

Associations between physical isolation and geographical variation within three species of Neotropical birds

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Abstract

We studied effects of physical isolation on geographical variation in mtDNA RFLP polymorphisms and a suite of morphological characters within three species of neotropical forest birds; the crimson-backed tanager *Ramphocelus dimidiatus*, the blue-gray tanager *Thraupis episcopus*, and the streaked saltator *Saltator albicollis*. Variation among populations within continuous habitat on the Isthmus of Panama was compared with that among island populations isolated for about 10 000 years. Putative barriers to dispersal were influential, but apparent isolation effects varied by species, geographical scale, and whether molecular or morphological traits were being assessed. We found no geographical structuring among the contiguous, mainland sampling sites. Migration rates among the islands appeared sufficient to maintain homogeneity in mtDNA haplotype frequencies. In contrast, variation in external morphology among islands was significant within two of three species. For all species, we found significant variation in genetic and morphological traits between the island (collectively) and mainland populations. Interspecific variation in the effects of isolation was likely related to differential vagility. These data generally corroborate other studies reporting relatively great geographical structuring within tropical birds over short distances. Behaviourally based traits – low vagility and high ‘sensitivity’ to geographical barriers – may underlie extensive diversification within neotropical forest birds, but more extensive ecological and phylogeographic information are needed on a diverse sample of species.

Keywords: birds, dispersal, gene flow, geographical variation, mitochondrial DNA, Neotropics

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Introduction

Geographical variation stems from historical events and some combination of ongoing processes such as restricted gene flow, selection, mutation, and drift (Slatkin 1987; Tamura *et al.* 1991; O’Reilly *et al.* 1993). And with the advent of modern molecular studies, evidence is emerging that the influence of historical factors can predominate (Zink & Remsen 1986; Endler 1986; Avise & Ball 1991). Reconstruction of a sequence of insular colonizations, for example, can explain contemporary geographical struc-

ture among island populations (e.g. Seutin *et al.* 1994; Klein & Brown 1994). Historical factors are generally expected to be more influential in species with comparatively low vagility (Phillips 1994). Thus, evolutionary importance of geographical barriers and restricted gene flow varies between terrestrial plants and more vagile organisms such as birds or marine species with planktonic larvae (Slatkin 1987; Avise 1993).

Barriers to dispersal may be especially influential on geographical variation among populations of birds in the Neotropics, however, where species appear to be relatively sedentary and geographical features such as rivers and disjunct habitats may inhibit avian dispersal more effectively than in temperate regions (Capparella 1988, 1992; Escalante-Pliego 1992; Peterson *et al.* 1992; Seutin *et al.* 1993; Seutin *et al.* 1994). Two biogeographic models of avian diversification within the Neotropics (especially Amazonia) – the refuge hypothesis (Haffer 1969) and the riverine hypothesis (Capparella 1988) – both assume the

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inability of neotropical landbirds to bridge discontinuities in habitat. Other models discount dispersal or gene flow and emphasize the importance of selection and parapatric differentiation along environmental gradients (Endler 1982).

At present, data for neotropical birds are too few to either corroborate or refute these different models of diversification. Comparing the magnitude of variation among isolated and contiguous populations is an effective means of assessing the importance of barriers to dispersal vs. simple isolation by distance (Jackson & Pounds 1979; Ashley & Wills 1987; Baker *et al.* 1990; Daly & Patton 1990). Published reports on the genetic structure of neotropical bird populations in different geographical settings are few, however, especially for DNA sequence variation and its covariation with geographical trends in morphological traits (but see Hackett & Rosenberg 1990).

We explored the importance of physical isolation and restricted dispersal on the magnitude of geographical structuring within neotropical landbirds. We compared the extent of molecular (mtDNA) and morphological variation among insular populations of three species on the Pearl Archipelago (Republic of Panama) with that among populations in continuous habitat (mainland Panama). Open water, like that among most of the Pearl Islands and between the archipelago and mainland, is thought to inhibit movements of tropical forest birds (MacArthur *et al.* 1972; Diamond 1975; Capparella 1992; Edwards 1993). Here we address three questions: What is the extent of geographical variation in mtDNA sequence polymorphism and morphological traits among the mainland sites, among the islands, and between the islands and mainland? How do the geographical patterns of variation in morphological and genetic traits covary? What do these results imply about the development of geographical variation and processes of speciation in the Neotropics?

Materials and methods

Sampling locations and study species

The Pearl Islands (PI) were most recently isolated from each other and the mainland (i.e. Isthmus of Panama-north-western Colombia) when sea levels rose between 9000–11 500 years BP (Golik 1968; Bartlett & Barghoorn 1973; Fairbanks 1989). Before this time, the present-day PI were likely high-elevation areas within a large, contiguous expanse of dry tropical forest (D. Piperno, personal communication, Fig. 1). Sixteen of the PI are now forested and located, on average, about 35 km from the mainland (Fig. 1). About 50 species of forest birds occur on the PI and

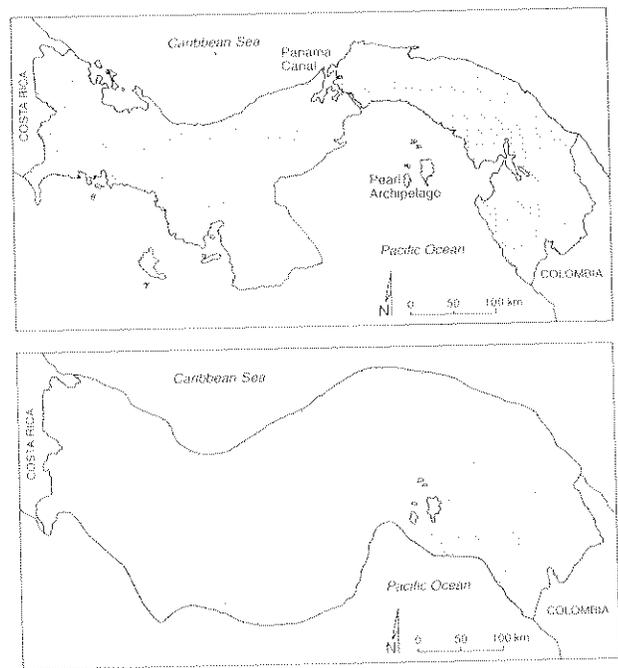


Fig. 1 Locations of Pearl Archipelago at present (a) and $\approx 14\,500$ years BP (b) when the contemporary islands were high elevation areas within the Isthmus of Panama (from D. Piperno, personal communication).

all but one are found on the nearby mainland (Wright *et al.* 1985). Biogeographical and palaeoecological evidence coupled with present-day habitat associations of the PI avifauna, strongly suggest the presence of these species on the PI during the most recent insularization event (R. Cooke, D. Piperno, personal communications).

We visited five islands that span the major axis of the archipelago (Fig. 2): Pacheca (62 ha), Saboga (288 ha), Chapera (178 ha), Bolanös (21 ha), and Rey (24 900 ha). We also established three sites for sampling mainland populations: 'Atlantic,' 'Gamboá,' and 'Summit' (Fig. 2). These sites were located in central Panama within relatively continuous habitat on the east side of the Panama Canal. The Pacific side of central Panama is about 56 km from the closest island on the PI (Fig. 1).

Distances among mainland sites (ranging from 8 to 43 km) were similar to the shortest (6 km) and longest (35 km) inter-island distances. Therefore, the potentially confounding effects of different intersite distances and isolation by distance were minimized.

We report on three species for which we obtained adequate sample sizes at most sampling sites: the blue-gray tanager *Thraupis episcopus*, the crimson-backed tanager *Ramphocelus dimidiatus*, and the streaked saltator *Saltator albicollis*. All three are songbirds (Order Passeriformes)

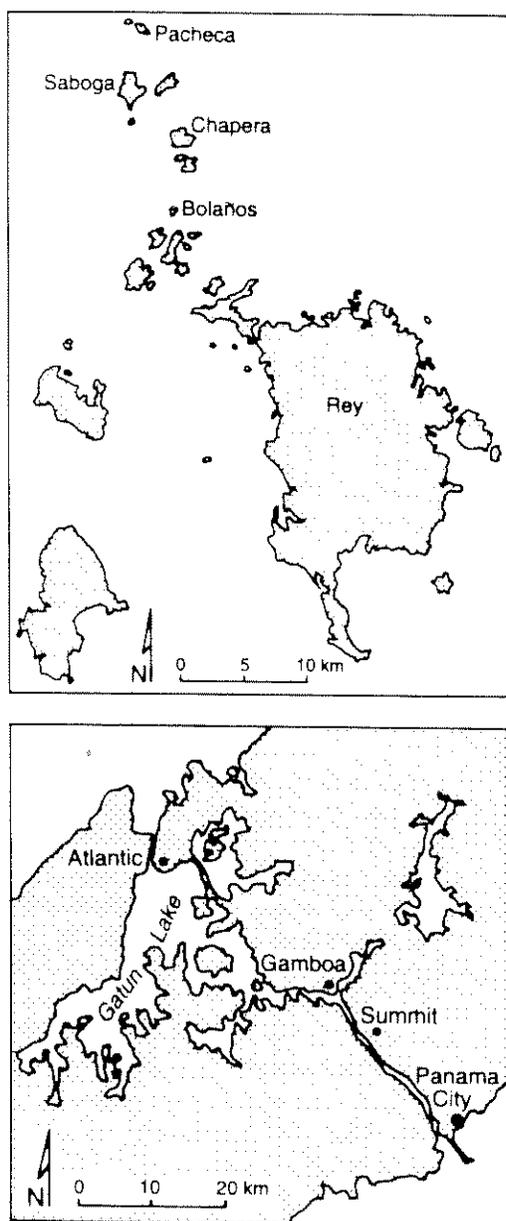


Fig. 2 Locations of five sampling sites within the Pearl Archipelago (a) and three sites in central Panama (b). Location of Panama City is included for reference.

with known omnivorous diets (Wetmore *et al.* 1984) and all are common to scrub, edges, and second-growth forests from lowland elevations up to 1500 m (Ridgely & Gwynne 1989). The crimson-backed tanager has the most restricted distribution of the three species and is found from western Panama through north-east Colombia (Isler & Isler 1987). At present, five subspecies are recognized based on plumage variation (Isler & Isler 1987). The blue-gray tan-

ager is widespread from southern Mexico through Central America and most of northern South America. Thirteen subspecies are currently recognized (Isler & Isler 1987). Unlike the other species, streaked saltators are found on the Lesser Antilles and also occur from south-western Costa Rica, south through Panama to northern Colombia/Venezuela, western Ecuador and Peru. Twelve subspecies are currently recognized and (Paynter & Storer 1970) and Seutin *et al.* (1993) reinforced earlier recommendations (Ridgeway 1901) that Antillean populations be treated as a distinct species. Wetmore *et al.* (1994) recognize subspecies unique to the Pearl Islands for the crimson-backed tanager (*R. d. limatus*) and the streaked saltator (*S. a. speratus*), but not for the blue-gray tanager.

Field and laboratory methods

Capture and processing of specimens

Birds were captured in mist nets. From each captured bird, we extracted tissue (commonly blood) for analyses of mtDNA and took a series of measurements (see James 1983) of external anatomy: bill length, width, and depth; tarsus length; toe length; wing (chord) length; and tail length. These characters were measured with electronic dial callipers or a graduated ruler (for wing and tail). Individuals released after processing were marked with uniquely numbered aluminium leg bands to identify recaptures. All but eight of 414 birds were measured by the senior author. Twenty-two recaptured specimens were remeasured to estimate the extent of measurement error. Differences between first and second measurements were not significant (paired *t*-tests, $P > 0.20$ in all tests) and ranged from 5% for bill length to 11% for wing length.

Tissue collection from most individuals involved puncturing the wing vein and extracting 50–150 μ L of blood with either a size 0 syringe (no needle) or a capillary tube. After extraction, the puncture was sterilized with denatured alcohol; when necessary, bleeding was stopped by application of a commercial styptic powder. Extracted blood was immediately placed in a 1.0-mL cryogenic vial with TE (10-mM Tris, 1-mM EDTA) and then stored in a portable container of liquid nitrogen. In cases of accidental death owing to capture and extraction of blood (5–7 individuals/species), we collected about 1 g of pectoral muscle tissue.

Preparation and extraction of DNA

For preparation of purified mtDNA samples, we also collected heart, liver and, pectoral muscle tissue from about 8 individuals/species. In the field, these tissues were immediately immersed in STEN buffer (25-mM Sucrose, 10-mM Tris, 100-mM EDTA and 10-mM NaCl) and stored up to 1 week at -4°C . Ultrapure mtDNA was obtained using a cytoplasmic enrichment protocol followed by caesium chlo-

ride-ethidium bromide (EtBr) gradient centrifugation and dialysis (see Lansman *et al.* 1981). High concentrations of nuclear DNA relative to mtDNA were a significant problem with the samples isolated from blood (see Quinn & White 1987). Therefore, we further purified the probe mtDNA, to be used for hybridization, by size fractionating fragments with electrophoresis on 1–2% NuSieve TAE agarose gels (FMC BioProducts) and staining with EtBr. DNA bands were visualized under long wavelength (preparative) UV, excised with glass cover slips, and purified using the GeneClean kit with the protocol provided (Bio 101).

Total DNA was extracted from 50–75 μ L of whole blood samples by an initial overnight digestion at 65 °C (with rotation in an environmental chamber) in 725 μ L of TE (10-mM Tris, 1-mM EDTA, pH 7.5), 20 μ L of 20% SDS, 25 μ L of proteinase K (10 mg/mL) and 2 μ L of 5-M NaCl. Overnight digestion was followed by two buffered phenol-chloroform-isoamyl alcohol (25:24:1) extractions and one chloroform-isoamyl alcohol (24:1) extraction. DNAs were precipitated at –20 °C for 2 h after addition of (1) 7.5-M ammonium acetate to a final concentration of 0.75 M, and (2) isopropanol (0.57–1.0 of volume resulting from step 1). Pellets were recovered by centrifugation at 4 °C for 30 min at 15 000 g. DNAs were resuspended in 350 μ L of TE and reprecipitated at 4 °C for 2 h after addition of 150 μ L of ammonium acetate and 1 mL of ethanol, followed by centrifugation as above, drying in a speed vac (Savant) and resuspension in 200 μ L of TE.

DNA restriction endonuclease digestion

Ten microlitres of DNA from the blood extractions, typically representing about 1 μ g of DNA, was digested with 20 units of restriction endonuclease following manufacturers recommendations (Bethesda Research Laboratories, New England Biolabs). For DNA extracted from muscle and for purified DNA, 5- and 1- μ L of samples were used, respectively (samples were brought up to volume with water). Digests were typically allowed to proceed overnight. DNAs were digested with the following endonucleases: *Ava*I, *Bam*HI, *Bcl*I, *Bgl*II, *Bgl*III, *Dra*I, *Eco*RI, *Eco*RV, *Hinc*II, *Hind*III, *Pvu*II, *Sac*I, *Stu*I, and *Xba*I. The enzymes *Ava*I and *Hinc*II recognize 5.3-base sequences; all other enzymes used recognize six bases.

Agarose gels and Southern blot transfers

Digested DNAs were electrophoresed on 25-cm-long 0.9% agarose gels in TBE running buffer (10X = 0.89-M Tris, 0.89 M boric acid, 0.11-M disodium EDTA, pH 8.3) at 57 V for about 15 h. Gels were then denatured in 0.5-M NaOH, 1.5-M NaCl and neutralized in 0.5-M Tris, 1.5-M NaCl, pH 7.0. DNA was transferred by capillarity to Zetabind membrane (CUNO) by placing gels on a southern blot transfer apparatus for 16–24 h. Following transfer, blots were washed in 2 \times SCP for 15 min and baked at 80 °C for 2 h.

Hybridization

Prior to hybridization, blots were wetted for 2 h in rotating canisters with 40 mL of 0.8 \times hybridization solution [2 \times hybridization solution: 300 mL 20 \times SCP (2.4-M NaCl, 0.3-M sodium citrate, 0.4-M NaPO₄), 100 g dextran sulphate, 40 mL of 25% *N*-lauryl-sarcosine and 1 mg/mL heparin to final volume of 540 mL]. Probe DNA was labelled with ³²P in a random priming reaction using a DNA labelling kit manufacturer's instructions (Boehringer Mannheim). Unincorporated nucleotides were separated from the labelled probe by spin dialysis through Sepharose CL-6b-200 (Sigma). Labelled probe was then mixed with carrier DNA (2 mg of salmon sperm) and 2 \times hybridization solution and added to prehybridized blots. Hybridization in rotating canisters at 65–72 °C proceeded for 24–48 h. Post-hybridization procedures involved 3 washes (2 \times SCP-1% SDS, 0.2 \times SCP-0.1% SDS and 0.1 \times SCP-0.05% SDS) each at 65–72 °C for 15 min. Washing blots under these stringent conditions further reduced contamination problems owing to hybridization with high concentrations of mitochondrial-like sequences of nuclear DNA.

Analyses

Geographical patterns in genetic and morphological traits

We estimated the extent of variation among sites in three different geographical settings; intermainland, interisland, and mainland–island. Genetic data were analysed in several ways. We first estimated nucleotide sequence divergence (*p*), as prescribed by Nei (1987; p. 101). We based our analyses on the proportion of shared restriction sites (using all haplotypes detected within sampling sites, not weighted by frequency) because in almost all cases we were able to unambiguously infer sites from fragment profiles (see Ball *et al.* 1988). The presence of fragments \leq 500 bp long could not be reliably visualized and these fragments were usually ignored. We did not include data from autoradiographs with ambiguous fragment patterns and individuals were often removed from analyses. Sample sizes for blue-gray tanagers were comparatively small owing to persistent technical problems in analysis of their mtDNA samples. Estimates of *p* among sampling sites were derived by pooling all haplotypes within a site rather than averaging pairwise distances among individuals.

We also assessed genetic diversity within population at different geographical settings (i.e. mainland and island) by estimating haplotype diversity (*h*, see Avise 1993). This metric ranges from 0 to near 1 with higher values indicating greater diversity.

Hypothesis tests for geographical subdivision in genetic structure among mainland sites, among islands, and

between the PI and mainland were first made with contingency table analyses of haplotype frequencies (Nei 1987). Independence of rows (sites) and columns (haplotypes) was evaluated with likelihood ratio statistics and significance values derived from exact tests using permutation procedures (Mehta & Patel 1992).

We further tested for geographical subdivision using a molecular analysis of variance (AMOVA) approach introduced by Excoffier *et al.* (1992) and estimated (genetic) variance components at hierarchical levels of population subdivision: (1) within populations (each island or mainland site); (2) among populations within regions (among islands or between mainland sites); and (3) between regions (between island and mainland populations). Significance of these variance components are derived using variance ratios (analogous to *F*-statistics) and permutation procedures. Sums of squares for our analyses were based on the number of restriction site differences between each haplotype (see Li 1976).

We refrained from characterizing gene flow with estimates based on F_{ST} because we could not assume that equilibrium conditions had obtained since isolation. Instead, we used procedures presented by Neigel *et al.* (1991) and Neigel & Avise (1993) that allow for non-equilibrium conditions and estimate dispersal distance per generation.

For external morphology, we compared sites with discriminant function analyses (DFA); our motivation was to assess differences among populations via formal hypothesis tests. All morphological measurements were log-transformed prior to DFA (see Mosimann 1970; Darroch & Mosimann 1985). Multivariate *F*-tests for equality of group

means (over all groups and variables) were derived from Wilk's lambda (Barker & Barker 1984). Tests for pairwise differences in group (i.e. site) means were derived from multivariate linear contrasts (Dixon 1990). Significance tests for the pairwise tests were adjusted with the Bonferroni inequality. We derived the proportion of sample variation explained by the discriminant models (i.e. r^2 owing to sampling location) based on: $r^2 = 1 - \text{Wilk's lambda}$ (see Cooley & Lohnes 1971; Barker & Barker 1984). Equality of group variance-covariance matrices was evaluated (and in all cases verified) by Box's *M* test (Barker & Barker 1984). All DFAs were run using the BMDP 7-M routine and default settings (Dixon 1990).

Based on the recommendations of Williams & Titus (1988) for sample sizes in DFA and demonstrated biases in use of Mahalanobis distances derived from small samples (Cherry *et al.* 1982), we deemed sample sizes to be insufficient for analyses of external morphology in three cases: blue-gray tanagers on Bolanós ($N=4$), streaked saltators on Saboga ($N=4$), and crimson-backed tanagers on Chapera ($N=7$).

Phylogeography

We examined the correspondence between phylogeny and the geographical distribution of mtDNA haplotypes by constructing approximate minimum length phylogenetic networks following methods outlined by Lansman *et al.* (1983). The geographical locations of the haplotypes were then plotted onto the minimum length networks. For streaked saltators, it was not always possible to determine with confidence that differences in the fragment profiles were the results of single cleavage site gains or losses, so a phylogenetic network was not constructed for this species.

Patterns of covariation in geographical patterns of morphological and genetic traits

Geographical patterns of variation in morphological and genetic data were compared with Mantel's test (see Douglas & Endler 1982). Elements in the matrices of morphological data were Mahalanobis distances between sites (i.e. group centroids). Elements of the genetic matrices were estimates of sequence divergence in mtDNA (p) between sites.

Results

Putative barriers to migration were apparently influential, but this effect varied by species, geographical scale, and whether morphological or genetic traits were being considered (Table 1). We found no morphological or genetic evidence of population subdivision over contiguous habitat within the mainland. On the PI, haplotype frequencies were homogeneous among islands, but we did detect morphological differences among island populations within

Table 1 Qualitative summary of results for study of isolation effects on geographical variation within three species of neotropical birds. A 'yes' indicates detection of significant variation among sampling sites; 'no' indicates no significant variation.

Species	Traits	
	external morphology	mtDNA
Inter-mainland		
crimson-backed tanager	no	no
blue-gray tanager	no	no
streaked saltator	no	no
Inter-island		
crimson-backed tanager	yes	no
blue-gray tanager	no	no
streaked saltator	yes	no
Island-Mainland		
crimson-backed tanager	yes	yes
blue-gray tanager	yes	yes
streaked saltator	yes	yes

crimson-backed tanagers and streaked saltators. Comparisons of the collective mainland and island samples were more consistent, except for one test of mtDNA in the blue-gray tanager, we found significant morphological and genetic variation.

Geographical variation in mtDNA

Estimated sizes of the mtDNA molecule varied slightly among species (mean over all endonucleases: crimson-backed tanager = 16.2 kb; blue-gray tanager = 16.7 kb; streaked saltator = 16.4 kb), but we found no evidence of intraspecific mtDNA size variation or heteroplasmy. We detected over 50 restriction sites within each species; the proportion of sites that were polymorphic was 12% ($N=59$) for the crimson-backed tanager, 16% ($N=55$) for the blue-gray tanager, and 20% ($N=54$) for the streaked saltator. On average, over all individuals, we sampled an estimated 0.43% of the mtDNA molecule. We analysed all island samples except blue-gray tanagers on three islands (Saboga, Chapera, and Bolanós). To achieve desired sample sizes from the mainland sites, we combined individuals from the Summit and Gamboa sites into a 'Pacific'

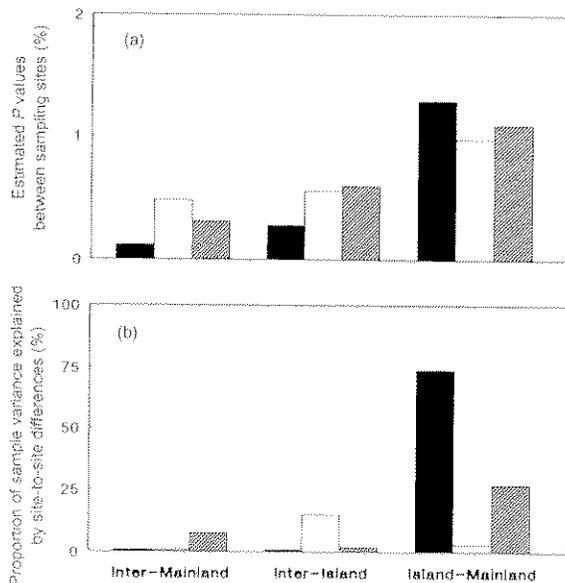


Fig. 3 (a) Estimated level of sequence divergence ($P \times 100$) among sampling sites in different geographical settings for three species, (b) proportion (%) of total sample genetic variation accounted for by site to site variation based on AMOVA (see text for explanation of technique): (■) crimson-backed tanager; (□) blue-gray tanager; (▨) streaked saltator. 'Inter-mainland' refers to the Atlantic vs. the pooled Gamboa-Summit sampling sites (see text), 'inter-island' refers to the average pairwise difference of all island samples, 'island-mainland' refers to combined island and mainland samples.

mainland category (no differences were apparent between these sites within any species).

Variation between mainland sampling sites

Estimated P -values ($\times 100$) between the Atlantic and Pacific sampling sites ranged from 0.14% for crimson-

Table 2 Distribution of haplotypes on mainland and island sampling sites. Letters indicate different fragment profiles for a given endonuclease (see Appendix 1 for order, a dash indicates that an enzyme did not cut). Blue-gray tanager mtDNA was analysed for only two island sites. Numerals indicate number of individuals. Haplotype diversity (h) was calculated for the pooled island and mainland sites

Species/haplotype	Sampling site*						
	Mainland		Island				
	Pa	At	P	S	C	B	R
Crimson-backed tanager							
CCCDCCC-CCCCC	3	6	0	0	0	0	0
DCCCCC-CCCBCC	1	1	0	0	0	0	0
DBCCCC-CCCCC	0	1	0	0	0	0	0
DCCCCC-CCCCC	2	2	0	0	0	0	0
DCCDCCC-CCCCC	1	1	0	0	0	0	0
CCCCCC-CCCCC	3	2	0	0	0	0	0
CCCDCCC-CCCDCC	0	0	8	4	3	4	7
CCCDCCC-CCCDCCB	0	0	0	0	0	0	1
CCCDCCC-CCCDDBC	0	0	0	0	0	0	1
Total	10	13	8	4	3	4	9
h	0.75		0.13				
Blue-gray tanager							
CBCCCC-CCCCCB-	0	1	0	-	-	-	0
CBCCCC-CCCCC-	2	2	0	-	-	-	0
CBCCCC-CCBCCC-	0	1	0	-	-	-	0
CBCBCC-CCCCC-	0	1	0	-	-	-	0
BCCCC-CCCCC-	0	1	0	-	-	-	2
CCCCC-CCCCC-	2	0	2	-	-	-	4
CCCCC-CCCCCB-	2	0	0	-	-	-	0
CCCCC-CCCCCE-	0	0	1	-	-	-	0
BBCCCC-CCCCC-	0	0	1	-	-	-	0
BCCCC-CCCCC-	0	0	2	-	-	-	0
Total	6	6	6	-	-	-	6
h	0.81		0.68				
Streaked saltator							
CCCC-CCCCCCC-	6	6	1	1	0	1	5
CCCB-CCCCC-	1	0	0	0	0	0	0
CCCC-CCCCC-	1	0	0	0	0	0	0
CCDC-CCCCC-	1	0	0	0	0	0	0
CACC-CCCCC-	1	4	10	3	3	2	4
CAEC-CCCCC-	0	0	0	0	0	0	1
CACC-CCCBCCC-	0	0	0	0	0	1	0
CBCC-CCCBCC-	0	0	0	0	1	0	0
Total	10	10	11	4	4	4	10
h	0.57		0.49				

*Pa, Pacific; At, Atlantic; P, Pacheca; S, Saboga; C, Chapera; B, Bolanós; R, Rey.

backed tanagers to 0.48% for blue-gray tanagers (Fig. 3). Haplotype distributions within each species (Table 2) revealed no evidence of subdivision between mainland sampling sites (χ^2 , $P > 0.10$ for all tests). Similarly, AMOVA derived estimates of variation between mainland populations were insignificant ($P > 0.15$) and interlocality variation accounted for less than 10% of total sample variation within all species (Fig. 3).

Variation among island sampling sites

Average estimated sequence divergence among the island sites ranged from 0.28% for crimson-backed tanagers to 0.6% for streaked saltators (Fig. 3). Each species was also characterized by dominance of a common haplotype on the PI (Table 2). For crimson-backed tanagers, this dominance was pronounced; all but two crimson-backed tanager individuals sampled throughout the archipelago shared the same haplotype. And with one exception (Rey), the

same streaked saltator haplotype dominated each island sample. For blue-gray tanagers, variation among islands was qualitatively greater than that among the mainland sites (Fig. 3), but contingency analyses and AMOVA indicated no subdivision among islands within any species.

Variation between island and mainland samples

Estimated sequence differences between the island and mainland samples were at or above 1% for all species, and greatest for crimson-backed tanagers (Fig. 3). We found significant and consistent differences in haplotype frequencies between the pooled island and mainland samples (Table 2 – crimson-backed tanagers $\chi^2_6 = 51.5$, $P < 0.001$; blue-gray tanager $\chi^2_4 = 15.6$, $P < 0.005$; streaked saltators $\chi^2_3 = 14.8$, $P < 0.005$). Within each species, the common haplotype on the mainland was different from that on the islands. For the crimson-backed tanager, this difference was extreme – the predominant haplotype on

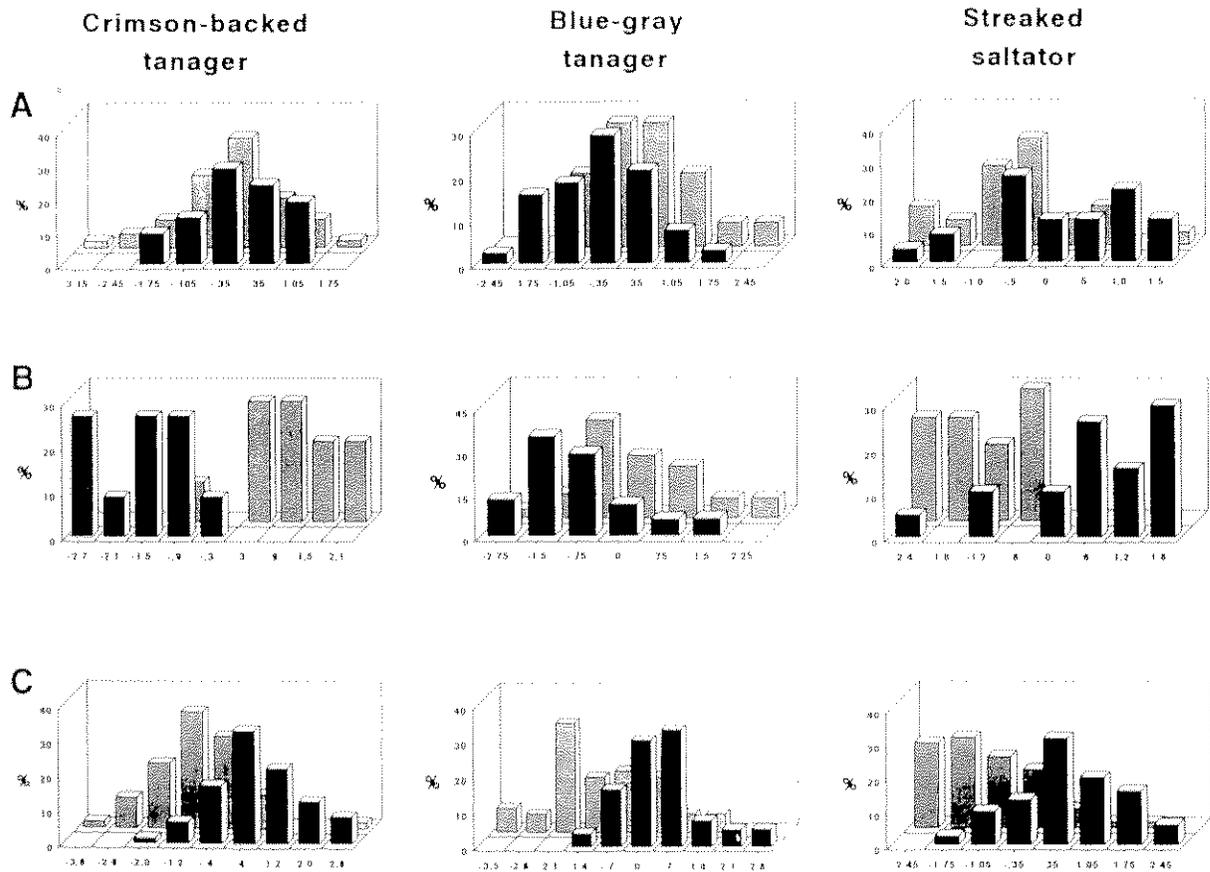


Fig. 4 Frequency histograms of individuals along first canonical axes illustrating variation in external morphology among populations at different geographic locations. Numbers on abscissas indicate scores along first canonical axis; in all analyses the first axis accounted for $\geq 70\%$ of the explained variation among groups. Different discriminant analyses were performed for: (A) Mainland comparisons illustrated with individuals from Atlantic (■) and Summit sites (▨). (B) Island comparisons illustrated with individuals from Pacheca (■) and Rey (▨). (C) Island (■) and mainland (▨) comparisons. For this latter analysis, all individuals sampled on islands and mainland sites were combined into two samples.

the PI was not detected on the mainland. Within all species, rare haplotypes were found only on the mainland or on the PI.

The proportion of sample variation attributable to differences between the mainland and the PI (Fig. 3) was significant within crimson-backed tanagers ($P=0.002$) and streaked saltators ($P=0.002$). Regional variation within blue-gray tanagers was slight (Fig. 3, $P=0.34$).

Estimated dispersal distances and haplotype diversity

Estimated dispersal distances per generation (using molecular data from all sampling locations) were 1.2 km for the crimson-backed tanager, 2.6 km for the streaked saltator, and 2.8 km for the blue-gray tanager.

Haplotype diversity (h) was estimated for the pooled island and mainland samples of each species. Values of h ranged from 0.57 to 0.81 on the mainland (Table 2) and were lower on the Pearl Islands than on the mainland for all three species (Table 2). The decrease in h in the Pearl Islands was greatest for the crimson-backed tanager, where, again, only one haplotype was found on most islands.

Geographical variation in morphological traits

Variation among mainland sampling sites

Morphological differences among populations at mainland sampling sites were slight (Fig. 4). Tests for overall differences in group means were not significant within any species (blue-gray tanager $F_{6,102}=2.9$, $P=0.07$; crimson-backed tanager $F_{2,64}=2.7$, $P=0.30$; streaked saltator $F_{10,84}=3.1$, $P=0.08$); in all cases eigenvalues associated with the first or second discriminant functions were <1 and location accounted for less than 20% of the sample variation. With the exception of blue-gray tanagers at the Atlantic and the Camba sites ($P=0.04$), contrasts testing pairwise site-differences were insignificant within each species ($P>0.05$, F -tests).

Variation among island sampling sites

Within crimson-backed tanagers and streaked saltators, interlocality variation among the islands was more pronounced than that among the mainland sites (Fig. 4). We observed no apparent effects of isolation within blue-gray tanagers. Differences among island populations were significant within crimson-backed tanagers ($F_{20,167}=3.32$, $P<0.005$) and location explained 76% of sample variation – a level over nine-fold greater than that for crimson-backed tanagers within the mainland. Similarly, intersite variation within streaked saltators was significant among the island samples ($F_{12,170}=8.4$, $P<0.001$) and more pronounced than that on the mainland (65% of total variance explained). For blue-gray tanagers, differences among the island sites was almost significant ($F_{9,166}=2.7$, $P=0.06$), and location accounted for 31% of the sample variation.

All pairwise-site contrasts within the crimson-backed tanager and three of six contrasts within streaked saltators were significant (F tests, $P<0.05$). No pairwise contrasts were significant within the blue-gray tanager island samples.

The magnitude of variation between a pair of island sites was associated with geographical distance. Correlations of Mahalanobis distances between group centroids (i.e. the average difference in morphological space between two island samples) and geographical distance between sites revealed a positive relationship within crimson-backed tanagers ($r=0.77$) and streaked saltators ($r=0.68$), but not within the blue-gray tanager ($r=-0.09$). No estimated correlations were significant, but samples sizes were small ($n=6$ within each species).

Variation between island and mainland samples

We found significant differences between the pooled island and mainland samples within all species (Fig. 4; crimson-backed tanager $F_{5,137}=10.45$, $P<0.001$; blue-gray tanager $F_{4,134}=19.1$, $P<0.001$; streaked saltator $F_{6,122}=13.7$, $P<0.001$). The proportion of sample variances explained in the island-mainland comparisons were similar to that found in the inter-island comparisons (crimson-backed tanager, 60%; blue-gray tanager, 39%; streaked saltator, 55%).

Phylogeny and geographical variation

The congruence between phylogeny (inferred from mtDNA parsimony networks) and the geographical distribution of mtDNA haplotypes also varied by species. With the crimson-backed tanager (Fig. 5), there was little evidence of correspondence between phylogeny and geography among mainland sites, but a split between the mainland and the PI was indicated. The haplotypes found on the PI were not detected on the mainland and these three haplotypes form a monophyletic clade in nearly all rootings of the parsimony network.

In contrast, for the blue-gray tanager, some of the PI haplotypes were found in the Pacific and Atlantic mainland sites (Fig. 5). The extremely reticulate nature of the blue-gray tanager network made possible rootings problematic, but no regions formed an obvious monophyletic clade. Although we refrained from constructing a network for the streaked saltator, all haplotypes that occurred in two or more localities are found in the PI and both mainland regions, thus indicating no strong phylogeographic pattern.

Comparisons of morphological and genetic patterns of geographical variation

We analysed correspondence between the geographical patterns of morphological and genetic variation in crim-

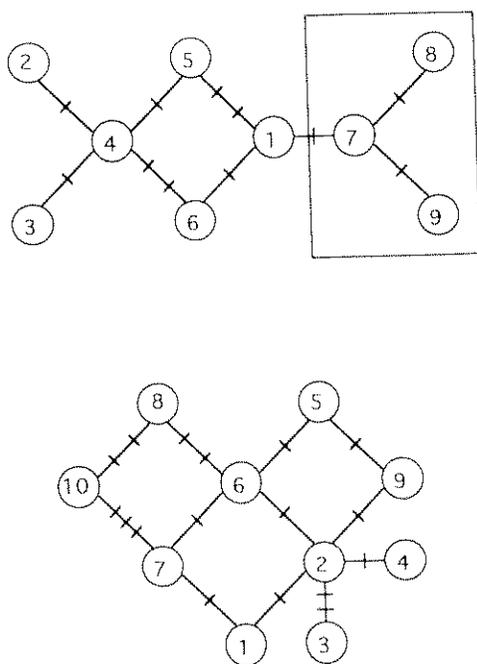


Fig. 5 Approximate minimum length phylogenetic networks interconnecting haplotypes. Numbers correspond to haplotype numbers in Table 2. Hachures represent number of inferred restriction changes along a branch. (A) Crimson-backed tanager where boxed in portion of network encloses the island populations, which are monophyletic in any rooting except along branches between 7–8 and 7–9. (B) Blue-gray tanager in which no region formed an obvious monophyletic clade.

son-backed tanagers and streaked saltators (blue-gray tanager data were not analysed because we could use only two island samples). Matrices of genetic and morphological comparisons were positively correlated for the crimson-backed tanagers character sets ($r=0.64$ for element-by-element correlation). Mantel's test, however, failed to reject the null hypothesis of no association between the two data sets ($t=1.23$, $P>0.05$). A slight negative association was found between the two matrices for streaked saltators ($r=-0.23$; Mantel test, $t=0.79$, $P>0.40$).

Discussion

Evolutionary processes on the Pearl Islands

We believe that the geographical structure we found stemmed from restricted dispersal over open water and, possibly, population bottlenecks within the PI. Our results also illustrate that the patterns and causes of geographical structuring can be diverse; apparent effects of isolation varied by species, trait, and scale of putative barriers to dispersal.

A key factor for understanding insular biogeographic patterns is whether island populations are relicts or established by small numbers of colonists (Berry 1986; Fleischer *et al.* 1991). Rates of evolutionary change within insular populations of small mammals such as house mice (*Mus musculus*), for example, are distinctly greater within populations founded by colonization (or introduction) than within relict populations on the same islands (Berry 1986). Similar findings have been presented for reptiles and mammals on the Galapagos islands (Patton 1984).

Ecological evidence indicates that founder events on the PI are unlikely for the species considered here. As explained above, our study species were likely present on the PI before and after insularization. Extinction-recolonization events since the terminal Pleistocene deglaciation also appear unlikely as all three species are extremely abundant throughout the PI (Wright *et al.* 1985; Brawn, unpublished data). Whereas, natural and anthropogenic disturbances on the PI may cause fluctuations in abundances and periodic disappearances of some species on some islands (see Wright *et al.* 1985), the species considered here are generally more abundant in disturbed habitat. Therefore, while population bottlenecks are possible and even indicated (see below), the extirpation of these species from the entire PI followed by recolonization would require an unusual (and unknown) ecological event.

One scenario with respect to structuring in mtDNA is that the diversity and frequency of haplotypes were similar throughout the Isthmus of Panama before deglaciation (as they appear on the mainland today). Dispersal was subsequently reduced over the wide water barrier, and frequencies of haplotypes changed on the PI owing to drift. Loss of haplotypes on the PI may have been particularly important with crimson-backed tanagers as only a single haplotype was found on nearly all islands. The near fixation of this haplotype suggests the occurrence of population bottlenecks since deglaciation with effects that persisted owing to low rates of dispersal between the PI and mainland. Dispersal over smaller water barriers by all three species was evidently sufficient to prevent inter-island differentiation in mtDNA.

Another important consideration is whether the sequence variation we detected between the PI and mainland arose in 10 000 years. If the commonly cited rate of mtDNA sequence divergence of 2% per million years (Brown 1983; Shields & Wilson 1987) is correct for birds, our results (Fig. 3) imply divergence between the PI and mainland populations before the terminal Pleistocene. By this scenario, reduced dispersal and drift also played a role, but contemporary geographical structure accumulated episodically during periods of isolation. This explanation assumes restricted gene flow during periods of reconnection as well. Numerous studies of plants and animals

report reduced rates mtDNA or cpDNA gene flow across contact zones (summarized in Avise 1993). Regardless of time scale, our results appear to corroborate theory (Birky *et al.* 1989) and add to growing empirical evidence (Avise 1993) that identifies reduced migration and random sampling of haplotypes as major sources of geographical structure in mtDNA variation.

The magnitude of isolation effects (mtDNA and morphology) was greatest with the crimson-backed tanager and least with the blue-gray tanager. Indirect evidence suggests this variation reflects interspecific differences in vagility. MacArthur *et al.* (1972) examined the distributions of PI birds throughout Central and South America and concluded that the crimson-backed tanager is the least vagile of our three species. Our estimates of dispersal distances per generation confirm that relative vagility varies among the three species. Personal observations (we often observed blue-gray tanagers over water) and anecdotal reports from residents on the PI also indicate interspecific differences in vagility. Whereas data on more species are needed, these results illustrate that differences in life histories may lead to different rates of diversification – even in a similar geographical setting and historical framework.

Geographical surveys of plants and animals commonly reveal different geographical patterns in genetic and morphological traits (summarized in Hillis 1987; Avise 1993). In the present study, interlocality variation was qualitatively greater in morphological than in genetic traits, especially among island populations of crimson-backed tanagers and streaked saltators. Greater variation in morphology is common among conspecific populations of aquatic and terrestrial vertebrates (Avise & Ball 1991; Zink & Dittman 1993), and expected with comparisons of organelle DNA (neutral variation from one locus in a haploid state) and morphological variation under polygenic control and at least partially responsive to selection (Hillis 1987). Demographic studies on the Pearl Islands assessing the influence of selective processes on morphology appear warranted.

Furthermore, the morphological variation we detected may have an environmental component. Variation in the external morphology of birds (James 1983), fish (Meyer 1987), and other animals (van Noordwijk 1989) can be environmentally induced. For some birds, such induction appears rooted in the microclimatic conditions of nestlings as they develop (James 1983; E. James, personal communication). Reduced migration among the PI with the crimson-backed tanager and the streaked saltator could therefore lead to detectable variation among the island populations even if morphological variation was not entirely genetically based. Transplant experiments of eggs among islands or between the PI and mainland (*sensu* James 1983) are required to evaluate the magnitude of environmental effects (if any).

Implications for avian diversification and speciation in the Neotropics

Recent studies report that geographical variation within birds is deeper and more finely structured in the Neotropics than at temperate latitudes (Capparella 1988, 1992; Hackett & Rosenberg 1990; Peterson *et al.* 1992; Escalante-Pliego 1992; Seutin *et al.* 1993, 1994). Comparatively great genetic divergence within (and among) neotropical species has prompted suggestions that neotropical taxa are older than their temperate-zone counterparts (e.g. Escalante-Pliego 1992). A finer scale of diversification suggests either greater sensitivity to discontinuities in habitat (Capparella 1992) and the importance of reduced gene flow, the selective influence of comparatively fine-scaled environmental gradients (Endler 1982), or both.

At the descriptive level, our results are partially consistent other surveys of tropical species. We sampled at a smaller geographical scale than have most previous tropical studies and, for two of three species, genetic distances between the PI and central Panama (again, separated by about 50 km) exceed those found in many continental surveys of temperate bird species (e.g. Ball *et al.* 1988; Moore *et al.* 1991; Zink 1991). The island-mainland divergence we observed also exceeds those found between island and mainland populations of North American songbirds (Hare & Shields 1992; Zink & Dittman 1993).

Our results therefore identify physical isolation and reduced dispersal as potentially key factors underlying geographical variation in at least some tropical species. Over similar distances, geographical structuring was greater with barriers to dispersal than without.

Importantly, our study species are more vagile than many species inhabiting the interiors of expansive forests in South America (J. Bates, A. Capparella, personal communications). Thus, the sensitivity of many tropical forest birds to geographical barriers and disturbances that interrupt habitat may be greater than those reported here. A realistic expectation is that the magnitude of geographical variation will vary among species within and between different latitudes. Latitudinal comparisons should also be based on samples and methods that generate similar levels of resolution for identifying geographical structure. Therefore, we join Seutin *et al.* (1994) in encouraging a careful, empirically based approach to latitudinal comparisons of geographical structure and processes of diversification.

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Appendix 1

Fragment profiles from RFLP analyses of mtDNA. Letters refer to arbitrary name for fragment profile. Numbers are estimated lengths (bp) of fragments. — indicates no cuts or a single cut

Enzyme	Crimson-backed tanager			Blue-gray tanager		Streaked saltator				
	C	D		C	B	C	B	A		
<i>AclI</i>	5300	5300		6000	5700	8500				
	3950	3950		5700	4000	5800				
	2400	2400		2650	2650	2400				
	2250	2250		2200	2200					
	550			2000						
<i>BamHI</i>	C	B		C	B	C	B	A		
	11000	12625		12000	12000	7000	12300	15800		
	4000	4000		3000	3000	5300	3500			
	1625			1650	1750	3500				
<i>BclI</i>	C			C		C	D	E		
	8000			7900		7200	7200	6900		
	4500			5400		5300	4900	5300		
	2800			3100		3600	3600	3600		
<i>BglII</i>	C	D		C	B	C	B			
	6108	6108		6600	3950	9000	11800			
	3850	3850		3950	3600	2900	2900			
	3500	2700		2600	3000	2800	1500			
	2200	2200		2500	2600	1500				
<i>BglIII</i>	C			C						
	11000			14000						
	2600			2800						
<i>DraI</i>	C			C		C				
	7700			8100		8150				
	4400			7150		7050				
	2500			1600		1825				
<i>EcoRI</i>	C			C		C				
	7300			---		9160				
	4400					7700				
<i>EcoRV</i>	C			C		C				
	---			11000		15000				
				3600		1750				
<i>HaeIII</i>	C			C		C	B			
	5800			6700		5400	6200			
	3950			3000		3600	3600			
	3100			2700		2200	2200			
	1450			1550		1450	1450			
	1200			1200		1150	1150			
	560			910		980	980			
			670		910	540				
<i>HindIII</i>	C			C	B	C	B			
	8800			8300	8300	6400	6400			
	4000			2300	2150	2300	2300			
	2400			2150	1725	2100	2100			
	1650			1725	1500	1950	1950			
				1025	1200	1900	1900			
				980	980	450	450			
<i>PvuII</i>	C			C		C				
	11500			8900		8600				
	4800			6100		5900				
				2050		2200				
<i>SacI</i>	C	B	D	C	B	C				
	7300	11700	7300	8900	12900	12900				
	4400	2800	2800	8000	3600	2900				
	2800	750	2750			950				
	750	510	1750							
	510	400	750							
	400		510							
<i>SnaI</i>	C	B		C	B	D	E	B	A	C
	8000	8000		4000	4000	4000	4000	5100	5100	5100
	3150	3150		2900	2900	2900	2900	3000	3000	2450
	2750	2650		1750	1750	1650	1550	1550	1750	1750
	2350	2350		1350	1650	1550	1450	1500	1550	1550
		100		1450	1550	1450	1350	1375	1375	1375
		1200		1450	1350	1200	1200	1200	1200	
		1100		1100	1100	1100	490	900	900	
		750		750	750	750	330	490	490	
		500		500	500	500	250	330	390	
		450		490	490	490		330		
<i>XbaI</i>	C	B								
	7400	7400								
	7400	6200								
	1550	1550								

Appendix 2

Mean values (\pm SE with coefficients of variation in parentheses) of morphological measurements on mainland and island sampling sites

Species/Location	n	Bill length (mm)	Bill width (mm)	Bill depth (mm)	Tarsus (mm)	Mid-Toe (mm)	Sternum (mm)	Wing (mm)	Tail (mm)	Mass (g)
Crimson-backed tanager										
Atlantic	21	12.11 \pm 0.11 (4.3)	7.91 \pm 0.14 (8.4)	7.88 \pm 0.08 (4.7)	21.61 \pm 0.21 (4.4)	13.95 \pm 0.19 (4.4)	21.10 \pm 0.22 (4.7)	74.20 \pm 0.57 (3.5)	71.60 \pm 0.49 (3.1)	35.90 \pm 0.39 (6.8)
Gambua	16	12.07 \pm 0.14 (4.6)	7.87 \pm 0.16 (8.0)	8.01 \pm 0.16 (7.8)	21.99 \pm 0.24 (4.4)	14.18 \pm 0.24 (4.4)	21.60 \pm 0.28 (5.1)	74.90 \pm 0.77 (4.1)	72.10 \pm 0.76 (4.2)	27.00 \pm 0.61 (9.0)
Summit	30	12.23 \pm 0.09 (5.8)	7.71 \pm 0.14 (9.8)	7.75 \pm 0.07 (5.0)	22.29 \pm 0.28 (6.9)	14.59 \pm 0.22 (8.2)	21.70 \pm 0.14 (3.6)	75.90 \pm 0.71 (5.2)	71.60 \pm 0.34 (2.6)	26.20 \pm 0.93 (19.5)
Pacheca	16	11.80 \pm 0.16 (4.3)	8.24 \pm 0.36 (14.4)	8.20 \pm 0.16 (6.7)	20.49 \pm 0.14 (2.3)	13.30 \pm 0.24 (6.0)	20.68 \pm 0.25 (4.0)	75.30 \pm 0.70 (3.1)	70.10 \pm 0.44 (2.1)	26.10 \pm 0.60 (7.9)
Suboga	15	11.66 \pm 0.11 (3.5)	8.87 \pm 0.20 (8.7)	7.88 \pm 0.12 (5.7)	20.25 \pm 0.20 (5.8)	13.15 \pm 0.25 (7.4)	20.70 \pm 0.16 (2.9)	75.80 \pm 0.66 (3.4)	70.00 \pm 0.48 (2.5)	26.40 \pm 0.39 (5.7)
Chopora	7	11.28 \pm 0.19 (4.3)	7.81 \pm 0.20 (6.9)	7.44 \pm 0.10 (3.7)	20.45 \pm 0.41 (5.3)	13.80 \pm 0.25 (4.9)	20.63 \pm 0.57 (7.2)	72.60 \pm 1.10 (4.1)	65.90 \pm 0.96 (3.9)	24.90 \pm 0.63 (6.7)
Bolafos	16	11.33 \pm 0.24 (9.0)	7.85 \pm 0.27 (14.6)	7.37 \pm 0.24 (13.6)	19.93 \pm 0.31 (6.7)	12.85 \pm 0.32 (10.0)	20.24 \pm 0.41 (8.7)	74.00 \pm 0.50 (2.9)	69.20 \pm 0.57 (3.5)	26.20 \pm 0.44 (7.1)
Rey	20	12.02 \pm 0.12 (3.3)	7.91 \pm 0.26 (11.0)	7.51 \pm 0.08 (3.3)	20.45 \pm 0.36 (5.6)	12.83 \pm 0.23 (5.9)	19.83 \pm 0.37 (6.1)	71.50 \pm 0.74 (3.4)	67.10 \pm 0.69 (3.4)	25.90 \pm 0.63 (6.7)
Mainland	67	12.13 \pm 0.06 (4.2)	7.82 \pm 0.05 (9.7)	7.83 \pm 0.06 (5.8)	22.00 \pm 0.15 (5.8)	14.29 \pm 0.13 (7.6)	21.48 \pm 0.12 (4.4)	75.10 \pm 0.43 (4.5)	71.70 \pm 0.28 (3.1)	36.30 \pm 0.45 (14.1)
Islands	76	11.84 \pm 0.08 (5.6)	8.23 \pm 0.10 (12.6)	7.68 \pm 0.09 (9.1)	20.29 \pm 0.15 (5.0)	13.10 \pm 0.13 (7.8)	20.43 \pm 0.16 (3.9)	74.60 \pm 0.36 (3.8)	66.80 \pm 0.33 (3.7)	26.00 \pm 0.24 (7.3)
Blue-gray tanager										
Atlantic	19	9.52 \pm 0.12 (5.6)	6.62 \pm 0.12 (7.9)	6.66 \pm 0.60 (3.9)	20.04 \pm 0.18 (3.9)	14.06 \pm 0.27 (8.1)	23.98 \pm 0.53 (9.6)	83.70 \pm 0.64 (3.3)	60.70 \pm 1.40 (9.8)	32.60 \pm 1.40 (18.9)
Gambua	22	6.30 \pm 0.10 (7.4)	6.31 \pm 0.10 (7.4)	6.72 \pm 0.05 (3.5)	20.21 \pm 0.37 (8.9)	13.46 \pm 0.23 (7.4)	24.15 \pm 0.44 (8.8)	85.70 \pm 0.39 (3.3)	62.50 \pm 0.53 (4.1)	31.60 \pm 0.60 (9.3)
Summit	16	6.60 \pm 0.09 (11.2)	6.60 \pm 0.20 (11.2)	6.84 \pm 0.24 (13.1)	20.03 \pm 0.37 (8.9)	14.36 \pm 0.27 (6.9)	24.68 \pm 0.19 (2.9)	83.17 \pm 1.00 (4.7)	61.60 \pm 0.53 (3.2)	29.10 \pm 0.57 (7.3)
Pacheca	25	9.83 \pm 0.09 (4.8)	7.45 \pm 0.10 (5.3)	7.09 \pm 0.05 (3.7)	20.31 \pm 0.39 (10.4)	14.95 \pm 0.31 (11.3)	25.36 \pm 0.20 (4.3)	87.40 \pm 0.42 (2.7)	63.50 \pm 0.46 (4.0)	33.80 \pm 0.39 (6.2)
Suboga	18	9.76 \pm 0.09 (4.2)	7.59 \pm 0.10 (5.3)	7.03 \pm 0.06 (3.7)	20.41 \pm 0.22 (4.5)	13.66 \pm 0.26 (7.9)	25.66 \pm 0.45 (7.5)	87.30 \pm 0.64 (3.1)	64.90 \pm 0.52 (3.4)	34.20 \pm 0.40 (5.0)
Chopora	17	10.10 \pm 0.23 (7.9)	7.48 \pm 0.22 (10.3)	7.29 \pm 0.36 (16.9)	20.33 \pm 0.28 (4.8)	13.83 \pm 0.27 (6.9)	24.70 \pm 0.37 (5.2)	87.80 \pm 0.76 (3.0)	65.30 \pm 1.80 (9.4)	32.80 \pm 2.90 (31.2)
Bolafos	4	7.81 \pm 1.10 (28.6)	6.50 \pm 1.00 (36.9)	6.20 \pm 1.04 (40.3)	18.27 \pm 1.20 (13.6)	11.60 \pm 0.83 (14.4)	22.73 \pm 1.20 (10.5)	87.80 \pm 1.00 (2.3)	62.80 \pm 0.48 (1.5)	32.30 \pm 1.30 (8.1)
Rey	16	9.48 \pm 0.19 (8.1)	7.10 \pm 0.14 (8.0)	6.74 \pm 0.08 (4.9)	20.10 \pm 0.28 (5.5)	13.72 \pm 0.23 (6.7)	25.36 \pm 0.18 (3.0)	86.10 \pm 0.67 (3.1)	62.30 \pm 0.65 (4.2)	33.10 \pm 0.73 (8.8)
Island	80	9.68 \pm 0.10 (8.3)	7.32 \pm 0.09 (11.0)	6.94 \pm 0.09 (11.4)	20.20 \pm 0.18 (8.0)	14.08 \pm 0.17 (10.9)	25.20 \pm 0.17 (5.9)	87.21 \pm 0.28 (2.9)	63.80 \pm 0.37 (5.2)	33.50 \pm 0.49 (13.0)
Mainland	57	9.40 \pm 0.07 (5.5)	6.55 \pm 0.07 (8.5)	6.73 \pm 0.06 (7.2)	20.11 \pm 0.21 (7.8)	14.73 \pm 0.17 (11.0)	24.23 \pm 0.26 (8.0)	84.40 \pm 0.43 (3.9)	61.60 \pm 0.52 (6.4)	31.30 \pm 0.37 (13.7)
Straw-colored saltator										
Atlantic	23	13.87 \pm 0.17 (5.8)	8.85 \pm 0.15 (7.9)	11.24 \pm 0.14 (5.9)	23.33 \pm 0.28 (5.0)	16.12 \pm 0.21 (6.4)	23.72 \pm 0.27 (5.5)	86.00 \pm 0.70 (3.9)	82.70 \pm 0.62 (3.6)	38.00 \pm 1.00 (12.8)
Gambua	17	13.83 \pm 0.20 (5.0)	9.47 \pm 0.29 (11.0)	11.69 \pm 0.12 (3.4)	23.70 \pm 0.32 (4.7)	16.60 \pm 0.36 (7.6)	24.31 \pm 0.26 (3.7)	85.80 \pm 0.96 (3.9)	83.30 \pm 1.20 (4.9)	37.30 \pm 0.70 (6.4)
Summit	15	13.48 \pm 0.14 (3.9)	9.10 \pm 0.18 (7.6)	11.49 \pm 0.12 (4.0)	23.37 \pm 0.64 (9.9)	16.76 \pm 0.34 (7.6)	23.50 \pm 0.26 (4.2)	86.60 \pm 0.61 (2.6)	82.10 \pm 1.10 (5.2)	36.50 \pm 0.98 (9.7)
Pacheca	19	13.20 \pm 0.12 (3.5)	9.78 \pm 0.18 (7.9)	11.67 \pm 0.09 (3.3)	24.26 \pm 0.18 (3.2)	16.25 \pm 0.29 (7.9)	23.86 \pm 0.41 (7.4)	88.60 \pm 0.58 (2.9)	83.10 \pm 0.59 (3.1)	40.00 \pm 0.52 (5.7)
Suboga	4	13.61 \pm 0.31 (5.1)	9.74 \pm 0.22 (5.1)	11.38 \pm 0.12 (2.4)	24.20 \pm 0.18 (1.7)	15.57 \pm 0.54 (7.7)	25.00 \pm 0.62 (5.6)	87.60 \pm 1.70 (4.4)	83.60 \pm 1.70 (4.5)	38.20 \pm 0.30 (9.0)
Chopora	17	13.26 \pm 0.11 (3.3)	9.24 \pm 0.14 (6.0)	11.17 \pm 0.12 (4.7)	23.87 \pm 0.37 (6.1)	15.90 \pm 0.20 (4.9)	24.38 \pm 0.29 (4.9)	87.40 \pm 0.66 (3.0)	82.50 \pm 0.62 (2.9)	36.70 \pm 0.65 (7.1)
Bolafos	21	13.66 \pm 0.23 (8.2)	9.20 \pm 0.25 (12.4)	10.95 \pm 0.22 (9.1)	23.30 \pm 0.24 (4.4)	14.98 \pm 0.29 (8.9)	23.15 \pm 0.35 (7.0)	86.10 \pm 0.75 (3.9)	81.40 \pm 0.64 (5.6)	36.00 \pm 0.44 (5.6)
Rey	18	12.84 \pm 0.12 (3.8)	9.04 \pm 0.15 (8.6)	10.74 \pm 0.12 (4.7)	23.30 \pm 0.24 (4.4)	14.90 \pm 0.20 (5.8)	23.60 \pm 0.27 (4.8)	82.90 \pm 0.66 (3.4)	78.90 \pm 0.70 (3.7)	34.60 \pm 0.50 (6.1)
Mainland	55	37.42 \pm 0.58 (0.11)	9.06 \pm 0.11 (8.8)	11.42 \pm 0.08 (5.0)	23.72 \pm 0.24 (7.1)	16.42 \pm 0.17 (7.1)	23.84 \pm 0.17 (4.9)	86.10 \pm 0.43 (3.5)	82.70 \pm 0.52 (4.4)	37.40 \pm 0.58 (10.7)
Island	79	36.94 \pm 0.34 (8.1)	9.34 \pm 0.09 (9.3)	11.15 \pm 0.08 (6.3)	23.58 \pm 0.14 (5.2)	15.49 \pm 0.14 (7.9)	23.83 \pm 0.17 (6.5)	86.30 \pm 0.40 (4.1)	81.60 \pm 0.36 (3.9)	36.90 \pm 0.34 (8.2)