Molecular Ecology (2007) **16**, 3634–3642 doi: 10.1111/j.1365-294X.2007.03422.x

Ancient differentiation in the single-island avian radiation of endemic Hispaniolan chat-tanagers (Aves: *Calyptophilus*)

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Abstract

The simple geographic structure of island systems often makes them tractable for studies of the patterns and processes of biological diversification. The *Calyptophilus* chat-tanagers of Hispaniola are of general evolutionary interest because their multiple lineages might have arisen on a single island, of conservation concern because several isolated populations are nearly extinct, and taxonomically ambiguous because they have been variously lumped or split into one to four species. To explore the context of diversification of the seven extant *Calyptophilus* populations, we conducted a multilocus coalescent analysis based on sequences of mitochondrial ND2 and three nuclear intron loci. We then compared patterns of phylogeographic genetic variation with the morphological differences that distinguish these populations. Mitochondrial haplotypes formed two reciprocally monophyletic groups separated by a large magnitude of nucleotide divergence. Intron structure largely paralleled the geographic grouping pattern of the mitochondrial DNA (mtDNA), but these groups were only reciprocally monophyletic at one of the three introns. Also, the magnitude of between-group divergence was much lower in the introns than mtDNA genealogies. Multilocus coalescent analyses inferred a nonzero divergence time between these two major geographic groups, but suggested that they have experienced a low level of gene flow. All four markers showed substantial allele sharing within each of the two groups, demonstrating that many now separated montane populations do not have long histories of isolation. Considered in concert, our multilocus phylogeographic reconstructions support the recognition of two species within the *Calyptophilus* complex, and raise the possibility that these taxa differentiated prior to the fusion of the two palaeo-islands that form present-day Hispaniola.

Keywords: *Calyptophilus*, chat-tanager, Hispaniola, island, phylogeography, speciation

Received 5 January 2007; revision accepted 10 May 2007

Introduction

Many oceanic archipelagos have been the setting of spectacular avian radiations (James & Olson 1991; Sato *et al*. 1999), but avian speciation *within* single islands appears to be extremely unusual. Although the existence of endemic sister species on remote islands has provided rare evidence for sympatric speciation in other taxa (Savolainen *et al*. 2006), a worldwide survey of small-island avifaunas failed to identify any single-island sister taxa that might have arisen sympatrically (Coyne & Price 2000). Mayr (1963) suggested that birds cannot speciate in sympatry, requiring geographic allopatry as a necessary precursor to differentiation. Following similar logic, Diamond (1977) suggested that endemic sister species of birds do not occur on single islands smaller than New Guinea.

Hispaniola is one of the smallest islands with multiple endemic sister pairs of birds, including the two or more potential species of chat-tanagers that we consider in this study (*Calyptophilus tertius* and *C. frugivorus*), as well as the todies (*Todus angustirostris* and *T. subulatus*), the palm-tanagers (*Phaenicophilus palmarum* and *P. poliocephalus*) and the green-tailed and white-winged warblers (*Microligea*

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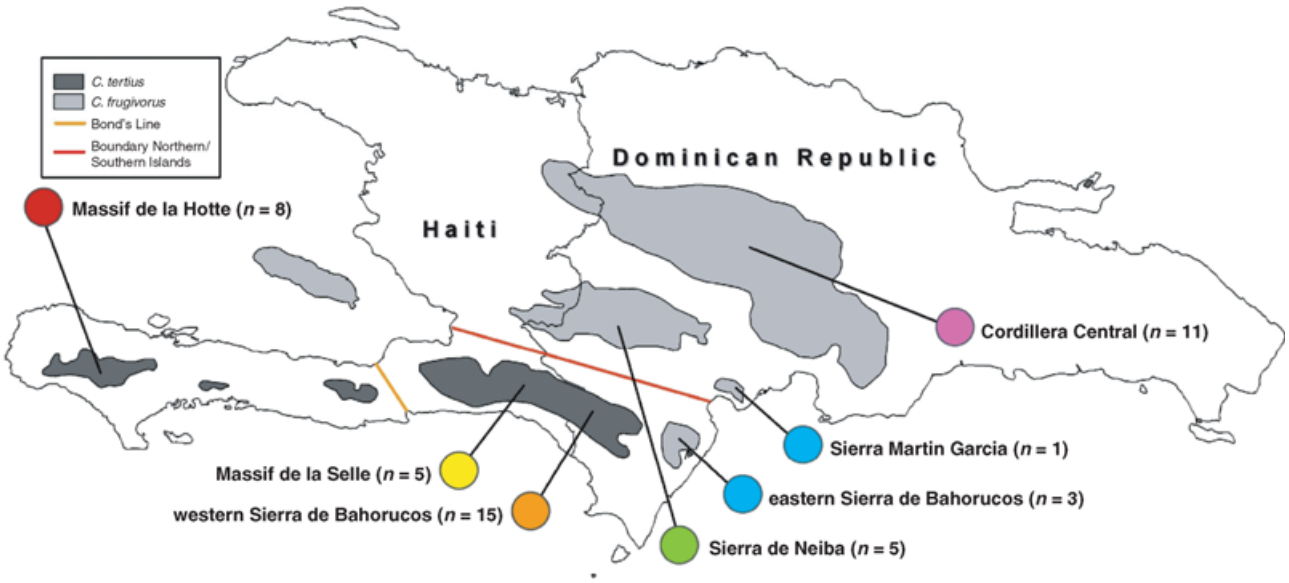

Fig. 1 Distribution of chat-tanagers on the disjunct mountain ranges of Hispaniola. *Calyptophilus tertius* populations shown in red (Massif de la Hotte; *C. t. tertius*), yellow (Massif de la Selle; *C. t. selleanus*) and orange (Western Sierra de Bahoruco; *C. t. selleanus*); *C. frugivorus* populations shown in blue (Eastern Sierra de Bahoruco and Sierra Martin Garcia; forms unnamed), green (Sierra de Neiba; *C. f. neibae*) and purple (Cordillera Central; *C. f. frugivorus*). Number of samples collected from each population is indicated. The line separating the 'northern/southern Island' indicates where the southern palaeo-island merged with the northern palaeo-island in the mid-Miocene. 'Bond's line' indicates a probable ancient ocean channel that closed in the Pleistocene.

palustris and *Xenoligea montana*). The presence of likely sister taxa on Hispaniola raises the question of whether these avian lineages arose *in situ* (and potentially in sympatry) or whether they reflect a more complex origin involving periods of within-island allopatry.

Although sympatric speciation in these Hispaniolan taxa is an intriguing possibility, the island's complex geological and biogeographic history has provided many opportunities for allopatric divergence (Fig. 1). First, the single modern island of Hispaniola was formed by the mid-Miocene fusion of separate northern and southern palaeo-islands (Graham 2003). Their corresponding landmasses have subsequently remained separated by a deep rift, the Neiba Valley, which was inundated repeatedly over the Pleistocene during periods of high sea level (Keith *et al*. 2003). At present, the Neiba depression is an arid plain dotted with large saline lakes. Second, the Haitian Peninsula of the south palaeo-island was itself bisected by a wide sea channel prior to the late Pleistocene. This channel would have separated the Massif de la Hotte in the west from the Massif de la Selle in the east: the resulting valley that now divides these mountain ranges represents a biogeographic breakpoint sometimes termed 'Bond's Line' (Keith *et al*. 2003). Finally, the topography of Hispaniola includes a series of parallel mountain ranges dissected by deep xeric valleys. There are three mountain ranges in each palaeo-island region, each with their own *Calyptophilus* population: the Massif de la Hotte, the Massif de la Selle and the Sierra de Bahoruco to the south; as well as the Sierra Martin Garcia, the Sierra de Neiba and the Cordillera Central to the north. This topography likely limits present-day dispersal of birds and other taxa restricted to high-elevation forests. However, dispersal across these valleys may have been more feasible during Pleistocene cold periods when high elevations were glaciated and the island's vegetational life zones were shifted to lower elevations (Schubert & Medina 1982).

Hispaniola supports endemic radiations of many taxa in addition to birds, but comprehensive phylogeographic studies have been conducted only for two groups of lizards: an *Anolis* species complex (Glor *et al*. 2003) and an *Ameiva* species complex (Gifford *et al*. 2004). Owing in part to their different elevational and habitat associations, these two lizard groups have dissimilar phylogeographic patterns within Hispaniola, but both are composed of a set of geographically structured populations separated by substantial mitochondrial DNA (mtDNA) divergence. The distributions of divergent lineages in both groups are associated with known biogeographic and topographic features, demonstrating the likelihood of allopatric differentiation in these lizard taxa. Although the greater dispersal ability of birds such as the *Calyptophilus* chat-tanagers might reduce phylogeographic structuring across the same landscape, the single published molecular study of

Calyptophilus found substantial mitochondrial cytochrome *b* divergence between two individuals from different mountain ranges (Klein 1999), indicating mitochondrial differentiation among at least some of the extant *Calyptophilus* populations.

The seven extant *Calyptophilus* populations are currently restricted to the high elevations of six mountain ranges (Fig. 1). These populations are known to vary substantially in body size and plumage characters (Bond 1956; Dod 1992; Woods & Ottenwalder 1992; BirdLife International 2000; Rimmer *et al*. 2005), which has led to long-standing speculation about their biogeographic history and magnitude of evolutionary differentiation (Wetmore & Swales 1931; Klein 1999; Keith *et al*. 2003). With two populations extinct and three more restricted to highly vulnerable forest remnants, the entire *Calyptophilus* complex is considered 'critically endangered' (Latta *et al*. 2006) and is ranked as 'vulnerable' by the International Union for Conservation of Nature and Natural Resources (IUCN). Clarification of the degree of evolutionary distinctiveness among populations may increase conservation rankings for the most at-risk populations (BirdLife International 2000).

Here, we investigate the historical pattern of diversification among all extant populations of *Calyptophilus*, comparing gene genealogies for one mitochondrial and three nuclear loci with patterns of morphological variation. Congruent breakpoints in the genealogical networks from these four unlinked loci allow us to identify the major phylogeographic groups within the *Calyptophilus* radiation and to evaluate the evolutionary independence of several populations at high risk of extinction. We use a multilocus, coalescent-based analysis to compare the history of lineage divergence between populations of the two currently recognized species of chat-tanagers. The goals of this study were (i) to determine the number of taxonomic lineages of chat-tanagers, which has been ambiguous because they have been variously lumped or split into one to four species; (ii) to determine, through the timing and pattern of lineage divergence, the likelihood of within-island speciation; and (iii) to identify genetically distinct populations at high extinction risk.

Materials and methods

Sampling and field measurements

Two species of chat-tanagers are currently recognized (A.O.U. 1998): *Calyptophilus tertius* and *C. frugivorus*. There are two extant forms named within each of these species, based on qualitative morphological distinctions (Wetmore & Swales 1931; Bond 1956), and these forms vary in conservation status. Within *C. tertius*, the single population of the nominate *C. t. tertius* is endemic to the Massif de La Hotte in the western Haitian peninsula, where it is critically endangered by forest habitat loss (Rimmer *et al*. 2005). The second form of *C. tertius*, *C. t. selleanus*, is represented by two populations: the first, in the Massif de la Selle of Haiti, is also critically endangered by habitat loss (Rimmer *et al*. 2005; CC Rimmer, personal communication), while the second, in the western Sierra de Bahoruco of the Dominican Republic, is locally common in a relatively well-protected national park (Latta *et al*. 2003). Within *C. frugivorus*, the single population of the nominate *C. f. frugivorus* is represented by a population in the relatively well-protected park of the Cordillera Central (AK Townsend, personal observation), while the second form, *C. f. neibae*, is critically endangered by habitat loss, represented by a single population in the Sierra de Neiba (Rimmer *et al*. 1998; Keith *et al*. 2003; Rimmer *et al*. 2003). Two extant populations within the range of *C. frugivorus* have never been categorized to form — those of Sierra Martin Garcia and of eastern Sierra de Bahoruco (Keith *et al*. 2003). There were also two lowland forms that may now be extinct. The paler and smaller form of Haiti's Ile de la Gonave (*C. f. abbotti*; Wetmore & Swales 1931) has not been recorded in recent years (Raffaele *et al*. 1998), and a population of *C. f. frugivorus* in the Samaná Peninsula of the northern Dominican Republic has not been detected since the 1960s, in spite of concerted efforts to relocate it there (Dod 1992).

As part of a broader field survey of Hispaniolan avian diversity during 2002–2006, we captured, measured and obtained blood samples from *Calyptophilus* individuals from all of the seven extant geographic populations (Fig. 1). Birds were captured in arrays of 32–36 mm mist nets. Sample sizes at each site varied (Fig. 1), owing to variability in sampling intensity. All birds were marked with permanent, individually numbered leg bands. From 46 of the 48 individuals sampled in total, we measured seven morphological characters: exposed culmen length (from top of bill to tip), bill length (from distal end of nares to tip), bill width and depth (measured at distal end of nostrils) to the nearest 0.1 mm, wing length (unflattened chord), tail length to the nearest 0.5 mm and weight to the nearest 0.5 g. To improve consistency, all measurements were taken or directly supervised by AKT and/or CCR.

From each individual we collected approximately 80 μL of blood in heparinized capillary tubes via brachial venipuncture, using sterile 27-gauge hypodermic needles. Blood samples were stored in 0.5 mL of blood lysis buffer [100 mM Tris–HCl, pH 8; 100 mM Na2 EDTA, 10 mM NaCl, 0.5% SDS; (White & Densmore 1992)]. The individuals sampled for this study were not collected and vouchered as museum specimens, in part because they were captured as part of an ongoing, multispecies, mark–recapture field study of demography and survivorship, and also because of concerns for their critical conservation status.

Laboratory methods

DNAs were extracted from blood samples using Perfect gDNA Blood Mini kits (Eppendorf) following the manufacturer's protocol. Because chat-tanagers are sexually monomorphic, we sexed all individuals genetically at diagnostic sex-linked alleles, using the P8/P2 primer set (Griffiths *et al*. 1998). Sexes were readily distinguishable by consistent length differences between the W and Z alleles.

From each of the 48 individuals, we amplified and sequenced 827 nucleotides of the mitochondrially encoded ND2 gene and three nuclear introns, using primers and protocols described in Lovette & Rubenstein (in press). We initially screened seven nuclear introns for successful amplification on five individual chat-tanagers: beta-fibrinogen intron 5 (hereafter Fib-5), the Z-linked aconitase 1 gene (hereafter Aco-9), intron 4 of the Z-linked muscle-specific tyrosine kinase (hereafter Musk-4), beta-fibrogen intron 7 (Prychitko & Moore 1997), transforming growth factor beta-2 intron, rhodopsin intron 1 (Primmer *et al*. 2002), and myoglobin intron 2 (Slade *et al*. 1993). Amplification primers of the first three introns were designed by F. K. Barker (personal communication). We chose these three introns for further work because they amplified successfully for all individuals, while Polymerase chain reaction (PCR) amplification was poor at the other four introns.

When intron sequences were heterozygotic at more than one nucleotide position, they were cloned using the pGEM-T Easy TA cloning kit (Promega, Madison, WI) to obtain clean sequences of the individual alleles. Three or more independent clones per sample were purified with the Sigma GenElute Plasmid Miniprep Kit (Sigma, St. Louis, MO) and then sequenced using the relevant locus-specific primers.

Molecular analyses

All intron sequences were resolved as individual alleles. Two intron alleles per individual were included in analyses, regardless of whether individuals were homo- or heterozygous, except for the Aco-9 and Musk-4 introns: females have only one copy of these Z-linked loci, and thus only the single allele was included for females. The resulting mtDNA haplotypes or intron-allele sequences were aligned and imported into PAUP* (Swofford 2000) for analysis of nucleotide variation and preliminary phylogenetic analysis. To facilitate comparisons with the many previous studies based on genetic distances derived from passerine mtDNA divergence, ND2-sequence divergence was calculated using two metrics: uncorrected; and under a K2+G model with the alpha parameter set at 0.25 and all sites considered variable.

Relationships among mitochondrial haplotypes or intron alleles were estimated using the statistical parsimony approach implemented in the program TCS 1.21 (Clement *et al*. 2000). Owing to the high similarity of many haplotypes or alleles at each locus, we did not reconstruct relationships using standard tree-based methods that force all observed variants to the termini, because it is probable that our datasets contain many haplotypes/alleles that are ancestral to other observed haplotypes/alleles. Likewise, we did not employ nested-clade approaches (Templeton *et al*. 1995; Templeton 1998) because the uneven sampling across populations — and particularly the small sample sizes from some endangered populations — render the resulting inferences suspect: a common haplotype could easily be missing from most of the populations solely due to a sampling artefact.

We used the isolation with migration (IM) program for multilocus coalescent analysis (Nielsen & Wakely 2001; Hey & Nielsen 2004) to compare the history of lineage divergence among populations of the two currently recognized species of chat-tanagers: *C. tertius* and *C. frugivorus*. The IM program assumes that each locus under consideration is free from recombination and selectively neutral. To determine the minimum number of recombination events in the history of each locus, we performed the four-gamete test using DnaSP 4.0 (Rozas *et al*. 2003). When there was evidence of recombination, we arbitrarily selected the 5′-most recombination-free segment of sequence with two or more segregating sites at that locus. We tested these segments for neutrality by performing the Hudson–Kreitman–Aguade test (HKA) (Hudson *et al*. 1987) and calculated F_{ST} values between lineages using DnaSP 4.0 (Rozas *et al*. 2003).

We fitted the IM model to our recombination-free segments of sequence data using the infinite-sites model. We ran five preliminary ($5*10^7$ steps) metropolis coupled Monte Carlo Markov chain simulations to optimize the duration of burn-in, heating mode and parameters, number of chains and parameter bounds. Our three final simulations were carried using $1*10^8$ steps with a burn-in of $1*10^7$ steps and a linear heat mode with eight chains. Estimated priors for mutation rate ranged from 0.16 to 0.25% per million years for nuclear introns and 1–3% per million years for ND2. IM converted the time parameter to divergence time in years using the geometric mean of estimated sequence divergence at all loci. Marginal posterior density distributions for all parameters were convergent in the final runs with different seed numbers.

Morphological analyses

A scatterplot matrix indicated positive correlations among all of our seven morphometric variables. Using principal components analysis (PCA), we reduced our

variables to a smaller number of meaningful components by a scree test (Cattell 1966) and the eigenvalue > 1 criterion (Kaiser 1960). We compared size differences between sexes with an independent-samples, one-tailed *t*-test, and we examined size differences among sites, while controlling for sex, with a two-way ANOVA on PC1 and PC2 scores with site and sex as between-group factors. When main effects were significant, we used Tukey's HSD multiple comparison test to determine which levels were different. All morphological analyses were performed using JMP 5.1 for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Mitochondrial and intron structure

Mitochondrial variation in *Calyptophilus* is highly but simply structured, with two clusters of nearly identical haplotypes separated by substantial genetic divergence (Fig. 2a). One haplotype cluster includes the three *Calyptophilus tertius* populations, among which the maximal ND2 divergence is 10 nucleotide substitutions (1.2% uncorrected). The other cluster includes the four populations of *C. frugivorus*, among which the maximum divergence is 12 substitutions (1.5%). The two clusters are separated by 97–106 nucleotide substitutions in our fragment, equivalent to 11.8–12.9% uncorrected ND2 sequence divergence, or 21.7–25.9% when divergence is calculated under the K2P+G model that better accounts for multiple substitutions at nucleotide sites.

Intron structure largely parallels the geographic pattern of mtDNA variation (Fig. 2c,e,g): each locus has two completely (Musk-4) or nearly (Fib-5, Aco-9) segregated clusters of alleles that match the geographic distribution of the two mtDNA clades. The exceptions in the nuclear dataset are (i) several rare alleles at the centre of the Fib-5 and Aco-9 networks, where alleles ancestral to both groups are most likely to be located; and (ii) several alleles in Fib-5 and Aco-9 that came from birds in *C. tertius* populations, but are typical of the *C. frugivorus* lineage. This lack of reciprocal monophyly may be evidence for immigration between lineages. Among the nuclear introns, the maximum uncorrected sequence divergences for Aco-9, Fib-5 and Musk-4, respectively, are 1.2%, 2.5% and 1.7% within the populations of *C. frugivorus*; 2.2%, 2.3% and 1.3% within populations of *C. tertius*; and 2.3%, 2.7% and 2.5% between populations of *C. frugivorus* and *C. tertius*.

All loci show significant values of F_{ST} between *C. tertius* and *C. frugivorus* lineages (0.77, 0.64, 0.83 and 0.95 for Aco-9, Fib-5, Musk-4 and ND2, respectively; $P < 0.05$). The magnitude and pattern of variation across loci are shown as histograms of the pairwise divergence (Fig. 2b,d,f,h).

Multilocus analysis: isolation with migration

To be appropriate for IM analysis, DNA sequences must be free of evidence of recombination, selectively neutral, and show polymorphic variation within or between populations (Nielsen & Wakely 2001). The four-gamete test indicated that there was evidence for recombination at all loci. Our arbitrary selection of the 5′-most recombination-free segment of sequence with two or more segregating sites at each locus reduced our ND2, Aco-9, Musk-4 and Fib-5 sequences from 827 to 88 bp, 975–536 bp, 485–241 bp and 524–145 bp, respectively. There was no evidence for selection in these segments: the HKA analysis for neutrality was not significant for any locus (d.f. = 1, $P > 0.05$). Marginal posterior density distributions for the IM-generated parameters of divergence time and migration are presented in Fig. 3. Peak posterior distribution estimates with 90% credibility intervals are presented in Table 1.

Morphological variation

A PCA of the seven morphological measurements generated eigenvalues for the first three PCs of 4.63, 0.95 and 0.59, which accounted for 66.3%, 13.8% and 8.4% of the total variance, respectively. We retained PC1 as composite 'size' variable in subsequent analyses, as it was positively correlated to each of the morphological traits. In contrast, in PC2, factor loadings on wing, weight and tail were negative, whereas those for bill measurements were positive. We retained PC2 as a factor explaining bill shape in relation to size ('bill shape'), while recognizing that both the eigenvalue-one and scree test suggested that PC2 explained relatively little of the overall variation.

An independent-samples, one-tailed *t*-test on PC1 and PC2 scores revealed that males were significantly larger than females (d.f. = 46, $t = -2.10$, $P = 0.02$), but that the sexes did not differ in bill shape (d.f. = 46; $t = 3.11$, $P = 0.08$). A two-way ANOVA on PC1 scores with site and sex as between-group factors revealed significant main

Table 1 Peak posterior distribution densities derived from the isolation with migration (IM) model. The 90% highest posterior density (HPD) interval for each parameter (the shortest interval containing 90% of the probability) is shown in parentheses

Parameter	HPD
Effective population size of *Calyptophilus frugivorus*	2.33 (1.35–3.39)
Effective population size of *C. tertius*	1.47 (0.80–2.2)
Time since divergence (in years)	9 744 889 (6 600 502–27 093 232)
Migration of *C. frugivorus* into *C. tertius*	0.065 (0.01–0.22)
Migration of *C. tertius* into *C. frugivorus*	0.445 (0.14–0.80)

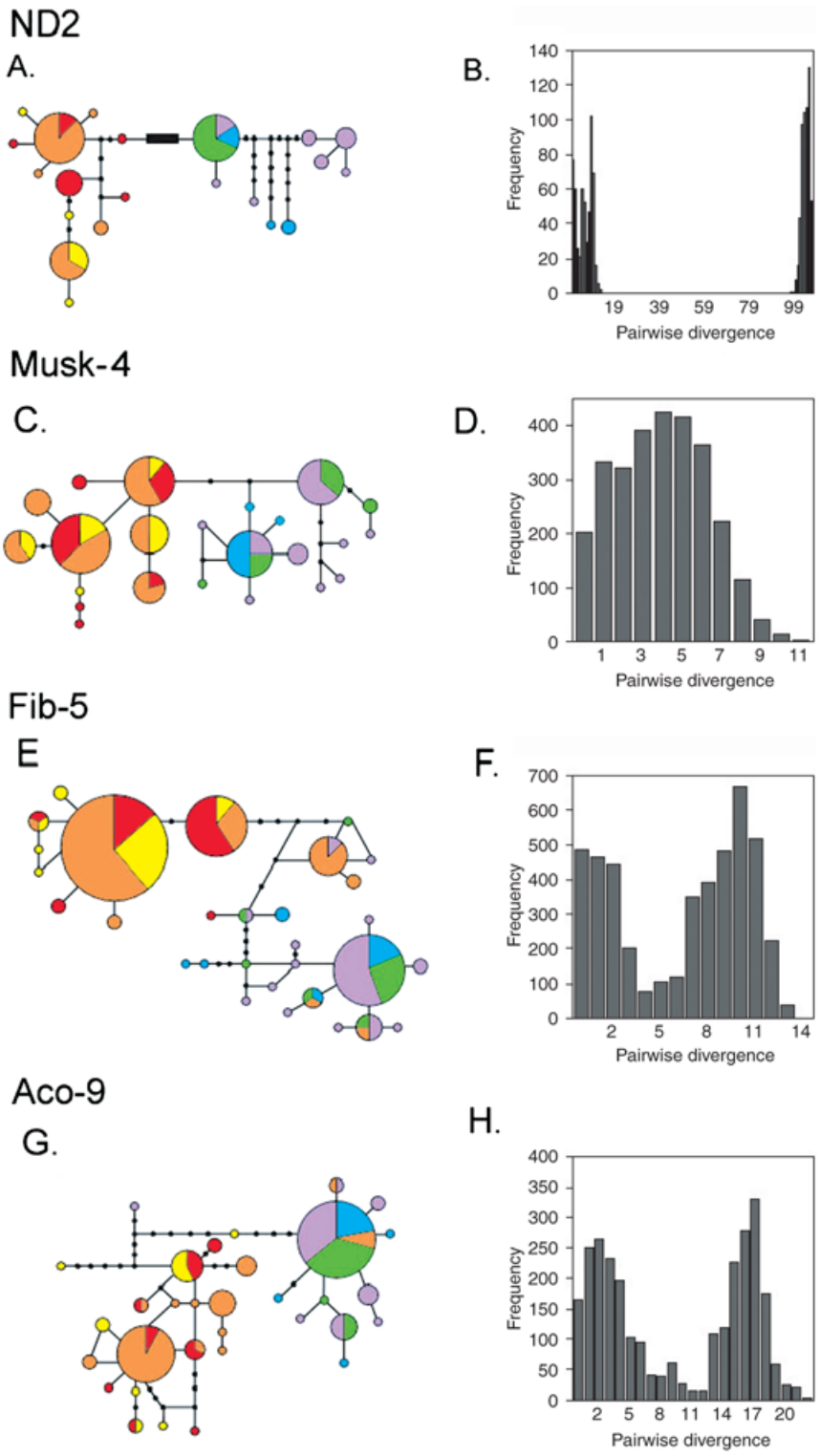

Fig. 2 Statistical parsimony haplotype networks and pairwise divergence histograms. Black dots indicate inferred intervening haplotypes separated by single substitutions. Colours correspond to sampling locations in Fig. 1.

effects of both site (d.f. = 5, $F = 53.08$, $P < 0.0001$) and sex (d.f. = 1, $F = 28.32$, $P < 0.0001$), which together had a large treatment effect ($R^2 = 0.90$; MSE = 0.60). There was no interaction between sex and site (d.f. = 5, $F = 0.42$, $P = 0.83$). Tukey's HSD test showed that birds from all measured populations of *C. frugivorus* were significantly smaller than all populations of *C. tertius* ($P < 0.05$). Within *C. tertius*, birds from the westernmost population at Massif de la Hotte were significantly larger than individuals sampled from the western Sierra de Bahoruco ($P < 0.05$;

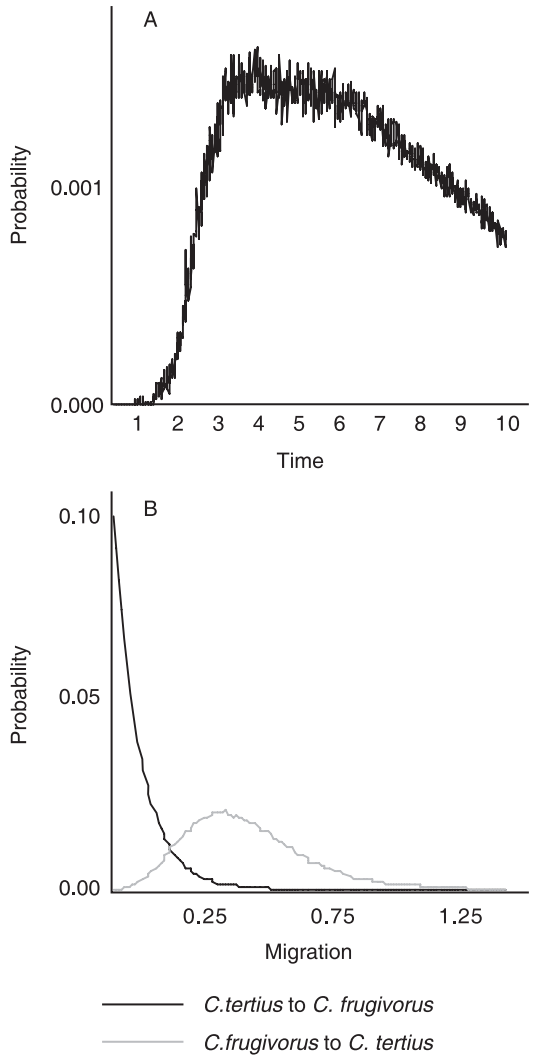

Fig. 3 Marginal posterior density distributions of the parameters (a) time since divergence, and (b) migration between lineages, derived from IM.

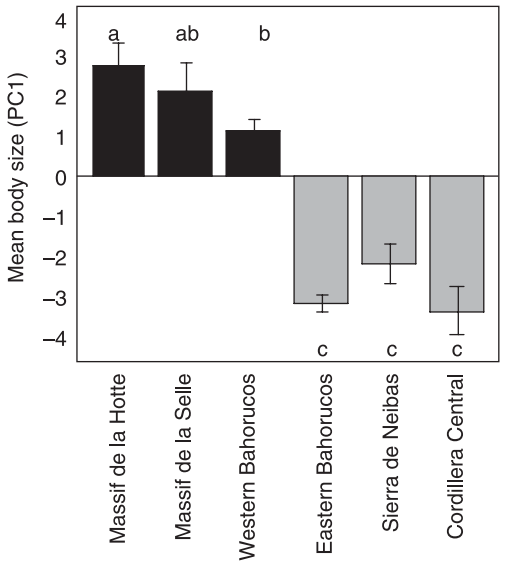

Fig. 4 Average size [Principal Component (PC) 1] of birds on each mountain range. Levels not connected by the same letter are significantly different. Black bars indicate *Calyptophilus tertius* populations, while grey bars indicate *C. frugivorus* populations.

Fig. 4). In contrast, a two-way ANOVA on PC2 scores with site and sex and between-group factors revealed no significant treatment effects or interactions ($R^2 = 0.19$; MSE = 0.96), suggesting that these *Calyptophilus* individuals and populations show simple allometric scaling, such that they differ in size but not in relative bill shape.

Discussion

There are three main findings of our study, which we discuss in turn: first, that there are two lineages of chat-tanagers, which correspond spatially and temporally to the ancient division of Hispaniola as two palaeo-islands; second, that this divergence was possibly precipitated by Hispaniola's previous configuration as two proto-islands, and therefore speciation in chat-tanagers was unlikely to have occurred in sympatry; and third, molecular data (in contrast to morphometric data) suggest that the populations within these two lineages are not evolutionarily distinct: the most endangered populations are closely allied phylogenetically to counterparts on other mountain ranges.

Phylogeography and multilocus genealogies

Our data support the current classification of two species of chat-tanager: the western *Calyptophilus tertius* and the eastern *C. frugivorus*. The nonzero divergence time yielded by our multilocus coalescent analysis, along with the similar geographic structure among the four independent *Calyptophilus* gene genealogies, is strong evidence that this divergence pattern was generated by extrinsic processes. The lack of reciprocal monophyly in both Fib-5 and Aco-9 suggests that there may be, however, limited, gene flow between lineages. The coalescent analysis similarly indicates gene flow between lineages (Fig. 3). This gene flow appears to be asymmetrical: there is a clear nonzero migration rate from *C. tertius* to *C. frugivorus*, in contrast to migration from *C. frugivorus* to *C. tertius*, which peaks at zero. Likewise, the HPD interval for migration rate from *C. frugivorus* to *C. tertius* includes the lowest possible value for migration (0.01, which is functionally zero) with the given parameter values (Table 1).

Biogeographic implications

Both the timing and pattern of lineage divergence suggest that the pre-Pliocene configuration of Hispaniola as two palaeo-islands (Fig. 1) has been the most important geographic barrier to gene flow of the chat-tanagers. Temporally, according to the IM analysis, the most likely divergence time between lineages was approximately 9.7 Ma, which corresponds to the approximate timing of fusion of the two palaeo-islands, estimated to have occurred in the mid-Miocene. Spatially, the breakpoint in the two *Calyptophilus* lineages generally corresponds to the boundaries of the northern and southern palaeo-islands. One exception to this distribution pattern occurs in the eastern Bahorucos of the southern Island, which supports a population belonging to *C. frugivorus*. This exception may be the result of a relatively recent colonization of that region from the adjacent northern highlands.

In contrast to the divergence between the two lineages, neither the isolation of the southwestern range via a now-closed sea channel nor the current isolation of populations on disjunct mountain ranges has left an imprint on the finer-scale phylogenetic structure of the chat-tanager: more recent gene flow appears to have homogenized genetic diversity within the lineages. This paucity of genetic structuring among populations is discordant with our morphological data, particularly in *C. tertius*, which demonstrates a significant decrease in size from western to eastern populations.

The lack of distributional overlap between congeners, as well as the overall phylogeographic concordance of differentiation with historical geographic separation, suggests that a sympatric mechanism of divergence is unlikely for *Calyptophilus*. Perhaps more surprisingly, differentiation is unlikely to have occurred *in situ*: phylogeographic data suggest that the only vicariant event extreme enough to drive divergence was the ancient division of the island. In contrast, the size, complex geological history and rugged landscape of Hispaniola has provided numerous opportunities for divergence in nonvolant taxa (Glor *et al*. 2003; Gifford *et al*. 2004). Thus, our results lend support to Diamond's (1977) assertion that avian speciation is unlikely to occur on single islands that are smaller than New Guinea, such as Hispaniola. However, we must test the generality of these results by conducting further phylogeographic investigations of other congeneric avian species pairs on Hispaniola.

Conservation implications

The ancient population structure that we report here suggests the existence of two *Calyptophilus* species, which will likely result in the listing of both species at a more critical level by the IUCN (BirdLife International 2000). Our molecular data suggest that the populations within these species are not evolutionarily distinct, and that the most endangered populations have genetically similar counterparts on other ranges. This information must be considered carefully, however, given the discordance with morphological data, which suggests that the critically endangered population of *C. tertius* in Massif de la Hotte may be morphologically distinct from the eastern population, which occupies more secure habitats of the Dominican Republic. One possible explanation for the discordance between the morphological and molecular data is that the genetic divergence among these populations may have occurred relatively recently, at a time scale too short for divergence detectable in the markers used in this study.

Acknowledgements

The authors are grateful to the many people who assisted with challenging field work on Hispaniola. We thank Elvis Cuevas and Jim Goetz for providing three samples from the Cordillera Central, and Laura Stenzler, Chris Makarewich and Amanda Talaba for help with various molecular analyses. We thank Kent McFarland for creation of the map in this manuscript and for GIS analyses. AKT's work was supported by the American Ornithologists' Union, the Mario Einaudi Center for International Studies, the Tinker Foundation/Latin American Studies Program and the Ecology and Evolutionary Biology Department of Cornell University. IJL's work was supported by NSF grants DEB-0515981 and DEB-0315218. CCR's work was funded by the Blake Fund, the Carolyn Foundation, the Conservation and Research Foundation, the National Geographic Society, the Stewart Foundation, the Thomas Marshall Foundation and friends of the Vermont Institute of Natural Science. Authorization to conduct work in the Dominican Republic was provided by the Subsecretaria de Areas Protegidas and the Departamento de Vida Silvestre. Field studies in Haiti were authorized by the Ministry of Environment and Agriculture. Statistical analyses were carried out, in part, using the resources of the Computational Biology Service Unit from Cornell University which is partially funded by Microsoft Corporation.

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