RESEARCH ARTICLE

Fine-scale genetic population structure of an understory rainforest bird in Costa Rica

Stefan Woltmann · Brian R. Kreiser · Thomas W. Sherry

Abstract We studied five populations of a rainforest understory insectivorous bird (*Myrmeciza exsul*, chestnut-backed antbird) in a fragmented landscape in northeastern Costa Rica in order to test hypotheses about the influence of forest fragmentation on population genetic structure using 16 microsatellite loci. Bayesian assignment approaches—perhaps the most conservative analyses we performed—consistently grouped the sites into two distinct groups, with all individuals from the smallest and most isolated population clustering separately from the other four sites. Additional analyses revealed (1) overall significant genetic structure; (2) a pattern of population differentiation consistent with a hypothesis of isolation by resistance (landscape connectivity), but not distance; and (3) relatively short dispersal distances indicated by elevated mean pairwise relatedness in several of the sites. Our results are somewhat surprising given the small geographic distances between sites (11–34 km) and the short time ($\sim$60 years) since wide-spread deforestation in this landscape. We suspect fine-scale genetic structure may occur in many resident tropical bird species, and in the case of the chestnut-backed antbird it appears that anthropogenic habitat fragmentation has important population genetic implications. It appears that chestnut-backed antbirds may persist in fragmented landscapes in the absence of significant migration among patches, but mechanisms that allow this species to persist when many other similar species do not are not well understood.

Keywords Antbird · Costa Rica · Deforestation · Dispersal · Population genetic structure · Microsatellites · Relatedness

Introduction

Population genetic structure of natural populations is an important aspect of ecological and evolutionary biology (Rousset 2004). The quantification of population genetic structure gives insight into ecological and evolutionary processes like dispersal, local adaptation and speciation, and the mechanisms (e.g., selection, genetic drift) that influence these processes at various temporal and geographic scales (Avise 2004; Hedrick 2005). A key factor to understanding population genetic structure is what has been called the genetic "patch size," or the minimal geographic scale at which significant population structure is present (e.g., Wilson et al. 2011).

Among studies of population structure in birds, there appears to be a pattern of smaller genetic patch size among non-migratory versus migratory species (e.g., Bates 2002; Veit et al. 2005; Coulon et al. 2008; Stenzler et al. 2009; Wilson et al. 2011). Evidence for this is especially convincing when migratory and non-migratory populations of the same species are considered (Arguedas and Parker 2000; Gibbs et al. 2000; Korfanta et al. 2005; Browne et al. 2008). Given that the majority of bird species live in tropical regions and are non-migratory (BirdLife International

S. Woltmann (✉) · T. W. Sherry
Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA 70118, USA
e-mail: stefan.woltmann@gmail.com

Present Address:
S. Woltmann
School of Renewable Natural Resources, Louisiana State University, Baton Rouge, LA 70803, USA

B. R. Kreiser
Department of Biological Sciences, The University of Southern Mississippi, Hattiesburg, MS 39406, USA

2000), we should expect fine-scale population structure to be relatively common in such species.

Several recent studies highlight both the presence of fine-scale genetic structure, and its ecological and evolutionary consequences at small geographic scales in non-migratory birds. In Ecuador, Andean valley populations of rufous-collared sparrow (*Zonotrichia capensis*) separated by only 25 km displayed significant population genetic structure, and considerably different breeding phenology (Moore et al. 2005). In Mexico, McCormack and Smith (2008) found morphological and genetic divergences related to foraging behavior among Mexican jay (*Aphelocoma ultramarina*) populations at different elevations. In the endangered habitat specialist Florida scrub jay (*A. coerulescens*), habitat fragmentation (both natural and anthropogenic) has led to significant population structure at small geographic scales (Coulon et al. 2008). Individuals are capable of dispersing across non-preferred habitat, but such individuals display both increased dispersal distances and reduced reproductive success compared to individuals that remain within non-fragmented habitats (Coulon et al. 2010).

A necessary first step in understanding ecological consequences of anthropogenic habitat fragmentation on genetic population structure is determining the relevant geographic scale over which genetic structure occurs. We studied populations of a rainforest understory insectivore in a fragmented landscape in northeastern Costa Rica in order to test hypotheses about the influence of forest fragmentation on population genetic structure of a non-migratory rainforest bird, the chestnut-backed antbird (*Myrmeciza exsul*). We used a suite of population genetic analyses to test four predictions: (1) the degree of population structure is greater between sites separated by deforested areas than between those in contiguous forest, (2) effective population size (N_e) is greater in populations within contiguous forest compared to those in fragments, and (3) mean pairwise relatedness of individuals is greater in forest fragments compared to populations in contiguous forest. Additionally, because sex-biased dispersal has important implications for overall gene flow, and because female-biased dispersal is prevalent in landbirds (although few tropical species have been evaluated), we predicted (4) dispersal is female-biased in chestnut-backed antbird.

Methods

Study organism and field methods

The chestnut-backed antbird (Thamnophilidae: *Myrmeciza exsul*) is a sub-oscine passerine found in forests and older second growth mainly below 1,000 m asl in southern

Central America and northwestern South America (Zimmer and Isler 2003). Individuals are paired and territorial year-round (Skutch 1969; Willis and Oniki 1972; Stutchbury et al. 2005), and individuals only uncommonly switch to another territory (Woltmann and Sherry 2011). The species prefers dense vegetation in closed forest, forest edges, and older second-growth, and has a largely invertebrate diet (Woltmann et al. 2010). Unlike many other antbird species, which tend to decline or disappear from small forest fragments (e.g., Sekercioglu et al. 2002; Lees and Peres 2006, 2008), chestnut-backed antbirds are often found in forest fragments (Wetmore 1972), and are commonly found in fragments as small as 4 ha in northern Costa Rica (Roberts 2007).

Birds were captured in mistnets and banded with a unique series of one aluminum and three plastic color bands. The sex of chestnut-backed antbirds is readily determined by underpart color. A blood sample (50–100 μl) was drawn from the brachial vein and stored in a lysis buffer (Seutin et al. 1991), and birds were released. Chestnut-backed antbirds exhibit extended parental care, thus we excluded from analyses all juvenile individuals associating with parents or suspected parents on a territory (Woltmann 2010).

We sampled five locations in both contiguous and fragmented forest in Heredía province in northern Costa Rica (Fig. 1). This landscape was completely forested until the 1950s, with deforestation occurring most rapidly after 1970 (Slud 1960; Joyce 2006). Currently, ∼30 % of the landscape consists of forest suitable for chestnut-backed antbirds (Sesnie et al. 2008). The La Selva Biological Preserve (hereafter La Selva, or abbreviated as LS) is a largely forested 1,600-ha research preserve surrounded on three sides by a largely agricultural matrix (mainly pasture, pineapple and bananas), but is contiguous with the >40,000 ha Braullio Carrillo National Park (BCNP) along its southern border. Plastico (PL) and Quebrada Gonzales (QG) are both within the Braullio Carrillo Park and are in forest contiguous with La Selva at distances of 14 (PL) and 31 km (QG). Tirimbina (TI) is a forested 300 ha private preserve 11 km southwest of La Selva, and is partially isolated from both La Selva and BCNP. The most isolated site (in terms of forest connectivity), Rio Frio (RF; 80 ha), is a privately managed forest in the town of Rio Frio, 16 km from both La Selva and Plastico.

As part of a longer-term study, we sampled at La Selva on a 300-ha focal plot every year from 2005 to 2009. Other sites were sampled in 2006 (TI), 2007 (PL), and 2009 (QG, RF). Because we found no evidence of temporal population structure within the La Selva samples (2005 vs 2009, $F_{ST} = 0.0022$, $P = 0.2218$), we present results from analyses using 2009 samples, for which sampling coverage of the focal study plot was greatest. However, results are
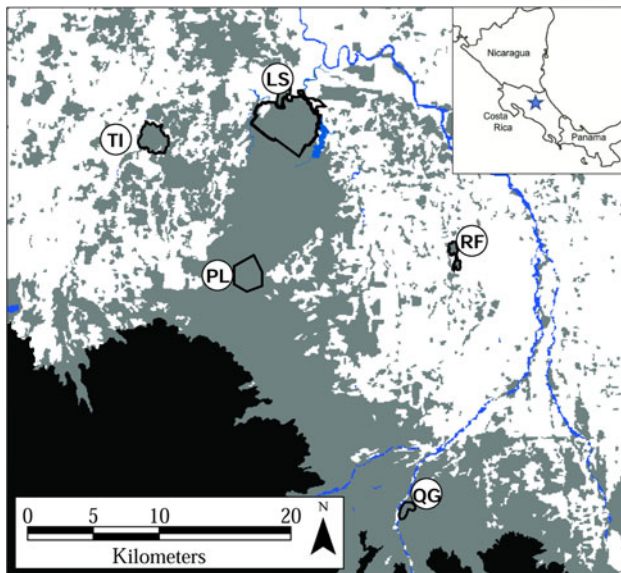
Fig. 1 Map of the study area in northeastern Costa Rica showing the location of the five study sites and landscape configuration. *Dark gray* indicates remaining forest, and *light shading* indicates non-forest (mainly agriculture and residential areas). The *area* in *black* is above 1,000 masl and beyond the altitudinal range of chestnut-backed antbirds. TI = Tirimbina Rainforest Preserve, LS = La Selva Biological Reserve, RF = Rio Frio, PL = "El Plastico," QG = Quebrada Gonzales. The latter two sites are in the Braullio Carrillo National Park (BCNP)

qualitatively the same regardless of which year of La Selva data were analyzed (results not shown); indeed, many of the same individuals were present in all five La Selva datasets.

Laboratory methods

Whole genomic DNA was extracted from the blood samples using DNEasy™ tissue kits from Qiagen, Inc. following the manufacturer's protocols. Individuals were genotyped at 15 microsatellite loci developed for chestnut-backed antbirds by Barnett et al. (2007) and Feldheim et al. (2010), and an additional locus developed for spotted antbird (*Hylophylax naevioides*) that cross-amplifies in chestnut-backed antbird (HyNa06; Woltmann et al. 2012; Table 1). We included loci that (1) could be scored consistently and had at least three alleles, (2) consistently met Hardy–Weinberg expectations (HWE; FSTAT v. 2.9.3.2; Goudet 2001), with no evidence for null alleles (MICRO-CHECKER v. 2.2.3; van Oosterhout et al. 2004), and (3) showed no consistent evidence of linkage disequilibrium (LD) with other loci (GENEPOP v.3.4; Raymond and Rousset 2004; all populations were tested for LD separately).

Amplifications were conducted using the PCR conditions in Barnett et al. (2007) and Feldheim et al. (2010);

annealing temperature for HyNa06 was 45 °C, with 32 cycles instead of 30 as for all other loci. Alleles were scored by eye with the aid of sizing standards, GeneImager IR™ software (LI-COR), and a subset of previously scored individuals. All gels were scored at least twice by the same observer. Potentially ambiguous genotypes were reamplified and run again adjacent to previously scored individuals until a consensus was reached. The proportion of individuals for which complete (16 locus) genotypes were obtained was 0.999.

Population analyses

Population differences in H_O, N_a (number of alleles standardized by sample size), and tests of HWE were calculated in FSTAT, and significance of F_{IS} was tested by 5,000 permutations in GENETIX (Belkhir et al. 1996–2004). We describe overall and pair-wise genetic differentiation in terms of F_{ST}, with significance determined by 5,000 permutations in GENETIX.

We used Mantel test (IBDWS v. 3.15; Jensen et al. 2005) to evaluate evidence of isolation by distance (IBD; $[F_{ST}/(1 - F_{ST})]$ against ln km) versus isolation by resistance (IBR; McRae 2006). IBR models incorporate factors other than distance (i.e., hypothesized ecological or physical barriers to dispersal), which in this case consisted of simple hypotheses of pairwise landscape connectivity based on our knowledge of habitat preferences of chestnut-backed antbird (1 = connected, 2 = moderately connected, 3 = isolated; see "Appendix Table 4" for distance and connectivity matrices). Significance was evaluated with 10,000 randomizations of the matrices.

Bayesian inference of population structure and models of gene flow

We used the Bayesian program STRUCTURE v. 2.3 (Pritchard et al. 2000) to infer the number of discrete genetic populations (K) among the sampled sites. We used both the original program implementation and the approach of Hubisz et al. (2009) to identify the best estimate of K from 1 to 7, using an admixture model with correlated allele frequencies and otherwise default parameter settings. For both analyses we ran 150,000 iterations (of which 50,000 were discarded as burn-in), and ran 20 replicates at each K. The most appropriate value of K was determined by examining the probability scores for each value of K and by examining the ΔK plot (Evanno et al. 2005) using CorrSieve v. 1.6–4 (Campana et al. 2011) in the R statistical environment (R Development Core Team 2011). We averaged all 20 runs at the best K with CLUMPP (Jakobsson and Rosenberg 2007), and visualized the results with DISTRUCT (Rosenberg 2004).

Table 1 Number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosity of 16 microsatellite loci within five populations of chestnut-backed antbird in Costa Rica

Locus	La Selva (46)			Plastico (29)			Quebrada Gonzales (20)			Tirimbina (34)			Rio Frio (35)		
	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e
HyNa06	17	0.891	0.910	14	1.000	0.915	12	0.850	0.900	14	0.971	0.904	13	0.943	0.893
Mex034	10	0.870	0.853	10	0.828	0.854	9	0.900	0.870	11	0.882	0.860	8	0.829	0.814
Mex080	5	0.826	0.756	5	0.862	0.694	4	0.600	0.654	6	0.676	0.612	5	0.629	0.591
Mex090	7	0.413	0.487	3	0.621	0.542	4	0.500	0.471	6	0.559	0.565	4	0.457	0.557
Mex140	12	0.891	0.858	12	0.897	0.875	13	1.000	0.903	13	0.882	0.833	10	0.914	0.805
Mex162	7	0.739	0.718	4	0.690	0.669	6	0.850	0.691	4	0.706	0.705	5	0.743	0.699
Mex176	9	0.717	0.723	9	0.690	0.803	10	0.850	0.810	7	0.735	0.747	8	0.800	0.796
Mex178	8	0.739	0.801	7	0.821	0.785	6	0.850	0.791	8	0.824	0.756	5	0.771	0.714
Mex191	9	0.783	0.815	11	0.828	0.833	9	0.950	0.831	8	0.824	0.807	9	0.829	0.794
MyEx19	13	0.891	0.882	11	0.862	0.863	14	0.900	0.886	14	0.941	0.896	9	0.886	0.818
MyEx20	4	0.609	0.702	4	0.759	0.735	4	0.600	0.681	4	0.765	0.692	4	0.600	0.609
MyEx27	9	0.652	0.692	7	0.690	0.637	5	0.700	0.586	6	0.471	0.616	3	0.657	0.591
MyEx41	9	0.848	0.768	6	0.862	0.769	7	0.900	0.780	7	0.824	0.808	7	0.629	0.721
MyEx46	3	0.587	0.578	3	0.724	0.651	3	0.750	0.645	3	0.706	0.639	3	0.514	0.558
MyEx52	15	0.978	0.886	12	0.828	0.839	12	0.950	0.900	17	0.912	0.881	12	0.912	0.862
MyEx61	10	0.804	0.819	11	0.862	0.838	8	0.650	0.705	11	0.882	0.859	8	0.886	0.831
Mean	9.2	0.765	0.765	8.1	0.801	0.769	7.9	0.800	0.757	8.7	0.785	0.761	7.1	0.750	0.728
F_{IS}	0.0115			−0.0246			−0.0318			−0.0161			−0.0149		

Sample sizes (number of individuals) are in parentheses following site names

To assess the robustness of inferences made using STRUCTURE (see e.g., Guillot et al. 2009), we also analyzed the data in another Bayesian clustering program, TESS (Francois et al. 2006; Chen et al. 2007), which uses a different approach to estimating the best K. For initial clustering in TESS we conducted 10 runs for each K_{max} 2–9 (admixture model), with 35,000 sweeps (5,000 discarded as burn-in), and otherwise default program parameters. The best K was chosen as the point of inflection on a plot of mean DIC (Deviance Information Criterion) against K_{max}, following Durand et al. (2009). We then ran an additional 200 replicates at the selected K_{max}, using the same parameters above, and selected the best 10 % of runs (lowest DIC) to export for analysis in CLUMPP and visualization in DISTRUCT.

We used the program 2MOD v.0.2 (Ciofi et al. 1999) to determine the more likely of two hypotheses of population divergence. 2MOD uses allele frequency distributions among sites to test the relative likelihoods of two general models: (1) drift only ("nonequilibrium" model), in which populations diverge in the absence of gene flow, and (2) drift-migration equilibrium ("equilibrium" model), in which allele frequencies drift in the presence of continued migration among (sub-)populations. For both hypotheses, it is assumed that the effects of mutation are small relative to the effects of drift and migration (Ciofi et al. 1999). We

analyzed both the entire five-site dataset, as well as various smaller subsets of the data to evaluate the effect of including putatively isolated sites. We conducted five separate runs consisting of 75,000 (5-site analyses) or 1×10^5 (subsets) MCMC iterations each, discarding the first 10 % of iterations as burn-in (Barnett et al. 2008). We follow the guidelines of Kass and Raftery (1995) in interpreting Bayes factors.

Effective population size (N_e) and relatedness within sites

We estimated N_e separately for each population using an unbiased LD approach using LDNE (Waples and Do 2008; monogamous mating model, lowest allele frequency = 0.05).

Groups of individuals in isolated habitat fragments often show increased pairwise relatedness, indicating that dispersal out of a patch is less likely than in large or contiguous areas (e.g., Stow et al. 2001; Miller-Sims et al. 2008). We calculated within-site pairwise relatedness (r of Queller and Goodnight 1989), and tested whether within-site relatedness was significantly different from 0 using 9,999 permutations and 10,000 bootstraps in GENALEX (v. 6.4; Peakall and Smouse 1996).

Sex-biased dispersal

Greater dispersal by females is the norm in landbirds (Greenwood and Harvey 1982), but few data exist for resident tropical birds (Clarke et al. 1997). We evaluated four commonly used lines of genetic evidence of sex-biased dispersal in FSTAT (see Goudet et al. 2002). If sex-biased dispersal is pronounced, mean pairwise relatedness (*r*) of individuals within each sex is expected to differ: the sex with shorter dispersal distances is expected to have higher pairwise relatedness. Assignment indices (*AI*) are generally used to assign individuals to source populations based on genotypic characteristics. If sex-biased dispersal leads one sex to disperse more frequently between populations, both mean corrected assignment index (*mAIc*), and the variance of corrected assignment index (*vAIc*) are expected to differ, with less success in assigning individuals to the correct population, and higher variance expected in the sex with greater dispersal. For similar reasons, when analyzed separately for each sex, F_{ST} values are expected to be greater in the more philopatric sex.

Results

Population analyses

We found no departures from HWE either within sites or as a whole, and no consistent evidence of LD between loci in any of the sites sampled. One pair of loci at LS (Mex140, Mex178) showed evidence of LD ($P < 0.0001$), but there was no suggestion of LD between these two loci in any other site. Mean overall H_O within populations was high (range 0.765–0.801), and differences in H_O between populations were not significant (Table 1). We found no indication of inbreeding in any site (all F_{IS} $P > 0.05$). Mean N_a among sites ranged from 7.1 (site RF) to 9.2 (site LS).

All five sites were significantly differentiated ($F_{ST} = 0.0184$, 95 % CI = 0.0118–0.0254, $P < 0.0001$). After excluding a potential outlier site (RF; see below), the remaining four sites were still significantly differentiated ($F_{ST} = 0.0112$, 95 % CI = 0.0058–0.0173, $P = 0.0112$),

indicating that site RF was not unduly influencing overall F_{ST}. All pairwise F_{ST} values remained significant ($P < 0.05$) after sequential Bonferroni correction, with the four highest values involving comparisons with the most isolated site (RF; Table 2). Combined with exact tests of differentiation (Goudet et al. 1996; Woltmann et al. 2012), the five sites could be considered separate populations (under the evolutionary paradigm considering panmixia) according to the criteria outlined by Waples and Gaggiotti (2006).

Simple Mantel tests indicated no significant correlation between genetic and geographic distance ($Z = 0.2390$, $r = 0.0863$, $P = 0.9732$), but a significant negative correlation between genetic distance and hypothesized connectivity ($Z = -0.4599$, $r = -0.8533$, $P = 0.0341$), supporting a hypothesis of IBR, but not IBD. A partial Mantel test of genetic distance and hypothesized connectivity was significant when controlling for geographic distance ($r = -0.8691$, $P = 0.0341$), again indicating that landscape features influenced population genetic structure far more than geographic distance.

Bayesian inference of population structure and models of gene flow

We expected that $K = 3$ (i.e., two isolated sites [TI], [RF], and three clustered contiguous sites [LS + PL + QG]). In contrast to this initial prediction, STRUCTURE analyses indicated the best $K = 2$, with only RF significantly differentiated from all other sites (Fig. 2). The delineation of RF as a distinctive population was evident in both STRUCTURE analyses, but was clearer using the Hubisz et al. (2009) approach (Fig. 3a). Subsequent analyses excluding RF failed to recover additional population substructure ($K = 1$). Analyses in TESS initially suggested $K = 4$, but visual inspection of ancestry coefficients in DISTRUCT showed that a more biologically meaningful interpretation was $K = 2$, as the additional two "populations" referred to small amounts of admixture in a few individuals randomly distributed among samples (figure not shown). We thus re-ran the analysis in TESS using $K_{max} = 2$, which produced a result similar to that from STRUCTURE (Fig. 3b).

Table 2 Pairwise F_{ST} values (and their statistical significance) for five populations of chestnut-backed antbird sampled in northern Costa Rica

	La Selva	Tirimbina	Plastico	Q. Gonzales
Tirimbina	0.013 (<0.001)			
Plastico	0.006 (0.022)	0.012 (<0.001)		
Q. Gonzales	0.013 (0.002)	0.013 (0.002)	0.012 (0.004)	
Rio Frio	0.029 (<0.001)	0.027 (<0.001)	0.022 (<0.001)	0.036 (<0.001)

P values were obtained using 5,000 permutations of the data. All *P* values are significant ($P < 0.05$) after sequential Bonferroni correction
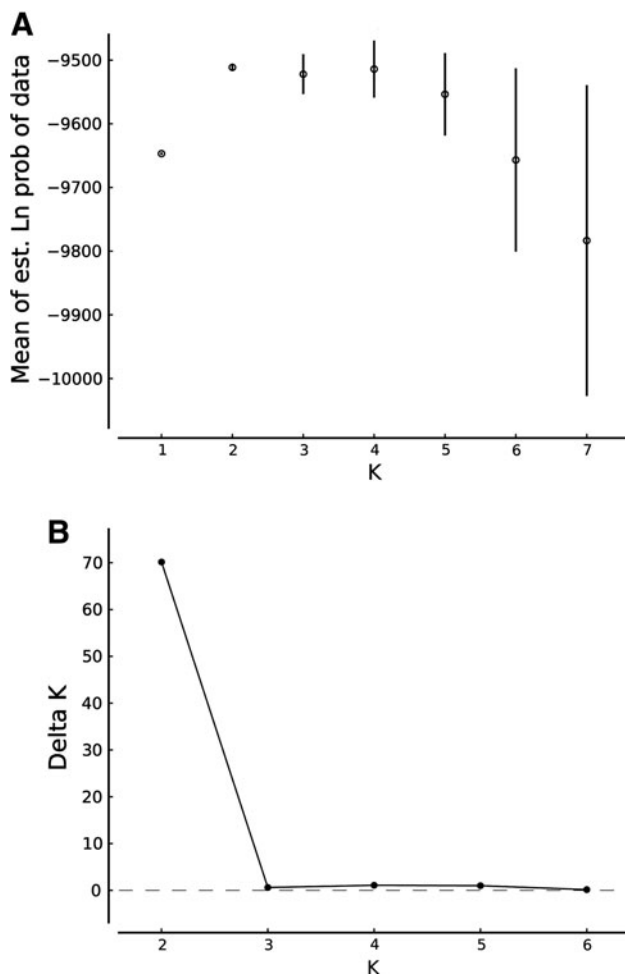
Fig. 2 **a** Plot of mean (±SD) penalized log likelihoods of Structure analyses for $K = 1$ through 7. The strongest and most consistent support is for $K = 2$. **b** ΔK plot (Evanno et al. 2005), showing greatest support for $K = 2$

Replicate runs in 2Mod produced highly congruent results, indicating that the MCMCs had converged, and we report here averaged results of the five replicate runs. In the five-population analyses, support was strong for the non-equilibrium model ($\overline{x}p$ (nonequilibrium) = 0.897 ± 0.019 SE, range 0.840–0.953; Bayes Factor = 10.5). Analyses of the three contiguous sites [LS, PL, QG] yielded no support for either model ($\overline{x}p$ (equilibrium) = 0.536 ± 0.013 SE, range 0.500–0.569; Bayes Factor = 1.2). Analyses of two other groupings ([LS, TI, PL] and [LS, PL]) also produced no support for either model. Longer runs (up to 3×10^5 iterations) on all datasets produced identical results. We conclude that the inclusion of the smallest and most isolated site (RF) provided strong signal for the nonequilibrium (drift only) model, but that the appropriate model of gene flow for other site groupings is not clear. Data from other groupings may fall further towards the equilibrium model in the continuum between the two models, or

alternatively the magnitude of genetic differentiation may be too weak in these datasets to provide strong evidence for either model.

Effective population size (N_e) and relatedness

Genetic effective population sizes were generally low (<200 in all but PL), but varied considerably among sites (Table 3). Using the criterion of non-overlapping 95 % CIs, N_e of PL was significantly greater than all other populations, and N_e of RF was significantly less than all other sites. Within-site pairwise relatedness varied among sites, being indistinguishable from 0 in PL and LS, but significantly >0 in QG and RF, and marginally >0 in TI (Fig. 4).

Sex-biased dispersal

Conventional genetic tests of sex-biased dispersal revealed no evidence of sex-biased dispersal (all sites pooled): mean pairwise relatedness ($P = 0.368$), $mAIc$ ($P = 0.351$), $vAIc$ ($P = 0.730$), F_{ST} ($P = 0.364$). Removal of the site RF (as a potential outlier) did not qualitatively change the results (data not shown).

Discussion

Population genetic characteristics

Considering the small geographic scope of our study, generally large populations, and the short (∼60 years) time since widespread deforestation (all factors acting against population differentiation), we found a relatively high level of population genetic differentiation among our sites—comparable to continent-wide and even subspecific population structure found in some temperate species (e.g., Stenzler et al. 2009; Bull et al. 2010). That all five sites were significantly differentiated from each other implies limited dispersal tendencies, even within contiguous forest at this small geographic scale (10s of km).

The patterns of differentiation we observed supported our hypothesis that population differentiation is greater in fragments in one isolated site (RF), but were somewhat ambiguous with respect to another isolated site (TI). Because TI is a much larger fragment than RF (300 vs 80 ha), and has a considerably larger population of chestnut-backed antbird, we cannot state unambiguously that the significant differentiation of TI from the other sites (in terms of F_{ST}) is due to forest loss and isolation of TI. However, isolation of TI is not complete, and it is possible that riparian corridors still help to maintain some connectivity with BCNP or LS. Even if forest loss has not

Fig. 3 Results of Bayesian admixture analyses for $K = 2$ for five populations of chestnut-backed antbird using **a** STRUCTURE (Hubisz et al. 2009 approach), and **b** TESS. Columns represent estimated membership coefficients for individuals. See text for details of each analysis
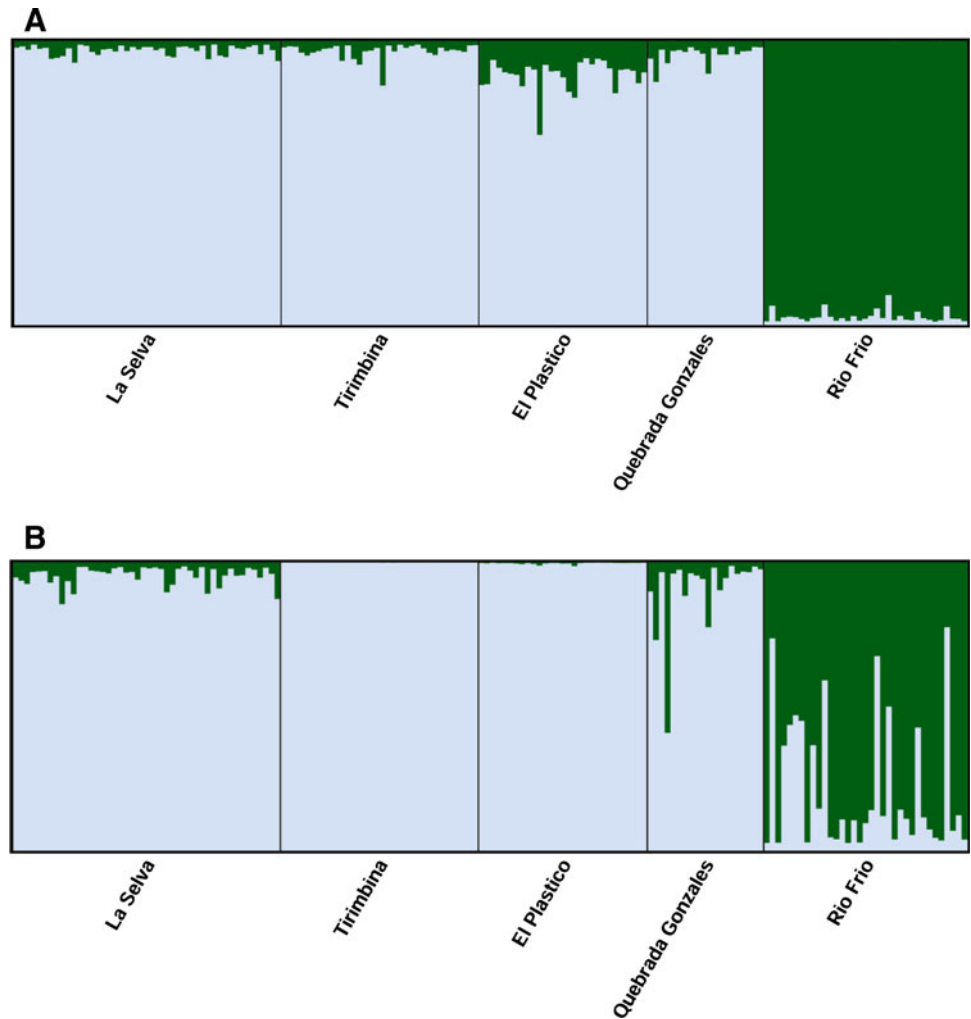
Table 3 Estimated effective population sizes N_e (95 % confidence interval) of five populations of chestnut-backed antbird sampled in northeastern Costa Rica

Site	N_e (95 % CI)
Plastico	$-$ (561 $- \infty$)
Quebrada Gonzales	170 (101–462)
Tirimbina	139 (97–228)
La Selva	126 (90–195)
Rio Frio	40 (32–50)

The inability to estimate N_e for site Plastico is likely the result of very large N_e

influenced population structure at TI, the observed structure is still indicative of generally limited dispersal, and any additional influence of decreased connectivity will only exacerbate genetic structuring in the future.

Our most conservative analyses (Bayesian clustering approaches) failed to support our hypothesis that the three contiguous sites would cluster separately from the two putatively isolated sites. Instead, only the smallest and

most isolated site was clearly differentiated from the other sites. However, in the STRUCTURE analyses, the hypothesis of $K = 3$ (in terms of mean log likelihood) was not strikingly less well supported than $K = 2$ (Fig. 2). Interestingly, STRUCTURE runs using $K = 3$ produced bar plots that grouped RF and LS + PL as two separate clusters, and grouped TI + QG as the third cluster (data not shown). Grouping TI and QG is not biologically intuitive (see Fig. 1), and suggests that some combination of population size and reduced immigration produced similar population genetic characteristics within these two sites.

Given that we found significant population genetic structure in our study area, a pattern of isolation by distance (IBD) was expected. We did not find IBD, but instead found a pattern of isolation by resistance (IBR). Two factors likely influenced the lack of an IBD pattern in our study. First, the range of pairwise distances (11–34 km) between our sites may not have been sufficient to provide an adequate test of IBD. Second, the complete lack of gene flow between RF and all other sites (2MOD analyses) could both (a) confound the test of IBD, and (b) render
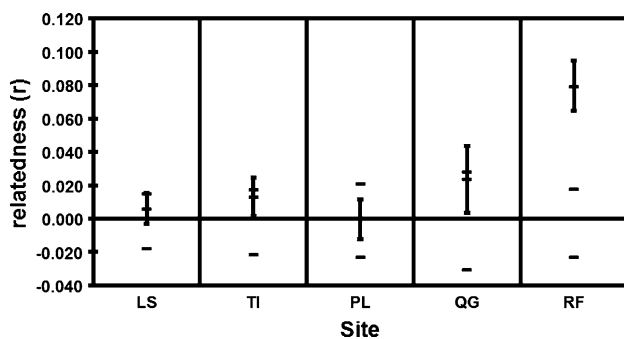
Fig. 4 Mean pairwise relatedness (*r* of Queller and Goodnight 1989) of chestnut-backed antbirds within five sites in northeastern Costa Rica. Whiskers represent the 95 % confidence interval (CI) of *r*, and dashes represent the 95 % CI of the null hypothesis of *r* = 0. *P* values of observed *r* for each site are: PL 0.388, LS 0.144, TI 0.061, QG 0.043, and RF < 0.001

hypotheses of IBD and IBR somewhat invalid, given the implicit assumption of at least some continuing gene flow in both IBD and IBR hypotheses. Thus, we suggest that isolated populations of chestnut-backed antbird may often be persisting in the absence of gene flow, and that their degree and rate of genetic differentiation will largely be a function of effective population size (i.e., small, isolated populations are more susceptible to rapid drift).

Effective population size (N_e) and relatedness

Perhaps the best explanation for the patterns of genetic structure in our landscape comes from analyses of relatedness among individuals within sites: mean pairwise relatedness was highest (0.079) in the smallest isolate (RF), and lowest (zero) in one of the control sites within contiguous forest (PL; Fig. 4). The most straight-forward interpretation of elevated within-site pairwise relatedness is that individuals born in a site are reluctant to disperse out of the site, particularly if the site is bordered by non-forest. The effect of this is most pronounced in RF, where mean pairwise relatedness of a sample of 35 individuals approached that of first cousins.

Effective population sizes (N_e) of the five populations we examined varied considerably, being lowest in the smallest site (RF) and largest in one of the sites (PL) well embedded within a large expanse of forest, as expected (Table 3). That RF had a very small N_e is perhaps not surprising given the small size of that forest patch and the apparent reluctance of birds to disperse out of the patch.

Sex-biased dispersal

We found no convincing pattern of sex-biased dispersal using commonly used population genetic approaches. We consider these results suggestive, but inconclusive, because

the disparity between typical male and female dispersal patterns may need to be quite strong in order to be detected using microsatellite data (Goudet et al. 2002). However, this lack of signal may be worth revisiting once more ecologically similar species are tested, because drivers of sex-biased dispersal remain relatively unexplored in species that maintain year-round pair bonds and territories.

Assumptions of population genetic structure analyses

Although our sampling sites qualify as discrete populations under the evolutionary paradigm following the ad hoc criteria (departures from panmixia) of Waples and Gaggiotti (2006), much of the underlying population structure (with the exception of QG) is likely the result of processes operating at ecological time scales (i.e., the past 60 years). That is, it is likely that accumulation of close relatives within most sites—exacerbated by a reluctance to disperse out of sites with extensive non-forest borders—drives the population differentiation we observed. One of the basic assumptions of most (if not all) analyses of population genetic structure is that there are no close relatives in the samples. However, violation of this assumption "becomes problematic only when it is mistaken for population structure in the more general sense" (Anderson and Dunham 2008). In our case, it is precisely this signal that supports hypotheses of generally short dispersal distances and restricted gene flow in this species.

Conservation implications

The removal and fragmentation of forests is the greatest threat to forest-dependent species world-wide, and nowhere does this process affect more species than in lowland tropical forests (Marini and Garcia 2005; Silvano and Segalla 2005; Sigel et al. 2006; Barlow et al. 2007). In general, understory insectivores are considered especially sensitive to forest loss and fragmentation (Stouffer and Bierregaard 1995; Canaday 1997; Canaday and Rivadeneyra 2001; Sekercioglu et al. 2002). The chestnut-backed antbird is thus unusual in that it is frequently found in forest fragments (Wetmore 1972; Roberts 2007), and it has seemed logical to infer that the species disperses well. Our results suggest that this is not necessarily the case, even within contiguous habitat. Our smallest and most isolated site (RF) is clearly an "island" experiencing essentially zero genetic connectivity with other populations in this landscape, and there are many similarly small forest patches in this landscape that contain chestnut-backed antbirds. In some cases, movements between patches may be facilitated by second-growth or riparian corridors (Losada-Prado 2009), but even in such cases gene flow is

likely greatly reduced compared to populations in large expanses of forest.

What allows populations of chestnut-backed antbirds to persist (at least in the short term) in highly fragmented landscapes while many other ecologically similar species do not is not clear. Relatively small home range sizes of chestnut-backed antbirds (ca. 2 ha; Losada-Prado 2009) may allow relatively higher densities, and thus larger populations within small forest fragments compared to many other understory insectivores, which not infrequently have home ranges 3–20 times as large (Willson 2004; Stouffer 2007). The ability of chestnut-backed antbird to use young second-growth (4–5 m high) to some degree also increases the possibility of movements in fragmented landscapes (Blake and Loiselle 2001; Losada-Prado 2009). Lastly, high renesting tendency following nest failure could also contribute to the species' persistence in fragments (Sieving 1992; Sieving and Karr 1997), where nest failure rates are higher than in intact forest (Young et al. 2008).

Future research

Greater replication, both in terms of number of sites (both intact and fragments), and in terms of species examined is needed to evaluate how general our results and interpretations are. Because forest loss and fragmentation of lowland tropical forests continues at an alarming rate, studies of other species within intact forest are especially important to help identify species most likely to be negatively affected by these processes and to design appropriate reserve systems in advance (Palumbi 2003). In fragmented landscapes, clarifying the relative influences of time-since-isolation, size of fragments, and degree of isolation will be important to prioritize conservation efforts to restore connectivity and increase the amount of available habitat for chestnut-backed antbird and other understory insectivores.

Appendix

See Table 4.

Table 4 (*A*) Distance (km) and (*B*) hypothesized connectivity (1 = connected, 2 = moderately connected, 3 = isolated) matrices of the five sites in Costa Rica

	LS	TI	PL	QG
A.				
TI	11			
PL	14	13		
QG	31	34	20	
RF	16	25	16	19
B.				
TI	2			
PL	1	2		
QG	1	2	1	
RF	3	3	3	3

References

Anderson EC, Dunham KK (2008) The influence of family groups on inferences made with the program Structure. Mol Ecol Resour 8:1219–1229

Arguedas N, Parker PG (2000) Seasonal migration and genetic population structure in House Wrens. Condor 102:517–528

Avise JC (2004) Molecular markers, natural history and evolution, 2nd edn. Sinauer Associates, Inc., Sunderland

Barlow J, Gardner TA, Araujo IS et al (2007) Quantifying the biodiversity value of tropical primary, secondary, and plantation forests. Proc Nat Acad Sci USA 104:18555–18560

Barnett JR, Woltmann S, Bogdanowicz SM, Lovette IJ (2007) Isolation and characterization of microsatellite markers from the Chestnut-backed antbird, *Myrmeciza exsul*. Mol Ecol Notes 7:1070–1072

Barnett JR, Ruiz-Gutiérrez V, Coulon A, Lovette IJ (2008) Weak genetic structuring indicates ongoing gene flow across White-ruffed Manakin (*Corapipo altera*) populations in a highly fragmented Costa Rica landscape. Conserv Genet 9:1403–1412

Bates JM (2002) The genetic effects of forest fragmentation on five species of Amazonian birds. J Avian Biol 33:276–294

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996) GENETIX, logiciel sous Windows^TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions CNRS UMR 5000, Université de Montpellier II, Montpellier

BirdLife International (2000) Threatened birds of the world. Lynx Edicions, Cambridge

Blake JG, Loiselle BA (2001) Bird assemblages in second-growth and old-growth forests, Costa Rica: perspectives from mist nets and point counts. Auk 118:304–326

Browne RA, Collins E, Anderson DJ (2008) Genetic structure of Galápagos populations of the Yellow Warbler. Condor 110:549–553

Bull RD, McCracken A, Gaston AJ, Birt TP, Friesen VL (2010) Evidence of recent population differentiation in Orange-crowned Warblers (*Vermivora celata*) in Haida Gwaii. Auk 127:23–34

Campana MG, Hunt HV, Jones H, White J (2011) CorrSieve: software for summarizing and evaluating structure output. Mol Ecol Resour 1:349–352

Canaday C (1997) Loss of insectivorous birds along a gradient of human impact in Amazonia. Biol Conserv 77:63–77

Canaday C, Rivadeneyra J (2001) Initial effects of a petroleum operation on Amazonian birds: terrestrial insectivores retreat. Biodivers Conserv 10:567–595

Chen C, Durand E, Forbes F, Francois O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. Mol Ecol Notes 7:747–756

Ciofi C, Beaumont MA, Swingland IR, Bruford MW (1999) Genetic divergence and units for conservation in the Komodo dragon *Varanus komodoensis*. Proc Roy Soc Lond B 266:2269–2274

Clarke AL, Sæther B-E, Røskraft E (1997) Sex biases in avian dispersal: a reappraisal. Oikos 79:429–438

Coulon A, Fitzpatrick JW, Bowman R, Stith BM, Makarewich CA, Stenzler LM, Lovette IJ (2008) Congruent population structure inferred from dispersal behaviour and intensive genetic surveys of the threatened Florida scrub-jay (*Aphelocoma coerulescens*). Mol Ecol 17:1685–1701

Coulon A, Fitzpatrick JW, Bowman R, Lovette IJ (2010) Effects of habitat fragmentation on effective dispersal of Florida Scrub Jays. Conserv Biol 24:1080–1088

Durand E, Chen C, Francois O (2009) TESS version2.3—reference manual. http://membres-timc.imag.fr/Olivier.Francois/tess.html

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Feldheim KA, Woltmann S, Kreiser BR (2010) Characterization of 20 additional microsatellite loci for *Myrmeciza exsul* (Aves: Thamnophilidae: chestnut-backed antbird). Mol Ecol Resour, vol, pp 10:404–408. In: MERPD Consortium, permanent genetic resources added to Molecular Ecology Resources Database, 1 Oct 2009–30 Nov 2009

Francois O, Ancelet S, Guillot G (2006) Bayesian clustering using hidden Markov random fields in spatial population genetics. Genetics 174:805–816

Gibbs HL, Dawson RJG, Hobson KA (2000) Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow? Mol Ecol 9:2137–2147

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from: http://www.unil.ch/izea/softwares/fstat.html

Goudet J, Raymond M, De-Meeus T, Rousset F (1996) Testing differentiation in diploid populations. Genetics 144:1933–1940

Goudet J, Perrin N, Waser PM (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. Mol Ecol 11:1103–1114

Greenwood PJ, Harvey PH (1982) The natal and breeding dispersal of birds. Ann Rev Ecol Evol Syst 13:1–21

Guillot G, Leblois R, Coulon A, Frantz AC (2009) Statistical methods in spatial genetics. Mol Ecol 18:4734–4756

Hedrick PW (2005) Genetics of populations, 3rd edn. Jones and Bartlett, Boston

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour 9:1322–1332

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. BMC Genet 13:v.3.16. http://ibdws.sdsu.edu/

Joyce AT (2006) Land use change in Costa Rica. Litografía e Imprenta LIL, S.A., San José

Kass RE, Raftery AE (1995) Bayes factors. J Am Stat Assoc 90:773–795

Korfanta NM, McDonald DB, Glenn TC (2005) Burrowing Owl (*Athene cunicularia*) population genetics: a comparison of North American forms and migratory habits. Auk 122:464–478

Lees AC, Peres CA (2006) Rapid avifaunal collapse along the Amazonian deforestation frontier. Biol Conserv 133:198–211

Lees AC, Peres CA (2008) Avian life-history determinants of local extinction risk in a hyper-fragmented neotropical forest landscape. Anim Conserv 11:128–137

Losada-Prado S (2009) Home range and movements of *Myrmeciza exsul* (Aves: Thamnophilidae) in two fragmented landscapes in Costa Rica: evaluating functional connectivity. Thesis, CATIE, Turrialba

Marini MÂ, Garcia F (2005) Bird conservation in Brazil. Conserv Biol 19:665–671

McCormack JE, Smith TB (2008) Niche expansion leads to small-scale adaptive divergence along an elevation gradient in a medium-sized passerine bird. Proc Roy Soc Lond B 275:2155–2164

McRae BH (2006) Isolation by resistance. Evolution 60:1551–1561

Miller-Sims VC, Gerlach G, Kingsford MJ, Atema J (2008) Dispersal in the spiny damselfish, *Acanthochromis polyacanthus*, a coral reef fish species without a larval pelagic stage. Mol Ecol 17:5036–5048

Moore IT, Bonier F, Wingfield JC (2005) Reproductive asynchrony and population divergence between two tropical bird populations. Behav Ecol 16:755–762

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. Ecol Appl 13:S146–S158

Peakall R, Smouse PE (1996) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258–275

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Raymond M, Rousset F (2004) GenePop on the Web v. 3.4. http://wbiomed.curtin.edu.au/genepop/. Updated from Raymond M, Rousset F (1995) GenePop (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86: 248–249

Roberts DL (2007) Effects of tropical forest fragmentation on ecology and conservation of migrant and resident birds in lowland Costa Rica. Ph.D. dissertation, University of Idaho, Moscow

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Mol Ecol Notes 4:137–138

Rousset F (2004) Genetic structure and selection in subdivided populations. Princeton University Press, Princeton

Sekercioglu CH, Ehrlich PR, Daily GC, Aygen D, Goehring DM, Sandi RF (2002) Disappearance of insectivorous birds from tropical forest fragments. Proc Nat Acad Sci USA 99:263–267

Sesnie SE, Gessler PE, Finegan B, Thessler S (2008) Integrating Landsat TM and SRTM-DEM derived variables with decision trees for habitat classification and change detection in complex neotropical environments. Remote Sens Environ 113:2145–2159

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analysis. Can J Zool 69:82–90

Sieving KE (1992) Nest predation and differential insular extinction among selected forest birds of central Panama. Ecology 73: 2310–2328

Sieving KE, Karr JR (1997) Avian extinction and persistence mechanisms in lowland Panama. In: Laurance WF, Bierregaard RO Jr (eds) Tropical forest remnants: ecology, management, and conservation of fragmented communities. University of Chicago Press, Chicago

Sigel BJ, Sherry TW, Young BE (2006) Avian community response to lowland tropical rainforest isolation: 40 years of change at La Selva Biological Station, Costa Rica. Conserv Biol 20:111–121

Silvano DL, Segalla MV (2005) Conservation of Brazilian amphibians. Conserv Biol 19:653–658

Skutch AF (1969) Life histories of Central American birds III. Pac Coast Avifauna 35:236–245

Slud P (1960) The birds of finca "La Selva," Costa Rica: a tropical wet forest locality. Bull Am Mus Nat Hist 121:49–148

Stenzler LM, Makarewich CA, Coulon A, Ardia DR, Lovette IJ, Winkler DW (2009) Subtle edge-of-range genetic structuring in transcontinentally distributed North American Tree Swallows. Condor 111:470–478

Stouffer PC (2007) Density, territory size, and long-term spatial dynamics of a guild of terrestrial insectivorous birds near Manaus, Brazil. Auk 124:292–306

Stouffer PC, Bierregaard RO Jr (1995) Use of Amazonian forest fragments by understory insectivorous birds. Ecology 76: 2429–2445

Stow AJ, Sunnucks P, Briscoe DA, Gardner MG (2001) The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellites. Mol Ecol 10:867–878

Stutchbury BJM, Woolfenden BE, Fedy BC, Morton ES (2005) Nonbreeding territorial behavior of two congeneric antbirds, *Myrmeciza exsul* and *M. longipes*. Ornitol Neotropical 16: 397–404

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Veit ML, Roberson RJ, Hamel PB, Friesen VL (2005) Population genetic structure and dispersal across a fragmented landscape cerulean warblers (*Dendroica cerulea*). Conserv Genet 6: 159–174

Waples RS, Do C (2008) LDNE: a program for estimating population size from data on linkage disequilibrium. Mol Ecol Resour 8:753–756

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol Ecol 15: 1419–1439

Wetmore A (1972) The birds of the Republic of Panama, part 3. Smithsonian Institution Press, Washington

Willis EO, Oniki Y (1972) Ecology and nesting behavior of the Chestnut-backed Antbird (*Myrmeciza exsul*). Condor 74:87–98

Willson SK (2004) Obligate army-ant-following birds: a study of ecology, spatial movement patterns and behavior in Amazonian Peru. Ornithol Monogr 55:1–67

Wilson AG, Arcese P, Chan YL, Patten MA (2011) Micro-spatial genetic structure in song sparrows (*Melospiza melodia*). Conserv Genet 12:213–222

Woltmann S (2010) Dispersal of a tropical rainforest understory bird, the Chestnut-backed antbird (Myrmeciza exsul). Ph.D. dissertation, Tulane University, New Orleans

Woltmann S, Sherry TW (2011) High apparent survival and stable territory dynamics of Chestnut-backed antbird (*Myrmeciza exsul*) in a large rain forest preserve. Wilson J Ornithol 123: 15–23

Woltmann S, Terrill RS, Miller MJ, Brady ML (2010) Chestnut-backed antbird (*Myrmeciza exsul*). In: Schulenberg TS (ed) Neotropical Birds Online. Cornell Lab of Ornithology, Ithaca. Retrieved from Neotropical Birds Online: http://neotropical.birds.cornell.edu/portal/species/overview?p_p_spp=393811

Woltmann S, Sherry TW, Kreiser BR (2012) A genetic approach to estimating natal dispersal distances and self-recruitment in resident rainforest birds. J Avian Biol 43:33–42

Young BE, Sherry TW, Sigel BJ, Woltmann S (2008) Edge and isolation effects on nesting success of Costa Rican forest birds. Biotropica 40:615–622

Zimmer KJ, Isler ML (2003) Family Thamnophilidae (Typical Antbirds). In: Del Hoyo J, Elliott A, Christie DA (eds) Handbook of the birds of the world, vol 8. Lynx Edicions, Barcelona