data revealed that 17 of 500 bases were phylogenetically informative, but only eight of those sites were phylogenetically informative within the ingroup of *P. tricarunculata*
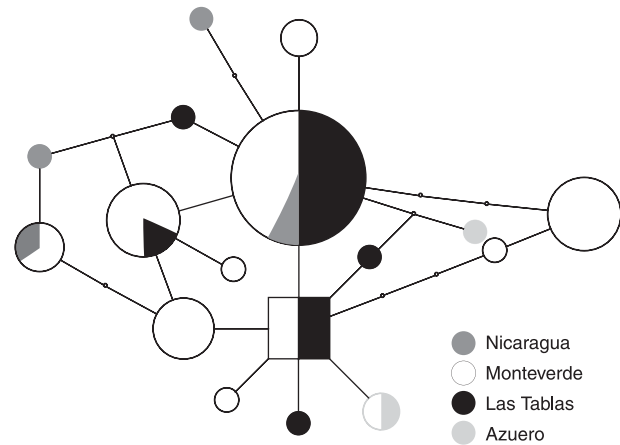

Fig. 4 Haplotype network of *Procnias tricarunculata* mtDNA control region sequences shows extensive haplotype sharing among different geographical populations. The size of circles represent the number of individuals sampled with that haplotype (from 1 to 10). The square represents the ancestral haplotype based on the relationship to the outgroup *Procnias alba* (not shown). Shading indicates the proportion of individuals of each haplotype from each sampled populations (shown in key). Each bar indicates a single nucleotide substitution. Small black dots denote unsampled haplotypes.

samples. Heuristic searches (with gaps coded as a new state) produced 1826 trees of length 32 (CI = 0.7143). The strict consensus tree identified only two resolved clades of two haplotypes each. The majority-rule consensus tree identified two additional clades of two haplotypes each and one large clade (found in 68% of the equally parsimonious trees) that includes all *P. tricarunculata* haplotypes except one Nicaraguan sample (Fig. 5). Four of the five supported clades included haplotypes of individuals from multiple localities and different song types. One of these clade included two haplotypes found only at Monteverde, but that locality included the largest number of ingroup samples. Using the HKY + G model of DNA evolution, maximum-likelihood analyses in PAUP identified only the same two clades that were supported in the strict consensus of the maximum-parsimony tree.

Additional phylogenetic analyses constraining geographical and behavioural groups to be monophyletic were performed to evaluate these alternative hypotheses. Assuming the monophyly of the four geographical populations or song types produces 25% or 53% increases in tree length, respectively, over the most parsimonious unconstrained tree. Likewise, assuming geographical or song type monophyly substantially decreases the log likelihood of the trees (from −827 in the unconstrained maximum-likelihood analysis to −860 and −877, respectively).

A Mantel test comparing interindividual mtDNA (pairwise) distances and geographical distances (kilometre) indicated that geographical distance explains only 2.9% of
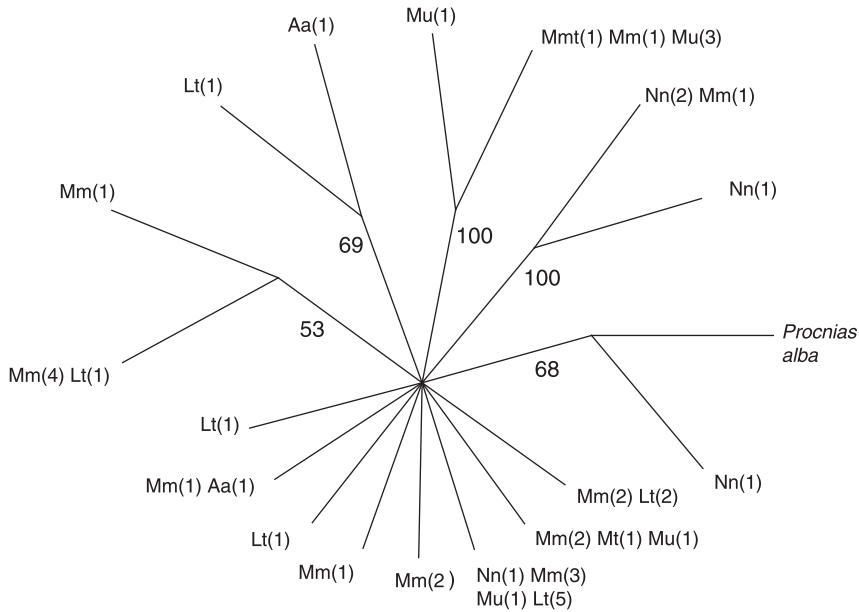
Fig. 5 Majority rule consensus tree for the 18 haplotypes found for a 500-bp sequence of the mitochondrial control region among the 44 three-wattled bellbirds *Procnias tricarunculata* and one white bellbird *Procnias alba* (Length = 32; CI = 0.7143). The five resolved clades are labelled with the percent of the 1826 equally parsimonious trees that include that clade. Haplotype labels indicate the locality (capital letters), song type (lowercase letters), and the number of individuals (in parentheses). There is no geographical or behaviour resolution to the mtDNA variation in the ingroup. Locality abbreviations: A, Azuero Peninsula, Panamá; L, Las Tablas, Cordillera de Talamanca, Costa Rica; M, Monteverde, Cordillera de Tilaran, Costa Rica; N, Matagalpa, Nicaragua. Song type abbreviations: a, Azuero; m, Monteverde; n, Nicaragua; t, Talamancan; and u, unknown Monteverde birds.

	N	Nicaragua	Monteverde	Talamancan	Azuero
Nicaragua	(5)	2.1333	3.1360	2.8182	4. 2000
Monteverde	(26)	**0.1157**	2.3077	2.0254	3.2400
Talamancan	(11)	**0.2845***	**0.0649**	1.0303	2.2727
Azuero	(2)	**0.1813**	**0.1176**	**0.1542**	1.3333

Table 2 Geographical population sample sizes (N), pairwise population mtDNA F_{ST} values (bold, below diagonal), and average pairwise genetic distances (in number of substitutions) (diagonal and above) for four breeding populations of the three-wattled bellbird *Procnias tricarunculata* (Cotingidae)

*F_{ST} values: significant; $P < 0.05$.

the variation in genetic distance (correlation coefficient, $r = 0.1719$), but that this relationship was significant ($P = 0.036$) (Table 2). However, a Mantel test of mtDNA distance and song types explained only 0.5% of the variation, and was not significant ($r = 0.0752$; $P = 0.153$). In a partial Mantel test examining the effect of song type on genetic distance while controlling for the effect of geographical distance, song type was only weakly correlated with genetic distance (partial correlation coefficient, $r = -0.1329$), and this association was not significant ($P = 0.989$).

The F_{ST} values calculated for mtDNA variation in *P. tricarunculata* show limited differentiation between the vocally distinct Monteverde and Las Tablas (0.0649), and values > 0.1 for all other population comparisons, with significant differentiation between Nicaragua and Las Tablas populations (0.2845) (Table 2).

In summary, there was a small but significant effect of isolation by distance on mtDNA genetic variation in *P. tricarunculata*, but there was no significant effect of song type on genetic variation. The mtDNA data show a weak association with geographical location, and no residual covariation with behavioural phenotype. Mitochondrial DNA haplotypes do not diagnose any monophyletic

geographical populations, and are not correlated with variation in vocal behaviour. The closest and best-sampled populations — Monteverde and Las Tablas — show strong vocal differentiation but low genetic differentiation.

Microsatellite loci

The amplification success of the seven nuclear loci for the 44 *P. tricarunculata* samples analysed was 95% (Table 1). Mean expected heterozygosity was 0.43 and observed heterozygosity was 0.39. All seven loci are in Hardy–Weinberg equilibrium (Table 1).

A Mantel test comparing the interindividual microsatellite genetic distances (D_{ps}, Table 3) and geographical distances (kilometre) indicated that geographical distance significantly explains 13.5% of the variation in genetic distance (correlation coefficient, $r = 0.3675$; $P < 0.001$). In a second Mantel test, song type significantly explains only 7.1% of the variation in genetic distance ($r = 0.2661$; $P < 0.001$). However, in a partial Mantel test controlling for the effect of geographical distance, song type was only weakly correlated with genetic distance (partial correlation coefficient, $r = -0.089$), and this association was not significant ($P =$

Table 3 Geographical population sample sizes (*N*), pairwise population F_{ST} values (bold, below diagonal), and average interindividual genetic distances (D_{ps}, diagonal and above) based on seven microsatellite loci for four breeding populations of the three-wattled bellbird *Procnias tricarunculata* (Cotingidae)

|	*N*	Nicaragua	Monteverde	Las Tablas	Azuero
Nicaragua	(5)	0.3850	0.4415	0.3848	0.5475
Monteverde	(26)	**0.0407**	0.3426	0.3506	0.6011
Las Tablas	(11)	**0.0028**	**0.0321***	0.3065	0.5736
Azuero	(2)	**−0.0048**	**0.2210***	**0.2615***	0.5

F_{ST} values: *significant; $P < 0.05$.

0. 93). Overall, there was a small but significant effect of isolation by distance on microsatellite variation in *P. tricarunculata*, but there was no residual effect of song type on genetic variation.

The microsatellite F_{ST} values between the Nicaragua, Monteverde, and Las Tablas were all low (< 0.04), and F_{ST} between Monteverde and Las Tablas (0.0321) was also statistically significant (Table 3). Thus, little of the observed microsatellite variation was explained by geography.

Bayesian analyses of the population structure in the microsatellite data using STRUCTURE identified a single population (K = 1) as the most likely partition of the genetic data with a large posterior probability under all combinations of assumptions (genetic admixture or no admixture, and correlated or uncorrelated allele frequencies). All other values of K from 2 to 7 had posterior probabilities close to 0. Under all combinations of assumptions, all specimens had essentially an equivalent probability of belonging to any particular subpopulation (Fig. 6a). Results of an alternative STRUCTURE analysis using only the larger samples from the Monteverde and Las Tablas populations yielded the same result (Fig. 6b).

In summary, microsatellite F_{ST} values indicated little geographical partitioning of genetic variation among the vocally distinct Nicaraguan, Monteverde, and Las Tablas populations.
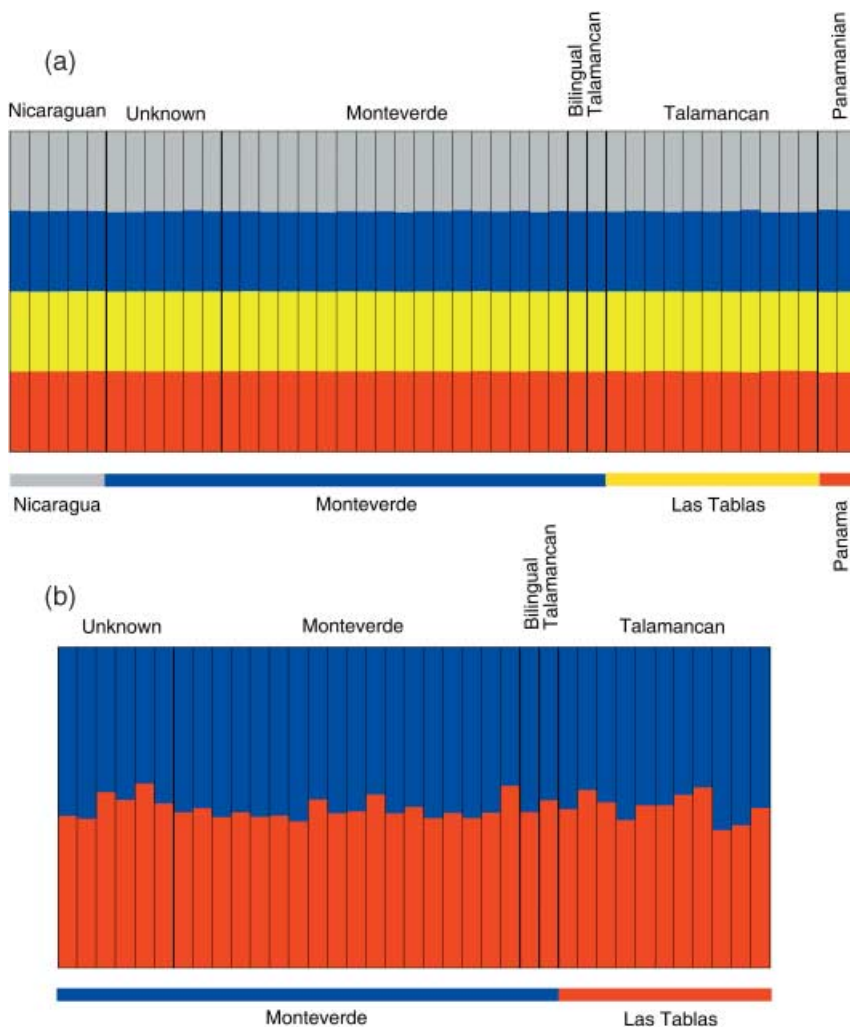
Fig. 6 Diagrams of the probabilities of population assignment of each individual estimated by the program STRUCTURE for (a) all microsatellite data assuming four subpopulations (K = 4), or the number of localities or song types, and (b) Monteverde and Las Tablas data only with K = 2. Each individual is represented by a vertical bar composed of coloured sections proportional to the probability of membership of that individual in each of the hypothesized subpopulations. Individuals are ordered by geographical locality from left to right (labelled at bottom and separated by thick lines), and by song type (labelled at top). All individuals are equally likely to be members of any population, documenting extensive genetic admixture without population substructure. Individuals with unusual songs for their locality (Talamancan or bilingual males at Monteverde) are not genetically distinct. The population structure with the highest likelihood is a single population [K = 1; for A, Pr(K) = 0.88; for B, Pr(K) = 0.63].

Table 4 Estimates of the selection coefficient, *s*, against nonlocal song types in Monteverde and Las Tablas populations using Wright's migration–selection balance model (Wright 1931; Hoekstra *et al.* 2004). *N*, sample size; N_e, effective population size; *s*, selection coefficient; and *h*, dominance coefficient (dominance: $h = 1$; semidominance: $h = 0.5$). Estimates of migration, *m* and *M*, are derived from both a symmetrical migration model using F_{ST} and the nucleotide diversity π, and from an asymmetrical migration model using MIGRATE (Beerli & Felsenstein 1999). MIGRATE analyses were conducted assuming variable *m* (shown) and variable N_e (not shown) with very similar results. The average 'deleterious' allele frequency for both populations were conservatively set to 0.01 (D.H. & D.E.K., unpublished observations). Asymmetric estimates of *s* for Las Tablas were not calculable because migration into Las Tablas was estimated as 0

Population	*N*	Data set	Migration model	N_e	*m*	*M*	*s*, $h = 1$	*s*, $h = 0.5$
Monteverde	26	mtDNA	symmetric	3310*	0.0022†	0.0022†	0.4291	0.8413
Monteverde	26	mtDNA	asymmetric	4600*	0.0016†	0.0000†	0.3097	0.6072
Las Tablas	11	mtDNA	symmetric	1460*	0.0049†	0.0049†	0.9675	1.8971
Monteverde	26	msat	asymmetric	3267	0.0012	0.0022	0.2518	0.4937
Las Tablas	11	msat	asymmetric	1973	0.0036	0.0021	0.7150	1.4021

*Effective female population size, N_f, for mtDNA.
†Female immigration, m_f and emigration, M_f rates for mtDNA loci.

Estimation of natural selection

An alternative explanation for the lack of congruence between neutral genetic variation and song type is (i) that song type is genetically determined by alleles that have not been assayed, and (ii) that genetic variation for song type is under strong, differential natural selection in different populations. To evaluate this hypothesis, we estimated the strength of natural selection that would be necessary to maintain the observed vocal differentiation between the Monteverde and Las Tablas populations assuming that the song types are determined genetically by alternative alleles at a single locus. Using Wright's migration–selection balance model (Wright 1931; Hoekstra *et al.* 2004), we estimated migration, *m*, using a symmetric model with F_{ST} and the nucleotide diversity (π), and with an asymmetric model using MIGRATE (Beerli & Felsenstein 1999).

Estimates of the selection coefficient, *s*, in the Monteverde population against a hypothesized Talamancan song type allele using mtDNA sequences and microsatellite loci with various alternative methods and dominance assumptions ranged from 0.2518 to 0.8413 (Table 4). Symmetric estimates of *s* in Las Tablas against the Monteverde song type were all extremely high (0.9675–1.8971), and could be inflated because of the overestimation of migration into Las Tablas resulting from the symmetrical method. However, asymmetric estimates of *s* in Las Tablas using microsatellites and MIGRATE were also extremely high (0.7150–1.4021) (Table 4).

The range of estimated *s* values in the Monteverde population against the Talamancan song type allele — 0.2518–0.8413 — are at high end of the range, or entirely exceed the range, of *s* values estimated for predation against integumentary colour polymorphisms in pocket mice (0.0002–0.39), peppered moths (0.19–0.33), ladybird beetles (0.1–0.67), and land snails (0.52–0.62) (reviewed by Hoekstra *et al.* 2004). The range of estimated *s* values in the Las Tablas populations are even more extreme — 0.7150 to undefined (>> 1). These estimates of natural selection on *P. tricarunculata* song types are unreasonably high. Because integumentary colour polymorphisms directly affect the probability of predator detection, natural selection on colour polymorphisms should be stronger than natural selection on song type in *P. tricarunculata*. All *P. tricarunculata* song types are extremely loud and include highly localizable, broad frequency syllable components (Fig. 3). Unlike integumentary colour polymorphisms, it is reasonable to hypothesize that the four *P. tricarunculata* song types can be detected with equal ease by potential predators. Furthermore, there is no obvious correlation between the vocal variation among *P. tricarunculata* song types and any environmental variable; males in all populations of *P. tricarunculata* call from emergent branches above biotically and structurally similar montane forest habitats. Therefore, it appears unlikely that natural selection for more efficient signal transmission in different acoustic environments could produce such strong selection coefficients in *P. tricarunculata*.

Lastly, a single Talamancan song type male at Monteverde called from the same territory for more than 20 years (D. Hamilton, G.V. N. Powell, unpublished observation), providing direct evidence against the hypothesis of extremely high natural selection against nonlocal song types there. We conclude that it is unlikely that the observed pattern of vocal variation among populations of *P. tricarunculata* is maintained by natural selection on genetic variation for song type. (The sexual selection alternative is discussed below).

Discussion

Barbara Snow (1970, 1973a, 1977) first suggested that the suboscine bellbirds may exhibit vocal learning like oscine passerines, and unlike other suboscines. Kroodsma (2005) documented a strong case for vocal learning in *Procnias tricarunculata* from field recordings. Because deprivation experiments on vocal learning in *P. tricarunculata* are not practical, we have tested the hypothesis of vocal learning in *P. tricarunculata* independently by examining whether genetic variation is concordant with vocal variation in the species.

Although our results depend upon some small sample sizes (unfortunately unavoidable for this threatened, rainforest canopy bird), and incorporate many simplifying assumptions, our analyses of the genetic structure and vocal behaviour of four allopatric breeding populations of *P. tricarunculata* indicate a lack of concordance between genetic and behavioural variation. The mtDNA control region data showed extensive haplotype sharing among localities and song types, little phylogenetic resolution, and little geographical structure (Figs 4 and 5). F_{ST} values for mtDNA variation show some significant isolation by distance, but no significant relationship between genetic distance and song type. Microsatellite distances indicate a small but significant effect of geographical distance, but no significant residual effect of song type. Microsatellite F_{ST} values were all quite low (< 0.05), except for those for the tiny sample from Azuero population (Table 3). Lastly, a Bayesian analysis of the genetic structure in the microsatellite data indicates that the most probable partition of the data is a single population (K = 1) with a high posterior probability, and all other population partitions (K > 1) have near zero posterior probabilities (Fig. 6).

We explicitly tested the alternative hypothesis that geographical variation in song is maintained by natural selection on genetic variation for song type despite weak population differentiation. We estimated the strength of selection required to produce the observed patterns of geographical variation if song types were determined by alleles at a single locus. The estimates of the selection coefficient, *s*, varied from the high end of the range to above the entire range of estimates of natural selection on colour polymorphisms for a range of vertebrate and invertebrate species (Hoekstra *et al.* 2004). Because natural selection on these antipredator colour polymorphisms should be much stronger that natural selection on the vocal variation in *P. tricarunculata*, these high estimates of selection further discredit the genetic basis for vocal variation in this species. Acoustic adaptation among the song types of *P. tricarunculata* to variations in the acoustic environment is also unlikely. Males of all populations sing from acoustically similar positions: dead, broken-off branches of emergent trees above the canopy of montane forests (Fig. 1).

Another reasonable, alternative hypothesis is that genetic variation for song types in *P. tricarunculata* is under strong sexual selection. *Procnias tricarunculata* is a lekking species, and sexual selection on male vocal advertisement is likely to be very high. However, other lekking suboscines with similar breeding systems and loud, vocal advertisement do not show similar geographical patterns of vocal variation. For example, the closely related, lekking, and extremely loud screaming piha *Lipaugus vociferans* shows extensive vocal uniformity across most of the lowland tropical forests of South America (R. O. Prum, unpublished data). Strong sexual selection may play a role in the rapidity of cultural evolution in vocal behaviour of *P. tricarunculata*, but strong sexual selection on genetic variation for song is unlikely to explain the observed pattern of geographical variation in song type in *P. tricarunculata*. Apparently, vocal evolution in other lekking suboscines may be constrained by the limited genetic variation for vocal behaviour.

Our genetic data support the hypothesis that song types in *P. tricarunculata* are learned from conspecific males rather than genetically transmitted from parents, and that variation in song types constitute culturally transmitted, geographically variable dialects. Similar genetic data have supported vocal learning in chimpanzees (Crockford *et al.* 2004). Furthermore, Kroodsma's (2005) data demonstrating rapid temporal change in vocal behaviour within a population of *P. tricarunculata* from Monteverde are comparable to the evidence used to support vocal learning in great whales (Noad *et al.* 2000). Thus, a combination of field recordings and these genetic data document a novel origin of vocal learning within the suboscine passerine clade that generally lacks vocal learning (Kroodsma 1984, 1985; Kroodsma & Konishi 1991). Now, a substantial body of evidence from multiple sources support vocal learning in *P. tricarunculata*.

Kroodsma (2005) documents ongoing learning and cultural evolution within a song type in a population during the lives of individual birds. Apparently, *P. tricarunculata* is among the very few avian species that can continue to learn and change vocal behaviour throughout its life (e.g. canary, *Serinus canaria*). In contrast, one individual *P. tricarunculata* male has sung the Talamanca song type at Monteverde for decades, indicating some individual vocal persistence even after years of exposure to another song type. Clearly, more research is needed on song learning processes in *P. tricarunculata*.

Phylogenetic limits of vocal learning in suboscines

Given evidence of vocal learning in *P. tricarunculata*, how broadly distributed is vocal learning within the cotingas and other suboscines? Some components of the vocal learning capacity of *P. tricarunculata* appear to be present

in other *Procnias*. All *Procnias* species exhibit similarly conspicuous vocal ontogeny, and a captive-raised *Procnias nudicollis* has evidently incorporated songs of a chopi blackbird into its vocal repertoire (Kroodsma 2005). Even without comparably detailed analyses, however, it is clear that geographical and interspecific variation in song of other *Procnias* species do not reach the level of the explosive regional vocal novelty found in *P. tricarunculata*. Snow (1970) documented that one element in the vocal repertoire of continental populations of *Procnias averano* is missing from the island population on Trinidad, and that this loss may have occurred since the late 19th century. However, mainland and Trinidad song types are otherwise highly comparable. Furthermore, songs of the sister species *P. averano* and *P. nudicollis* share strikingly similarities (e.g. a long accelerated series of similar *bong!* notes) that are greater than those shared by song types of *P. tricarunculata*. Although some components of vocal learning capacity are apparently shared by all *Procnias,* the process of cultural evolution in vocal behaviour that produces dialects is apparently unique to *P. tricarunculata* within the genus. It is unknown what constrains cultural evolution in other members of the genus.

All other species of cotingas have highly stereotyped vocal behaviour and limited geographical variation within a species like typical suboscines (Snow 1982; Ridgely & Tudor 1994). Although experimental data are scant, extensive song learning is known to be, or thought to be, absent in all other suboscine birds (Kroodsma 1984, 1985; Kroodsma & Konishi 1991; Gahr *et al*. 1993). Furthermore, the genus *Procnias* is phylogenetically embedded within the central, core clade of Cotingidae (Prum *et al*. 2000; Ohlson *et al*. 2007), and the cotingas are themselves phylogenetically embedded within suboscine birds (Barker *et al*. 2004; Ericson *et al*. 2006). Thus, the hypothesis that song-learning capacity in *Procnias* is phylogenetically plesiomorphic and retained from a shared common ancestor with the song-learning oscine clade is highly unparsimonious.

The only evidence of limited song learning in the suboscines comes from the long-tailed manakin (*Chiroxiphia linearis,* Trainer & McDonald 1993; Trainer *et al*. 2002). Duetting male *C. linearis* match the frequency of their social partners after years of cooperative display. This duetting behaviour is unique to *Chiroxiphia*, and this potentially learned component of vocal behaviour is likely to be unique to its unusual obligate cooperative lek breeding system. There is also good evidence of strong genetic basis for other song variables in *Chiroxiphia.* The advertisement songs of the all *Chiroxiphia* manakins are highly stereotyped and recognizable (Ridgely & Gwynne 1989; Ridgely & Tudor 1994). Furthermore, a natural intergeneric hybrid between *Chiroxiphia caudata* and *Antilophia galeata* had a strikingly intermediate song that included obvious vocal

elements of both parental species (Archivo Sonoro Neotropical, UNICAMP, Campinas, Brazil).

Capacity for extensive vocal learning in suboscines appears to be limited to the genus *Procnias.* Within *Procnias*, dynamic cultural evolution in vocal behaviour and dialects appear to be unique to *P. tricarunculata*. Because of their unusual ecology, natural history, and their threatened status, *P. tricarunculata* will never become a model, laboratory organism for the study of song learning. However, for purposes of understanding the comparative evolutionary origins of vocal learning, it is important to recognize all evolutionarily independent examples of vocal learning — such as great whales and *Procnias* bellbirds — even if they cannot be tested practically with developmental deprivation experiments in the laboratory.

Conservation of Procnias tricarunculata

Procnias tricarunculata is a threatened species throughout its range (Powell & Bjork 2004), and the Azuero Peninsula breeding population may be endangered. The estimates of effective female population size, N_f, from the mtDNA data indicate that substantial genetic variation remains: 3310–4600 for Monteverde and 1460 for Las Tablas (Table 4). The microsatellite estimates of total effective population size, N_e including males and females, are very similar: 3267 for Monteverde, 1973 for Las Tablas. Interestingly, the lower-than-expected effective population size for males and females may not be due to error, but could reflect the substantial effects of sexual selection reducing the effective male population size, N_m, in the species.

Although the small sample sizes from the Nicaraguan and Azuero breeding populations limit the power of our conclusions, current data indicate that the majority of genetic variation in *P. tricarunculata* is within, not among, the four breeding populations (Tables 2 and 3). The existence of genetic continuity among breeding populations is good, but it is important to realize that the population numbers are low and that the montane forest breeding habitat and lowland forest wintering habitats are increasingly fragmented. Furthermore, these birds are very long lived, and recent habitat change has been extremely rapid compared to average generation time in these populations. These relatively optimistic data may not yet reflect the genetic effects of current habitat and population fragmentation.

The biology of *P. tricarunculata* creates complexities for its conservation. *Procnias tricarunculata* is an obligate frugivore, and has a complex seasonal migratory cycle (Powell & Bjork 2004). All populations nest in highland areas, and migrate down to the lowlands during the non-breeding season (Slud 1964; Wetmore 1972; Ridgely & Gwynne 1989). Recently, Powell & Bjork (2004) established that, after breeding at mid-elevations on the Pacific slopes

of the Cordillera de Tilarán, the Monteverde population migrates down to the lowland Atlantic forest. After several months there, they migrate again over the Cordillera to the Pacific lowlands, where they remain for several more months before returning to the breeding grounds. This complex annual migration means that *P. tricarunculata* will require multiple ecosystem size protected areas in the central highlands, and the Atlantic and Pacific lowlands in order to maintain its life history.

It is unknown whether the migratory natural history of the species facilitates gene flow among breeding populations. Regardless, our still quite limited results indicate that even the most isolated and possibly endangered population in the Azuero Peninsula is not very distinct genetically, and that successful conservation of the Tilarán population would retain most of the genetic variation of the whole species.

The existence of distinct cultural phenotypes in each population raises the interesting question of cultural conservation. As with human linguistic diversity, avian cultural diversity provides an additional criterion for assessment of conservation priorities. The cultural diversity of isolated populations, like those of the Azuero Peninsula, provides further justification of high conservation status.

Acknowledgements

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