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Genetic structure in a tropical lek-breeding bird, the blue manakin (*Chiroxiphia caudata*) in the Brazilian Atlantic Forest

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Abstract

Determining the genetic structure of tropical bird populations is important for assessing potential genetic effects of future habitat fragmentation and for testing hypotheses about evolutionary mechanisms promoting diversification. Here we used 10 microsatellite DNA loci to describe levels of genetic differentiation for five populations of the lek-mating blue manakin (*Chiroxiphia caudata*), sampled along a 414-km transect within the largest remaining continuous tract of the highly endangered Atlantic Forest habitat in southeast Brazil. We found small but significant levels of differentiation between most populations. F_{ST} values varied from 0.0 to 0.023 (overall F_{ST} = 0.012) that conformed to a strong isolation by distance relationship, suggesting that observed levels of differentiation are a result of migration–drift equilibrium. N_em values estimated using a coalescent-based method were small (≤ 2 migrants per generation) and close to the minimum level required to maintain genetic similarity between populations. An implication of these results is that if future habitat fragmentation reduces dispersal between populations to even a small extent, then individual populations may undergo a loss of genetic diversity due to an increase in the relative importance of drift, since inbreeding effective population sizes are relatively small (N_e ~1000). Our findings also demonstrate that population structuring can occur in a tropical bird in continuous habitat in the absence of geographical barriers possibly due to behavioural features of the species.

Keywords: effective population size, gene flow, microsatellite DNA, Neotropical passerine birds, Pipridae

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Introduction

Determining the genetic structure of vertebrate populations in the tropics is essential for testing hypothesis about factors promoting diversification (cf. Mortiz *et al*. 2000). For species living in habitats that are experiencing high levels of fragmentation and destruction, information on genetic structure can also help identify the appropriate spatial scales for defining conservation units (Moritz 1994; Paetkau 1999; Paetkau *et al*. 2004) and provide insights as to the potential genetic effects of future fragmentation that would reduce gene flow between populations. For these reasons, birds living in the tropics are of special interest. However, information on tropical bird population structure remains scarce despite the exceptionally high diversity of species and the severe destruction which is ongoing in particular species-rich habitats, such as the Brazilian Atlantic Forest (Myers *et al*. 2000) (but see Smith *et al*. 1997; Bates *et al*. 1999; Bates 2000; Brown *et al*. 2004; Galbusera *et al*. 2004; Smith *et al*. 2005).

Neotropical passerine birds that inhabit forest understorey are thought to be highly sedentary, which might lead to greater genetic differentiation among populations than in temperate species (Bates *et al*. 1999; Bates 2000;

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Brown *et al*. 2004). In support of this claim, Bates (2000) found substantial levels of divergence based on allozymes among populations of four Amazonian passeriform birds, all located within 200 km of one another in continuous habitats. Studies using mitochondrial DNA variation have also revealed significant intraspecific differentiation in a set of Amazonian (Bates *et al*. 1999) and southern Costa Rican rainforest species (Brown *et al*. 2004). Nevertheless, the types of genetic markers that have previously been used may not be informative about the ecological and evolutionary processes that generate structure in these birds. In particular, more detailed insights into these processes can be gained when data from highly variable nuclear markers, such as microsatellites, are combined with analyses of gene flow and effective population size based on coalescent theory (Beerli & Felsenstein 1999, 2001). However, such an approach has only rarely been applied to Neotropical birds (but see Höglund & Shorey 2003; McDonald 2003). Here, we use microsatellite DNA markers to analyse population structure in blue manakin (*Chiroxiphia caudata*) sampled from within the largest remaining continuous tract of the highly endangered Atlantic Forest in southeast Brazil.

Manakins are small, nonmigratory, frugivorous birds endemic to the Neotropics. They are widely distributed in tropical and subtropical forest understorey habitat and are remarkable for their lek-mating behaviour and striking sexual dimorphism in plumage (Ridgely & Tudor 1994; Sick 1997; Brumfield & Braun 2001). Species within the genus *Chiroxiphia* perform highly specialized courtship displays in which males aggregate at traditional arenas, or 'leks', where they perform a precopulatory dance while courting visiting females. Each lek consists of between two and six males that form a dominance hierarchy with respect to copulations with females but are cooperative with respect to courtship displays (Foster 1981; McDonald & Potts 1994). Subordinate males expend substantial energy courting, which increases the fitness of the alpha male, but does not yield an immediate benefit to the subordinates (Foster 1981). It has therefore been hypothesized that kin selection could be involved, providing indirect genetic benefits to subordinate individuals (McDonald & Potts 1994; Shorey *et al*. 2000; Höglund & Shorey 2003). However, genetic analyses of one species in this genus (*Chiroxiphia linearis*) has shown that cooperating males are unrelated (McDonald & Potts 1994), which suggests that direct but long-delayed benefits, such as access to rare copulations and inheritance of the alpha position may better explain the evolution of this type of cooperation (Foster 1981; McDonald & Potts 1994). The generality of these results is unclear since relatedness among males has not been examined within any other species of the genus *Chiroxiphia*. Besides, other lekking birds, including manakins (*Manacus manacus*), have shown evidence for kinship among males at the same lek (Höglund & Shorey 2003).

These details of social behaviour are important, since they could have a significant impact on the genetic structure of these birds. Because leks remain in the same locations over long periods of time, if kin selection occurs, then male dispersal is expected to be limited, although females that are presumed to visit different leks could disperse more widely. More significantly, because the position of the alpha male in the hierarchy can persist over many years (Foster 1981; McDonald 1993; McDonald & Potts 1994), the variance in male mating success is one of the highest observed in vertebrates (McDonald 2003). If mating success is correlated with genetic success, the genetically effective population size of these birds might be substantially smaller than that of other species with mating success more evenly distributed among males. These features, combined with reduced dispersal of birds in understorey habitats, lead to the prediction that, in general, manakin populations should show high levels of genetic structure (McDonald 2003). However, contrary to this prediction, McDonald (2003) did not find significant divergence between two populations of long-tailed manakins (*C. linearis*), 115 km apart, in Costa Rica. Likewise, Höglund & Shorey (2003) failed to detect genetic structuring among representatives of nine leks of another complex lek-mating manakin, the white-bearded manakin (*Manacus manacus*), sampled over a 16 km^2 area in Trinidad. However, limitations present in one or both studies include the small number of populations sampled, low levels of variation present in the microsatellite loci used for the analyses, and the restricted geographical range over which populations were sampled.

As mentioned above, information on population structure can be useful from a conservation perspective if species studied are found in highly threatened habitats. This applies here because the populations of blue manakins we studied live in a critically endangered ecosystem, the Brazilian Atlantic Forest. This habitat is classified as one of the five most important diversity hotspots in the world due to an exceptionally high diversity of organisms including birds (Myers *et al*. 2000). Before significant human impacts occurred, the Atlantic Forest used to be the second most important humid forest habitat in South America (Oliveira-Filho & Fontes 2000). However, this ecosystem has been extensively exploited over the past 500 years, with most of the original forest logged for sugar cane and coffee plantations (Willis 1979; Willis & Oniki 1992; Aleixo & Galetti 1997). Currently, only 7.5% of the original 1 million km^2 area remains (Myers *et al*. 2000) and is distributed in many small isolated fragments and a few large tracts (SOS Mata Atlântica & INPE 1992), which continue to experience anthropogenic pressure. Despite the need to understand how fragmentation will impact species that currently exist within the few remaining large tracts of this habitat, little is known about the population structure of vertebrates in the Atlantic Forest (but see Grazziontin *et al*. 2006).

Here we describe the first analyses of the population genetic structure of an Atlantic Forest bird based on samples from populations that span the entire range of the last remaining continuous tract of this habitat. Our goals were to: (i) measure levels of genetic differentiation and variation for five populations of blue manakins sampled along a transect within a continuous tract of Atlantic Forest; (ii) use this information to make inferences about the evolutionary and ecological mechanisms that generate the observed patterns of structure; and (iii) discuss the potential evolutionary and conservation implications of these results for Neotropical birds that live in Atlantic Forest habitats.

Materials and methods

Study area

From 2003 to 2005, we collected blood samples from 143 birds at five sites within the largest remaining tract of the Atlantic Forest largely located in São Paulo state, southeastern Brazil. This tract consists of a set of contiguous conservation units and nonprotected areas that are distributed on the slopes of the mountains that face the Atlantic Ocean and represents the last large continuous corridor of this ecosystem. Although this corridor is narrower at the Cubatão region, the two major north–south forest blocks are still connected by suitable habitat (Fig. 1). This area contains more than 17 300 km² of humid forests (Secretaria do Meio Ambiente 1998; Morellato & Haddad 2000) and represents the only location in which the genetic structure of Atlantic Forest bird populations can be studied in the absence of local habitat fragmentation. Specifically, birds were sampled from multiple leks at Picinguaba (23°21′S, 44°50′W), Caraguatatuba (23°35′S, 45°25′W), Cubatão (23°54′S, 46°29′W), Carlos Botelho (24°04′S, 47°57′W) and PETAR (24°32′S, 48°41′W) (Fig. 1). The linear distance between sample locations ranged from a minimum of 67 km (Picinguaba–Caraguatatuba) to a maximum of 414 km (Picinguaba–PETAR).

Fig. 1 (A) Original distribution of the Atlantic Forest in Brazil (in grey) and the current remaining forested locations (darker areas). Source: Ministério do Meio Ambiente (2000). (B) Study areas in the largest remaining Atlantic Forest corridor, mostly located in São Paulo State (forest remnants in grey). Modified from: SinBiota/FAPESP (http://sinbiota.cria.org.br).

Bird sampling and DNA extraction

At each site, we captured individual birds using two to 15 12 × 2.5 m mist nets placed in front of the leks. Four to seven leks were sampled in each study area. We only sampled leks that were at least 500 m apart from another sampled lek to avoid duplicate sampling of the individuals from the same lek. All sampled birds were banded with metal rings for permanent identification. Upon capture, a 10–20 μL blood sample was obtained from each bird by cutting the nail tip. Blood was then mixed with an amount of 0.5 M EDTA that was three times the blood volume, and the blood/EDTA mixture was immediately added to a 1.5 mL tube containing 1 mL of 100% ethanol. After returning from the field, samples were placed in a –20 °C freezer for long-term storage. DNA was extracted using a standard phenol/chloroform protocol (Sambrook *et al*. 1989).

Genetic identification of sex

Since juvenile males retain a female-like plumage (Foster 1987), all green-plumaged individuals were sexed using the primers P2/P8 described by Griffiths *et al*. (1996, 1998). These primers amplify the homologous copies of the CHD (chromo-helicase-DNA-binding) genes, located in the Z and W sex chromosomes. In birds, the CHDZ and CHDW sequences differ in length due to a variation found in an intronic region, such that amplification products result in one band for males and two bands for females. Polymerase

chain reactions (PCRs) were performed according to Anciães & Del Lama (2002), and the amplification products run on 3% agarose gels. As a control, PCR products of 15 males identified by the definitive blue plumage were compared to the green individuals.

Microsatellite genotyping

DNA samples from all individuals were amplified at 10 species-specific microsatellite loci (*CHIR*1-16, *CHIR*1-18, *CHIR*2-9, *CHIR*3-2, *CHIR*3-15, *CHIR*3-22, *CHIR*3-27, *CHIR*4-21, *CHIR*4-33 and *CHIR*4-34 — see Francisco *et al*. 2004) using fluorescently labelled primers. Details on loci isolation and PCR conditions used to amplify individual loci are described in Francisco *et al*. (2004). Amplified products were run on an ABI 377 sequencer, and allele sizes scored using the software programs GENESCAN 3.0 and GENOTYPER 2.5 (Applied Biosystems).

Analyses of genetic variation and differentiation

For each population, we tested whether genotypes for each locus and over all loci were in Hardy–Weinberg equilibrium in the following two ways. First, we used the online version of the program GENEPOP (version 3.4; Raymond & Rousset 1995) to calculate expected (H_E) and observed (H_O) levels of heterozygosity, and then test for differences in these values using an exact test. Second, we used the program FSTAT (version 2.9.3.2) (Goudet 1995) to calculate the inbreeding coefficient (F_{IS}) as defined by Weir & Cockerham (1984), and then test if the observed value was significantly different from zero using a randomization procedure.

To assess levels of differentiation among populations, we used FSTAT to estimate F_{ST} as defined by Weir & Cockerham (1984) across all loci for each pairwise comparison of populations. To test if the values were significantly different from zero, we used the permutation procedure implemented in FSTAT, not assuming Hardy–Weinberg equilibrium. Pairwise population differentiation in genotype frequencies was assessed using an exact test, as implemented in GENEPOP.

Finally, we tested for the existence of a significant isolation by distance by using the Isold subroutine in GENEPOP to determine if there was significant positive relationship between the values of F_{ST} and the linear distance between all pairs of populations.

When multiple tests were conducted, we followed the suggestions of Narum (2006) and reassessed the critical level for significance for a given set of tests using both the more conservative approach of controlling experiment-wide error (Bonferonni correction) and the less conservative approach of controlling the false discovery rate as described by Benjamini & Yekutieli (2001). Critical values for both corrections are presented.

Effective population size and migration rates

We simultaneously estimated genetically effective migration rates between populations and long-term inbreeding effective population sizes for each population using the coalescent-based program MIGRATE, version 2.0 (Beerli & Felsenstein 2001). The parameters θ ($4N_e\mu$) and M (m/μ) (where μ and m are, respectively, mutation and migration rates), as well as their 95% confidence intervals (CI), were obtained using the likelihood option, and assuming stepwise mutation model for microsatellite loci. One advantage of MIGRATE is that it allows asymmetric values for M between pairs of populations. We estimated these parameters under the following two migration models that represent two plausible biological scenarios for gene flow in these birds. First, we ran a full migration model that allows the possibility of migration between any pair of populations, as assumed under the classic island model of migration (Wright 1943). This is justified by the relatively small geographical scale over which populations were sampled, which makes movement between any two populations hypothetically possible. Second, we also ran a restricted migration model in which migration could occur only between adjacent populations. This matches two key features of the manakin system: the linear spatial distribution of populations and our finding of a strong isolation-by-distance relationship in this system (see below). A run for each model consisted of 10 short chains of 10 000 steps each followed by three long chains of 1 million steps each with the first 10 000 steps of each of the long-chain runs discarded before parameter estimation. Each model was run twice from different starting random seeds to check whether the results were consistent. As described in Beerli (2004), N_e for each population was then estimated as $N_e = \theta/4\,\mu$, where μ was assumed to be 10^{-3} or 10^{-4} mutations/generation/locus, which are plausible rates (see Ellegren 2000) for the di-, tri- and tetranucleotide repeats responsible for the variation in these microsatellites (Francisco *et al*. 2004). To calculate values for N_em (number of migrants exchanged per generation), we multiplied θ ($4N_e\mu$) by M (m/μ).

Results

Levels of variability

Overall, we detected a mean of 16.4 alleles per loci (SD = 6.9, range: 5 [CHIR4-21] to 28 [CHIR1-16]) among the 143 birds that were genotyped (121 males and 22 females). Table 1 and the Appendix show observed and expected heterozygosities and F_{IS} values within each population averaged across all loci (Table 1) and on a locus-by-locus basis (Appendix). The pattern revealed, using either measure of variation, is similar: for all populations,

Table 1 Observed heterozygosities (H_O), expected heterozygosities (H_E), probability of deficit of heterozygotes calculated using score test (P_d), inbreeding coefficient (F_{IS}) and the probability that F_{IS} differed significantly from zero (P) using permutation procedure (10 000 randomizations) found in five populations of the blue manakin using 10 microsatellite loci

	Overall H_O	Overall H_E	H_O–H_E	P_d	F_{IS}	P
Picinguaba	0.783	0.827	–0.044	0.000	0.054	0.019
Caraguatatuba	0.745	0.809	–0.064	0.000	0.082	0.003
Cubatão	0.786	0.815	–0.029	0.001	0.037	0.111
Carlos Botelho	0.772	0.815	–0.043	0.007	0.054	0.008
PETAR	0.750	0.886	–0.111	0.000	0.054	0.007

Table 2 Pairwise measures of genetic differentiation among five populations of blue manakins. F_{ST} values and associated P values (in parentheses) are given above the diagonal whereas the P values for exact tests of genotypic differentiation across all loci are given below the diagonal. Critical value for $n = 10$ tests under Bonferroni correction is 0.005 and under false discovery rate (Benjamini & Yekutieli 2001) is 0.017

	PIC	CAR	CUB	CB	PET
PIC	—	0.0052 (0.35)	0.089 (0.02)	0.016 (0.005)	0.0227 (0.005)
CAR	0.325	—	0.000 (0.185)	0.0144 (0.005)	0.0171 (0.005)
CUB	0.027	0.296	—	0.0076 (0.005)	0.0117 (0.005)
CB	0.000	0.000	0.000	—	0.0108 (0.005)
PET	0.000	0.000	0.000	0.000	—

there was a small (mean = 5.8%) but significant difference in observed and expected heterozygosities, whereas four of five populations showed small but significantly positive F_{IS} values (mean overall $F_{IS} = 0.056$). When detected in microsatellite data, heterozygosity deficits or positive F_{IS} values are explained as due to biological causes such as inbreeding or the presence of null alleles. We feel that much of the deficit is due to inbreeding because: (i) no locus showed a significant heterozygosity deficit in all of the populations analysed as expected if null alleles were common at that locus; (ii) loci which showed a significant heterozygosity deficit in some populations showed an excess in others, as shown by negative F_{IS} values (i.e. *CHIR*1-16, *CHIR*3-2, *CHIR*3-27 and *CHIR*4-33); (iii) individuals that failed to amplify completely (potentially homozygous for null alleles) were not found, suggesting that null alleles, if present, were at low frequencies; and (iv) analysis using other measures of individual relatedness between males at particular leks suggest that within each population, a small number of them may be close relatives (M. R. Francisco, H. L. Gibbs, P. M. Galetti Junior, unpublished data).

Population differentiation and isolation by distance

Three related results demonstrate moderate but significant genetic structure among these bird populations. First, the overall F_{ST} value across all populations and loci was small (0.012) but positive and statistically significantly different from zero ($P = 0.00083$). Most pairwise F_{ST} values, which ranged between 0.0 and 0.023, were also significant (Table 2) with the exception that Caraguatatuba did not differ from adjacent populations to the north (Picinguaba) or south (Cubatão). Second, analyses of differentiation in genotypic frequencies using the exact test implemented in GENEPOP also showed that most populations are genetically distinct from each other (Table 2). Third, except for the locus *CHIR*4-21, private alleles (alleles found only in a single population) were observed for the nine other loci. Of the 164 alleles found in this study, 39 (23.8%) were only found in a single population. The average number of private alleles per locus was 3.90 (SD = 2.51) and the within- population frequencies of these alleles ranged from 0.013 to 0.068. Across loci, individual populations had between four (Cubatão) to 12 (PETAR) private alleles (7.80, SD = 3.19), which represented from 4.1 to 10.4% of the total number of alleles in a population.

Finally, there was a significant positive correlation ($r = 0.88$) between pairwise values of F_{ST} and the linear distance between populations (Mantel test, $P = 0.008$) (Fig. 2). Based on an r^2 value of 0.78, linear distance between populations explains an exceptionally high proportion (78%) of the variation in F_{ST}, demonstrating a strong pattern of isolation by distance in these birds.

Estimates of gene flow and genetically effective population sizes

Comparison of parameters estimated using MIGRATE under the full ('island') and restricted ('stepping stone') migration models were qualitatively similar (data not

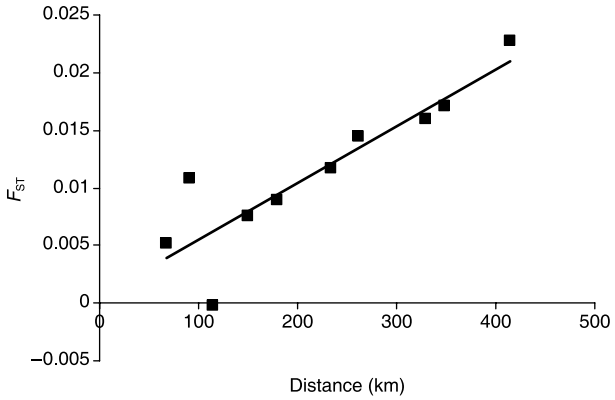

Fig. 2 Isolation- by-distance plot for F_{ST} vs. the linear geographical distance between five blue manakin populations. A Mantel test provides evidence for a strong effect of isolation by distance (P = 0.008; 100 000 randomizations). The best fit linear equation was F_{ST} = 0.00066 + 0.00005 (km).

Table 3 Estimates of N_e for individual blue manakin populations based on values for two plausible mutation rates (see Ellegren 2000)

		Mutation rate	
Population	θ ($4N_e\mu$)	10^3	10^4
PET	1.26	420	4200
CB	0.33	82.5	825
CUB	0.27	67.5	675
CAR	0.45	112.5	1125
PIC	0.61	152.5	1525
Mean	0.67	167	1670

shown). Because we judge the restricted model to be more biologically realistic due to the strong isolation-by-distance pattern that was observed, we present parameter estimates from this model only. Here, the mean value for θ across all populations was 0.67 (range: 0.27–1.26) (Table 3). The high value (from PETAR) seems anomalous and is not matched by a similarly high value for this population under the full-migration model (value under island model = 0.43), and so we suspect that in general, the most plausible values for θ are ≤ 1.0. Nonetheless, using the given estimates, the effective population sizes implied by these values ranged from 67 to 420 (mean = 167, SD = 145) under a mutation rate of 10^{-3} across all loci, and 675–4200 (mean = 1670, SD = 1451) under a mutation rate of 10^{-4}. Given the numerous assumptions about mutation rates, mutation process and consistency across loci, we can only conclude that local inbreeding effective population size of these birds is on the order of 10^3 individuals.

Migration rates (M) between populations averaged 15.92 (SD = 5.89) and were approximately symmetric between populations, although there was about threefold greater migration from PETAR to Carlos Botelho than in the opposite direction and greater migration from Caraguatatuba to Picinguaba than in the opposite direction. When translated into number of effective migrants per generation (N_em), all values were < 2.3 and averaged 1.60 (SD = 0.84) (Table 4). Of particular note is that several values were < 1.0. Thus, despite the geographical closeness of these populations, per-generation migration rates were low and approached the 'one migrant per generation', minimum value that is necessary to maintain genetic cohesiveness between populations under different models of population structure (Wright 1931; Wang 2004).

Table 4 Pairwise estimates of the migration rate (M) and numbers of genetically effective migrants per generation (N_em) between adjacent populations of blue manakins under a stepping-stone migration model. Km is the linear geographical distance between a given pair of populations

	M a→b b→a			
Pairs of populations (a × b)		95% CI	N_em	Km
Caraguatatuba × Picinguaba	10.53	9.53–11.60	3.29	67
	24.85	22.59–27.29	0.71	
Caraguatatuba × Cubatão	12.22	10.63–13.87	0.98	114
	14.06	12.06–16.12	0.95	
Cubatão × Carlos Botelho	21.26	19.32–23.29	1.65	150
	18.16	16.42–20.03	1.45	
Carlos Botelho × PETAR	4.46	6.63–8.00	2.30	91
	20.39	17.16–20.94	1.47	

Discussion

Population structure, N_e *and gene flow in a lek-breeding tropical bird*

This study is one of the first to use microsatellite DNA variation to assess population structure in a Neotropical passerine bird in an unfragmented habitat (see also McDonald 2003 and Höglund & Shorey 2004). We found moderate but significant genetic structure among populations of these birds over a much smaller spatial scale than those observed for temperate zone passerine species which are migratory and have broad geographical distributions (see McDonald 2003 for review). This supports the idea that, in general, tropical species may show more structure due to sedentary behaviour. However, it is clear that generalizations about genetic structure in tropical vs. temperate birds are overly simplistic. As shown in Table 2 in McDonald (2003), there are a number of temperate species with similar social behaviour to manakins (e.g. year-round resident lek breeding grouse) that also show similar levels of genetic structure. An important

contribution of our study is that it shows that population structuring can occur in a tropical bird even in continuous habitats, possibly due to behavioural features of the species. As such, it contrasts with most studies on South American birds which have focused on the role of geographical barriers, such as rivers and mountains or habitat differences as causes of diversification (Brumfield & Capparella 1996; Bates *et al*. 1999; Bates 2000; Aleixo 2004).

Our findings that manakin populations have small long-term effective population sizes, limited gene flow between adjacent populations, and strong isolation-by-distance relationships in a habitat that has remained undisturbed for at least 13 000 BP (Viadana 2002), support the idea that populations of this species are at or very close to genetic equilibrium. Hence, the observed differentiation is likely the result of drift–migration equilibrium (cf. Hutchinson & Templeton 1999). As a consequence, we feel that the observed levels of differentiation are not influenced by retained ancestral polymorphism as is the case for at least some temperate species with large population sizes which inhabit recently glaciated habitats (cf. Gibbs *et al*. 2000; Bulgin *et al*. 2003). This satisfies a key assumption (populations being at genetic equilibrium) of the coalescent model incorporated in MIGRATE that we used to estimate gene flow and population size.

The effective local genetic population size of these birds was on the order of 10^3, while for some temperate passerine species analysed using similar coalescent-based methods, it was on the order of 10^4–10^5 (Gibbs *et al*. 2000; Griswold & Baker 2002; Bulgin *et al*. 2003). As suggested previously, we feel that there may be two major causes for reduced population sizes in these birds: an exceptionally high variance in male reproductive success in this lek-breeding species (McDonald 2003) and inbreeding. It is a standard result from population genetic theory that increases in variance in either male or female reproductive success can lead to increased genetic drift by reducing local population effective sizes (Crow & Kimura 1970; Wang 2004). As described above, detailed behavioural work by McDonald (2003) has shown that males in a closely related *Chiroxiphia* species (*C. linearis*) show the highest variance in male reproductive success recorded in vertebrates because of the disproportionately high success obtained by dominant alpha males relative to subordinates. This factor could explain reduced population size in *Chiroxiphia caudata* assuming similar reproductive behaviour of males, and also that measures of reproductive success (copulation frequency) used by McDonald (2003) translate into differences in genetic measures of success.

Inbreeding may also lead to a reduced effective population size (Crow & Kimura 1970; Wang 2004) and increase differentiation between local populations. The small but significant reductions in observed relative to expected heterozygosity found in most blue manakin populations indicate a small amount of inbreeding, which would reduce N_e. The mechanisms by which inbreeding occurs is unknown at present but additional analyses of patterns of relatedness of males and female captured at specific leks demonstrates that a small proportion of the males are close relatives of each other and some of the females are also related to the males displaying at a lek (M. R. Francisco, H. L. Gibbs, P. M. Galetti Junior, unpublished data). The relative importance of kin selection favouring relatedness among males at leks (Höglund *et al*. 1999; Petrie *et al*. 1999; Höglund & Shorey 2003; Bouzat & Johnson 2004; Krakauer 2005; but see McDonald & Potts 1994) and strong philopatry by both sexes leading to matings between relatives both seem plausible causes of inbreeding in the blue manakin that could lead to a reduced effective population size.

Significantly, genetically effective migration rates as estimated using MIGRATE were low and close to or marginally below the 'one migrant per generation' (OMPG) threshold originally proposed by Wright (1931, 1943) as necessary to maintain genetic cohesion between populations under the classic island model. Wang (2004) has recently shown that this model is relatively robust to violations of the long list of assumptions required by the island model (see Whitlock & McCauley 1999). In this situation, the OMPG rule may represent a minimum level of gene flow required since under a stepping-stone migration model (assumed to better represent manakin population structure), higher levels of genetically effective migration are required to maintain genetically connected populations (see Fig. 2 in Wang 2004). At face value, our results suggest that manakin populations experience just sufficient gene flow to maintain genetic cohesion within these populations and prevent strong differentiation from occurring.

We are cautious about this conclusion because the observed level of differentiation as measured by F_{ST} seems too low, given our estimates of N_em implying that we are underestimating the amount of migration that is actually occurring. Under the ideal island model, an N_em value of 2 (our approximate upper limit) should lead to an overall F_{ST} value of 0.12 using Wright's formula, $F_{ST} = 1/(4N_em + 1)$. However, our observed F_{ST} value is one order of magnitude smaller (0.012). We are not sure about the reason(s) for this discrepancy. One possibility is that because of the different assumptions which underlie the classic and coalescent-based models for estimating gene flow, the parameter estimates are not directly comparable. However, we are uncertain if this is the case, and until this issue is resolved, we need to be cautious in our conclusion that levels of gene flow between manakin populations are at the threshold level specified by the OMPG rule.

In addition, our results from MIGRATE are based on two other important assumptions. One, that the microsatellite loci used in the analyses evolve via a stepwise mutation model (Beerli 2004), and two, that our male-biased sample

of individuals is representative of structure shown by both sexes. Although we did not conduct any formal tests of which mutation model best describes the allelic variation at each locus (cf. Becquet *et al*. 2007), all loci showed a continuous distribution of allele sizes with in most cases, one to two peaks in allele frequencies (data not shown) which suggests that no locus shows a strong deviation from a stepwise mutation model. Nothing is known about sex-specific patterns of dispersal in these birds but analysis within each of these populations suggest that males and females may show similar levels of dispersal between leks within populations (M. R. Francisco, H. L. Gibbs, P. M. Galetti Junior, unpublished data). If this pattern also holds for between-population dispersal, then it suggests that a sample dominated by one sex (males in our case) will not give a biased view of the overall structure in this species.

Unlike many current studies, we did not use Bayesian clustering methods such as those implemented in the program STRUCTURE (Pritchard *et al*. 2000) that assigns individuals to populations, while simultaneously estimating the number of populations and their allele frequencies. Our reason is that such methods do not perform well in situations where the pattern of differentiation is one of isolation by distance as was observed in our data. In such situations, many individuals would have mixed membership in multiple populations, which usually results in a *K* (number of populations) estimate that is biologically unrealistic (Pritchard *et al*. 2000; Pritchard 2003).

Implications for conservation

We see two main implications of our results for conservation of vertebrates in the Brazilian Atlantic Forest. On the one hand, the fact that estimated levels of gene flow are sufficient to maintain genetic variability and prevent inbreeding within populations of these birds argues that there is a significant level of genetic connectedness between manakin populations in this largest remaining tract of this highly threatened tropical forest habitat. To the extent that manakins are representative of other birds present in this tract of forest, it suggests that there is a high level of genetic cohesion among populations of vertebrates that are found in this forest and that, from a genetic perspective, they can be managed as a single genetic unit.

On the other hand, if the gene flow estimates that we calculate are accurate, then they suggest that if any future anthropogenic effects disrupt migration rates between manakin populations to even a small extent, then local populations of these birds may suffer significant losses of genetic diversity and experience inbreeding depression. If these results are at all general to other vertebrates that live in this habitat, then they provide yet another compelling reason to preserve the integrity of this largest remaining tract of one of the most imperiled tropical forest ecosystems left on Earth. To determine how general these results are for other Atlantic Forest birds, we need to assess population genetic structure using similar genetic markers. In particular, it would be of great interest to determine the structure of species which are thought to have more limited (e.g. antbirds) and less restricted (e.g. thrushes and flycatchers) dispersal behaviour than manakins.

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M. R. Francisco, H. L. Gibbs and P. M. Galetti Junior have broad interests in the application of molecular markers to conservation and evolutionary questions in various organisms. The research of M. R. Francisco focuses on Brazilian Atlantic Forest birds, and the data presented here is part of his PhD thesis on the population genetic structure of birds in the largest remaining area of this highly endangered ecosystem. M. Galetti and V. O. Lunardi are field ecologists who are interested in conservation and behavioural aspects of Brazilian birds.

Appendix

Number of alleles per loci, number of individuals analysed (N), observed heterozygosities (H_O), expected heterozygosities (H_E), probability of deficit of heterozygotes calculated using score test (P_d), inbreeding coefficient (F_{IS}) and the probability that F_{IS} differed significantly from zero (P) using permutation procedure (10 000 randomizations) found in five populations of the blue manakin using 10 microsatellite loci. Critical value for n = 10 tests under Bonferroni correction is 0.005 and under false discovery rate (Benjamini & Yekutieli 2001) is 0.017

	No. of alleles (N)	H_O	H_E	P_d	F_{IS}	P
Picinguaba						
CHIR 1–16	20 (30)	0.766	0.928	0.002	0.177	0.006
CHIR 1–18	14 (30)	0.833	0.915	0.012	0.090	0.111
CHIR 2–9	16 (30)	0.966	0.925	0.897	–0.045	0.915
CHIR 3–2	5 (30)	0.666	0.608	0.535	–0.097	0.846
CHIR 3–15	10 (30)	0.733	0.846	0.048	0.135	0.049
CHIR 3–22	5 (30)	0.833	0.723	0.928	–0.155	0.963
CHIR 3–27	12 (30)	0.666	0.871	0.000	0.238	0.001
CHIR 4–21	5 (30)	0.766	0.658	0.687	–0.167	0.982
CHIR 4–33	14 (30)	0.900	0.889	0.646	–0.012	0.685
CHIR 4–34	13 (30)	0.700	0.907	0.001	0.232	0.001
Caraguatatuba						
CHIR 1–16	12 (20)	0.750	0.862	0.011	0.133	0.085
CHIR 1–18	13 (20)	0.850	0.863	0.315	0.016	0.527
CHIR 2–9	15 (20)	0.850	0.865	0.497	0.018	0.512
CHIR 3–2	5 (20)	0.750	0.696	0.063	–0.080	0.789
CHIR 3–15	10 (20)	0.750	0.877	0.030	0.148	0.068
CHIR 3–22	10 (20)	0.950	0.793	0.998	–0.203	0.996
CHIR 3–27	11 (20)	0.550	0.785	0.000	0.304	0.007
CHIR 4–21	4 (20)	0.700	0.665	0.093	–0.053	0.723
CHIR 4–33	12 (20)	0.800	0.854	0.127	0.065	0.290
CHIR 4–34	9 (20)	0.500	0.827	0.000	0.402	0.001
Cubatão						
CHIR 1–16	14 (22)	0.682	0.921	0.001	0.264	0.001
CHIR 1–18	13 (22)	0.954	0.898	0.923	–0.064	0.915
CHIR 2–9	13 (22)	0.863	0.868	0.197	0.006	0.564
CHIR 3–2	3 (22)	0.409	0.630	0.012	0.356	0.033
CHIR 3–15	10 (22)	0.773	0.883	0.066	0.127	0.098
CHIR 3–22	6 (22)	0.909	0.784	0.960	–0.163	0.967
CHIR 3–27	11 (22)	0.818	0.777	0.349	–0.054	0.793
CHIR 4–21	4 (22)	0.818	0.628	0.991	–0.310	0.992
CHIR 4–33	12 (22)	0.864	0.895	0.191	0.036	0.406
CHIR 4–34	11 (22)	0.773	0.869	0.013	0.114	0.132
Carlos Botelho						
CHIR 1–16	14 (39)	0.923	0.889	0.717	–0.039	0.835
CHIR 1–18	13 (39)	0.923	0.880	0.933	–0.049	0.882
CHIR 2–9	18 (39)	0.846	0.903	0.284	0.064	0.158
CHIR 3–2	5 (39)	0.641	0.678	0.306	0.056	0.342
CHIR 3–15	11 (39)	0.769	0.830	0.101	0.075	0.187
CHIR 3–22	12 (39)	0.846	0.790	0.754	–0.071	0.883
CHIR 3–27	10 (39)	0.692	0.842	0.025	0.180	0.015
CHIR 4–21	4 (39)	0.641	0.639	0.478	–0.003	0.576
CHIR 4–33	12 (39)	0.820	0.846	0.309	0.031	0.388
CHIR 4–34	13 (39)	0.615	0.849	0.000	0.278	0.001
PETAR						
CHIR 1–16	19 (32)	0.812	0.953	0.001	0.150	0.003
CHIR 1–18	14 (32)	0.937	0.896	0.855	–0.047	0.867
CHIR 2–9	15 (32)	0.968	0.883	0.795	–0.099	0.990
CHIR 3–2	4 (32)	0.594	0.666	0.166	0.110	0.257
CHIR 3–15	12 (32)	0.812	0.869	0.239	0.067	0.231
CHIR 3–22	10 (32)	0.812	0.775	0.826	–0.049	0.761
CHIR 3–27	11 (32)	0.750	0.802	0.099	0.066	0.284
CHIR 4–21	5 (32)	0.625	0.705	0.217	0.116	0.187
CHIR 4–33	12 (32)	0.781	0.871	0.005	0.104	0.086
CHIR 4–34	13 (32)	0.750	0.861	0.062	0.131	0.057