

Associations between forest fragmentation patterns and genetic structure in Pfrimer's Parakeet (*Pyrrhura pfrimeri*), an endangered endemic to central Brazil's dry forests

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Abstract When habitat becomes fragmented, populations of species may become increasingly isolated. In the absence of habitat corridors, genetic structure may develop and populations risk reductions in genetic diversity from increased genetic drift and inbreeding. Deforestation of the Cerrado biome of Brazil, particularly of the dry forests within the Paranã River Basin, has incrementally occurred since the 1970s and increased forest fragmentation within the region. We performed landscape genetic analyses of Pfrimer's parakeet (*Pyrrhura pfrimeri*), a globally endangered endemic to the region, to determine if forest fragmentation patterns were associated with genetic structuring in this species. We used previously generated satellite imagery that identified the locations of Paranã River Basin forest fragments in 1977, 1993/94, and 2008. Behavioral data quantifying the affinity of Pfrimer's parakeet for forest habitat was used to parameterize empirically derived landscape conductance surfaces. Though genetic structure was observed among Pfrimer's parakeet populations, no

association between genetic and geographic distance was detected. Likewise, least cost path lengths, circuit theory-based resistance distances, and a new measure of least cost path length complexity could not be conclusively associated with genetic structure patterns. Instead, a new quantity that encapsulated connection redundancy from the 1977 forest fragmentation data provided the clearest associations with pairwise genetic differentiation patterns (Jost's D : $r = 0.72$, $P = 0.006$; F_{ST} : $r = 0.741$, $P = 0.001$). Our analyses suggest a 35-year or more lag between deforestation and its effect on genetic structure. Because 66 % of the Paranã River Basin has been deforested since 1977, we expect that genetic structure will increase substantially among Pfrimer's Parakeet populations in the future, especially if fragmentation continues at its current pace.

Keywords Landscape genetics · Cerrado biome · Dry forests · Habitat fragmentation · Connection redundancy · *Pyrrhura pfrimeri*

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Introduction

Habitat loss can have numerous cascading effects in natural populations, ranging from changes in ecosystem function down to changes in individual behavior (Lindenmayer and Fisher 2006; Noss and Csuti 1997). At the population level, habitat loss and fragmentation generally result in reduced population sizes and increased population isolation. These two repercussions are particularly relevant, as they are directly related to two important population genetic parameters: effective population sizes and migration rates (Lowe et al. 2005; Young et al. 1996). Reductions in effective population size can exacerbate the effects of diversity-reducing processes such as genetic drift and

inbreeding, which in turn can lead to reduced individual fitness (Reed and Frankham 2003; Westemeier et al. 1998). Likewise, increased isolation may limit opportunities for gene flow and movement and increase the demographic independence of populations. Appreciable gene flow may help counteract the effects of genetic drift (Mills and Allendorf 1996; Van Dongen et al. 1997) and minimize loss of fitness (Newman and Tallmon 2001). Furthermore, the physical movement of individuals between populations may be an important process that minimizes local extinction risks (Dunham et al. 1997; Reed 2004).

Over the past several decades, molecular genetic approaches have frequently been used to assess the effects of habitat fragmentation on genetic structure and diversity patterns (Keyghobadi 2007). In most cases, analyses have relied on pre-specified population contrasts involving sets of “fragmented” populations and “unfragmented” control habitats. Though a useful framework, recent conceptual developments and analytical frameworks from the field of landscape genetics (Manel et al. 2003) promise even more refined insights. Landscape genetic analyses have evolved to the point where variation in the distributions of landscape features, as encapsulated in GIS-based raster maps, can be directly examined using correlational or causal modeling-based analysis frameworks (Cushman et al. 2006; Lee-Yaw et al. 2009). Thus, the complete continuum of habitat fragmentation across a landscape may be considered in analyses and nominally provide much more pertinent information about the effects of habitat variation than studies based on pre-selected groups of populations that qualitatively differ from one another.

The Cerrado biome of Brazil is a global biodiversity hotspot (Myers et al. 2000) and is considered the richest savannah in the world (Klink and Machado 2005). Its historical area was estimated to be $\sim 2 \times 10^6$ km² (Klink and Machado 2005), but nearly 50 % of this area has been modified or developed for human activities (Machado et al. 2004). There are two primary climates in the Cerrado, dry and rainy seasons, and the landscape includes a mixture of open grasslands, shrublands, riparian forests, and deciduous dry forests (Ribeiro et al. 1998). Dry forests are the most threatened tropical forest formation in the world (Janzen 1988; Sanchez-Azofeifa et al. 2005) and populations of many species associated with this ecosystem are potentially in decline. One of the most significant formations of dry forests in the Cerrado is located in the Parana River Basin in the states of Tocantins and Goias (Scariot and Sevilha 2005; Fig. 1a), where there has been a 66 % decrease in the dry forest area since 1977 (Bianchi and Haig 2012; Fig. 1b–d). Across Brazil, only 3.9 % of the dry forests are protected (Espirito-Santo et al. 2008).

Pfimer’s Parakeet (*Pyrrhura pfrimeri*) is one of two endemic parrot species occupying the Cerrado biome in Brazil. The species is restricted to dry forests and is listed as Endangered at the global level (Birdlife International 2008)

and Vulnerable at the national level (Ministerio do Meio Ambiente 2003). Details of the species’ life history in the wild are unknown, but lifespan is believed to be at least 8 years with an age of first reproduction at 2 years of age (C. Bianchi, personal communication). Habitat loss to grazing and agriculture is considered the main cause of potential population declines, which is aggravated by the species’ strong dependence upon dry forests within the Parana River Basin (Bianchi 2008; Birdlife International 2008; Olmos et al. 1997; Silva 1997) and its avoidance of non-forested areas (Bianchi 2010). The severe reduction in dry forest habitat within the Parana River Basin suggests that there may be potential impacts of forest fragmentation on gene flow and genetic structure within Pfimer’s Parakeet.

Numerous review papers focusing on landscape genetics emphasize the importance of the field for conservation and management efforts (Balkenhol et al. 2009; Haig et al. 2011; Manel et al. 2003; Storfer et al. 2007). Thus, we used landscape genetic concepts and analytical approaches to determine if forest fragmentation is associated with genetic structure among Pfimer’s Parakeet populations. We used empirically derived landscape resistance surfaces and data on the locations of forest fragments from 1977, 1993–94, and 2008 to determine if genetic structure patterns were better associated with older versus newer fragmentation patterns. We likewise considered numerous different measures of habitat connectivity to identify appropriate approaches that may hold greatest utility in our study system. We initially applied least-cost path modeling and circuit theory-based resistance distances as connectivity measures in our analyses. These approaches subsequently served as the basis for deriving two new quantities that encapsulated least-cost path complexity and connection redundancy, which were also incorporated into our analyses.

Methods

Microsatellite analyses

We obtained blood samples from 104 specimens at six sites in the Parana River Basin (Table 1; Fig. 1) during September through December of 2007 and 2008. Birds were trapped using mist nets, and blood was transported back to the laboratory in cryogenic vials containing a storage buffer (100 mM Tris–HCl, 100 mM EDTA, 10 mM NaCl, 0.5 % SDS, pH 8.0). DNA extractions were performed as described in Haig et al. (1994). Multilocus microsatellite genotypes were obtained for each individual using seven loci previously isolated for the species using an Illumina GAIIx Genome Analyzer paired-end 80 bp run (Jennings et al. 2011). An eighth locus (Mmgt105) originally designed for the monk parakeet (*Myiopsitta monachus*; Russello et al. 2007) was also applied.

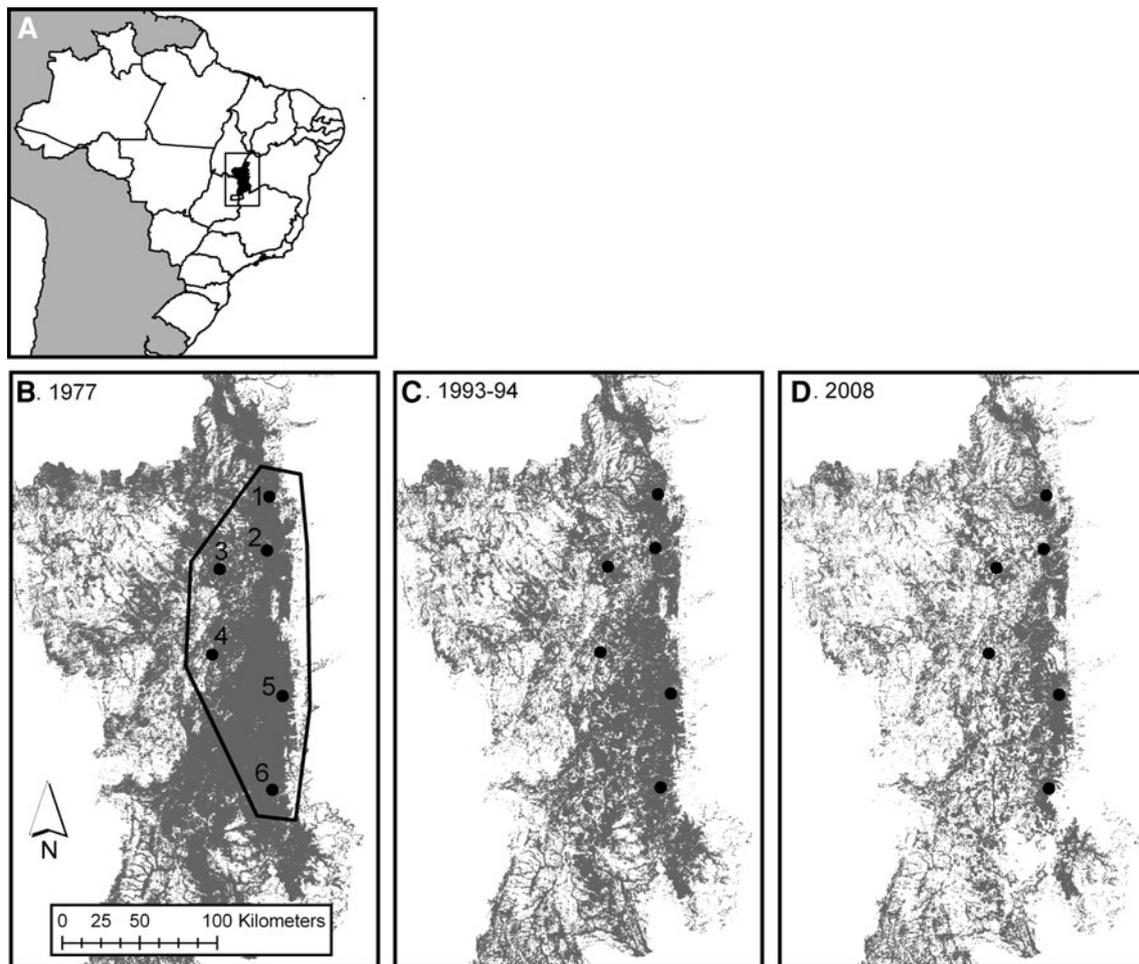


Fig. 1 **a** Map of Brazil highlighting the location of the Parana River Basin. **b–d** Maps highlighting the locations of six sampling areas analyzed in this study overlaid upon a temporal series of forest fragmentation data from 1977, 1993–94, and 2008. The convex

bounding polygon illustrated in **b** was used to mask out non-essential habitat data and reduce computer memory requirements for some analyses. See “[Methods](#)” for more information. Forest fragmentation data are from Bianchi and Haig (2012)

Primer sequences and polymerase chain reaction conditions are provided in Appendix I in Electronic Supplementary Material. Program GDA version 1.1 (Lewis and Zakyin 2002) was used to test for Hardy–Weinberg disequilibrium and linkage disequilibrium between pairs of loci. Composite Hardy–Weinberg test results for each sampling region were obtained by combining locus-specific P values using the Z -transform test (Whitlock 2005). GDA was also used to quantify genetic diversity patterns within populations based on observed (H_o) and expected (H_e) heterozygosities, whereas program HP-Rare (Kalinowski 2005) was used to calculate a rarefied estimate of allelic richness (A_r) that accounted for sample size differences among locations.

Quantifying genetic structure patterns

Genetic structure was quantified for the full data set and for all pairwise combination of populations using Weir and Cockerham’s (1984) analog of F_{ST} as implemented in the

computer program Arlequin ver. 3.5.1.2 (Excoffier and Lischer 2010). To complement the conventional F -statistics, we also calculated global and pairwise estimates of genetic differentiation (D) between sampling locations using Eq. 13 of Jost (2008) in a short computer program written by MPM (see Draheim et al. 2010). Jost’s D is unaffected by the level of diversity at a locus, which can influence the maximum value that statistics such as F_{ST} or G_{ST} can achieve (Hedrick 2005). Ten thousand randomizations were used to evaluate the significance of D and F_{ST} values.

Quantifying landscape conductance

Temporal deforestation trends in our study region were previously illustrated using LANDSAT images that identified the locations of dry forest fragments at three time points: 1977, 1993–1994, and 2008 (Bianchi and Haig 2012). Increased forest fragmentation over time was readily apparent (Bianchi and Haig 2012; Fig. 1b–d). The

Table 1 Names and locations of six Pfrimer's Parakeet populations included in our analyses

Population	Name	Longitude	Latitude	Average sample size over loci	A_r	H_o	H_e
1	Taguatinga	-46.459	-12.351	8.00	3.13	0.453	0.458
2	Aurora	-46.477	-12.665	27.88	3.14	0.392	0.407
3	Arraias	-46.759	-12.773	13.50	3.67	0.494	0.508
4	Monte Alegre	-46.808	-13.278	30.00	3.31	0.556	0.544
5	Terra Ronca	-46.389	-13.525	12.75	3.41	0.542	0.534
6	Posse	-46.454	-14.078	9.00	2.96	0.500	0.484

Population numbers (first column) correspond to numbers on Fig. 1b. Sample sizes represent the average number of individuals genotyped at each location for eight microsatellite loci. Actual sample sizes at each locus are listed in Appendix III in Electronic Supplementary Material. A_r , H_o , and H_e represent rarefied allelic richness, observed heterozygosity, and expected heterozygosity

binary (presence/absence) information contained in these maps, combined with established information on Pfrimer's Parakeet habitat affinities, allowed us to produce empirically-derived landscape conductance surfaces for use in our statistical analyses. Based on observational data, Pfrimer's Parakeet is most frequently found within forest habitat and is rarely detected at distances greater than 300 m from the forest's edge (Appendix II in Electronic Supplementary Material; Bianchi 2010). We fit a generalized exponential decay model to these observations (Appendix II in Electronic Supplementary Material, and used the resulting model parameters to quantify landscape cell conductance (the reciprocal of landscape resistance) as a decreasing function of the probability of use by Pfrimer's parakeet at different distances away from forest stands. More specifically, new raster maps were generated for each time point that reflected the relative use of habitat by Pfrimer's Parakeets at different distances from forest edges and the distances of individual raster map cells from the closest forest fragment. All analyses were performed at the resolution of the original forest maps (100 m pixel sizes; Bianchi 2010) using the Raster Calculator in ArcMap 9.3.1 (ESRI, Inc. Redlands, CA). The landscape was restricted to the subsection of the Parana River basin within the convex polygon in Fig. 1b to reduce the computer memory requirements of the raster map in subsequent analyses.

Calculating least-cost path lengths and resistance distances

Connectivity between sampling locations was initially quantified three ways. First, we calculated the matrix of pairwise geographic distances. Analyses based solely on Euclidean distances (or the logarithm of Euclidean distances) ignored habitat quality between locations and only allowed us to determine if physical distances between locations influenced genetic differentiation. These analyses served as a benchmark for comparison with more refined measures of connectivity that explicitly incorporated the

landscape conductance surfaces described above: least-cost path lengths (Douglas 1994) and resistance distances (McRae 2006; McRae and Beier 2007).

Least-cost paths were calculated for each time period (Fig. 1) using the Landscape Genetics ArcGIS toolbox (Etherington 2011). Prior to analysis, conductance values of map pixels were transformed to resistances using the reciprocal of each pixel's conductance. Resistance distances between sampling locations were calculated for each time period using program Circuitscape (Shah and McRae 2008). Analysis options included the "Connect eight neighbors" connection scheme and "Average conductance" connection calculations. Though both least-cost paths and resistance distances incorporate information on habitat conditions, each quantifies fundamentally different attributes (McRae et al. 2008). Least-cost paths represent a single route that theoretically represents the optimal path that an organism would follow when moving between locales. In contrast, resistance distances integrate information on all pathways between locations and incorporate the resistances associated with the myriad routes that could be taken.

The correlation of geographic distances, least cost path lengths, and resistance distances with pairwise measures of genetic differentiation (Jost's D or F_{ST}) were determined using Mantel tests (Mantel 1967) as implemented in program *zt* (Bonnet and Van de Peer 2002). Analyses based on F_{ST} were performed using the quantity $F_{ST}/(1 - F_{ST})$ as recommended by Rousset (1997). Because we generated three pairwise least-cost path length matrices and three resistance distance matrices (one matrix of each type for each time point, Fig. 1), separate tests were used to determine if genetic structure was more highly correlated with fragmentation patterns at any date. Note that P values produced by program *zt* are exact: it enumerates all possible permutations for matrices of size 12×12 and smaller (there are precisely 720 permutations possible for each 6×6 matrix analyzed here; Bonnet and Van de Peer 2002). This approach differs from conventional Mantel tests which rely on random selections of possible matrix permutations.

Quantifying least-cost path complexity and connection redundancy

In our analyses, we observed no associations between geographic distances and our measures of genetic differentiation (see “Results”). However, least cost path lengths and resistance distances are generally correlated with their respective geographic distances (McRae and Beier 2007; Fig. 2). This notion lead us to consider derivatives of least-cost path lengths and resistance distances that removed the effects of geographic distance on each variable while still providing insights about connectivity between sampling locations. We refer to these quantities as least-cost path complexity and connection redundancy and describe their interpretation in more detail below.

Logically, least-cost path lengths cannot be smaller than their corresponding geographic distances. A least-cost path length that is similar to its corresponding geographic distance will approximate a simple straight line (Fig. 2a). In contrast, a least cost path length that deviates substantially from its corresponding geographic distance will reflect a meandering, complex path between locations. Therefore, relative measures of least-cost path complexity can be obtained by taking the residuals from the regression of least-cost path length on geographic distance. Negative residuals reflect straighter least cost paths, whereas large positive residuals reflect more complex paths (Fig. 2a). Note that residuals from the regression of least-cost path lengths on their respective linear geographic distances form a pairwise matrix that can be used in Mantel tests.

Resistance distances were used to derive a measure of connection redundancy between sampling locations. As with least-cost path lengths, resistance distances are also strongly influenced by geographic distances. Assuming a uniform landscape, the resistance distance between geographically distant locations will be proportionally greater than the resistance distance between proximate sites (McRae and Beier 2007). However, distant sites that can be connected through numerous routes may have a comparable effective resistance distance as a pair of geographically proximate locations joined by only a few pathways (McRae 2006; McRae et al. 2008). A relative measure of connection redundancy can therefore be obtained from the residuals of the regression of resistance distance on geographic distance (Fig. 2b). We refer to this measure of connection redundancy as R_{resid} . Small negative values of R_{resid} indicate pairs of points that have low resistance distances relative to their geographic separation. More specifically, negative values indicate greater connection redundancy. In contrast, pairs of points that generate large positive values of R_{resid} indicate resistance distances that are high relative to their geographic separation. Consequently, these data points indicate location pairs that have relatively less redundancy of the pathways that connect them. As with our calculation of pairwise least-cost path complexity matrices, values of R_{resid} also take the form of a pairwise distance matrix.

McRae et al. (2008) provided an alternate measure of connection redundancy based on the ratio of a least-cost distance and its corresponding resistance distance. Specifically,

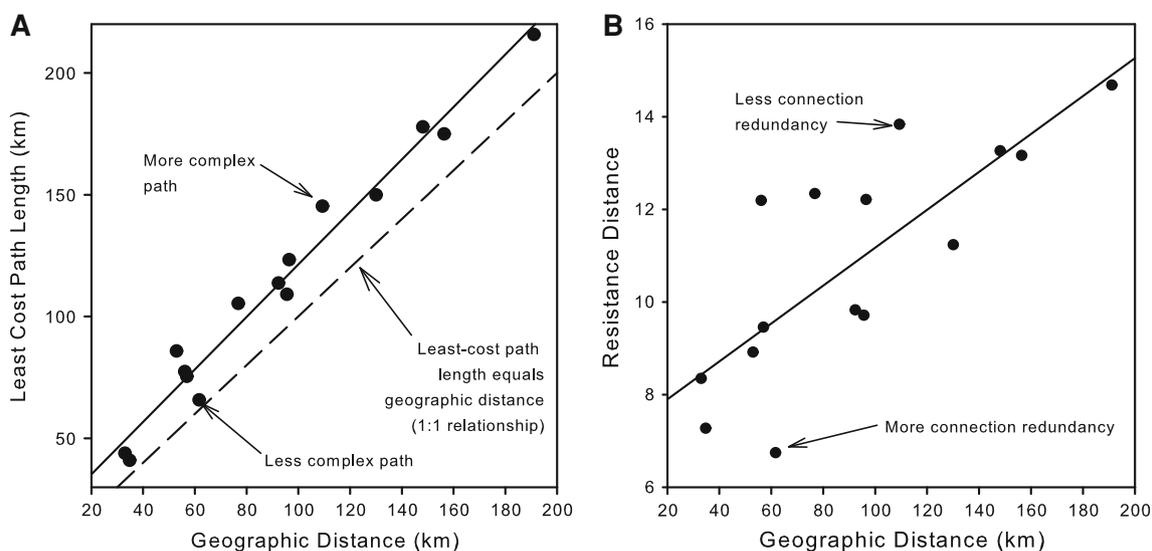


Fig. 2 Scatterplots illustrating relationships between pairwise geographic distances and least-cost path lengths (a) or resistance distances (b). In this example, least-cost paths and resistance distances were obtained using the 2008 forest fragmentation data. These relationships were used to derive two alternative measures of

landscape connectivity. Residuals from the regression of least-cost path length on geographic distance can be used to quantify least-cost path complexity, whereas residuals from the regression of resistance distance on geographic distance can be used to quantify connection redundancy between locations. See “Methods” for more information

$$R_{\text{McRae}} = (\text{least-cost distance})/(\text{resistance distance})$$

In contrast to R_{resid} , the R_{McRae} quantity scales in the opposite direction such that low values represent sampling location pairs with low levels of connection redundancy. When testing for correlations with genetic distances, we therefore expect to see R_{McRae} generate negative correlations, whereas R_{resid} is expected to generate positive correlations with genetic distances.

Pairwise measures of path complexity and connection redundancy (based on R_{resid} or R_{McRae}) were obtained for each of the three time points analyzed in this investigation (Fig. 1). As with previous analyses described above, the significance of correlations between these quantities and pairwise measures of genetic differentiation were obtained using Mantel tests.

Results

We detected between two and 13 alleles across the set of eight loci examined in this study (Appendix III in Electronic Supplementary Material). No evidence for deviations from Hardy–Weinberg equilibrium or linkage disequilibrium between locus pairs was noted after Bonferroni corrections. Estimates of genetic diversity levels within each population are provided in Table 1.

Our analyses identified significant genetic structure among populations of Pflimer's Parakeets. The overall value of Jost's D was moderately high and highly significant ($D = 0.174$, $P < 0.001$). Pairwise values of D ranged from 0.009 to 0.318 (Table 2), and all were highly significant ($P < 0.001$) with the exception of the contrast between Terra Ronca and Posse (populations 5 and 6; Fig. 1). Similar results were obtained when using F_{ST} , where a global value of $F_{\text{ST}} = 0.140$ and a range of pairwise values from 0.004 to 0.220 were observed (Table 2).

Considerable variation in the correlations between different connectivity measures and genetic structure patterns was apparent (Table 3). In general, analyses based on a specific connectivity measure approach (geographic distance, least-cost path length, resistance distance, path complexity, R_{resid} , or R_{McRae}) tended to cluster based on the magnitude of the correlation coefficient observed in analysis outcomes. Results of analyses based on either F_{ST} or D were very similar (Table 3). Among the measures examined, connection redundancy as quantified by R_{resid} was most closely associated with variation in genetic structure patterns among sampling locations (Table 3). The pairwise matrices of R_{resid} for each time point are provided in Appendix IV in Electronic Supplementary Material. Though values of R_{McRae} produced expected negative correlations, the overall magnitude of associations was substantially lower. Despite

Table 2 Pairwise values of Jost's D (below diagonal) and F_{ST} (above diagonal) calculated for all pairwise combination of Pflimer's Parakeet populations illustrated in Fig. 1

Population	1	2	3	4	5	6
1 (Taguatinga)	–	0.201	0.154	0.213	0.163	0.164
2 (Aurora)	0.241	–	0.086	0.220	0.059	0.079
3 (Arraias)	0.244	0.123	–	0.144	0.056	0.076
4 (Monte Alegre)	0.290	0.318	0.213	–	0.114	0.096
5 (Terra Ronca)	0.274	0.102	0.068	0.174	–	0.004
6 (Posse)	0.220	0.130	0.085	0.119	0.009	–

Values in bold are highly significant ($P < 0.001$)

non-significant individual test results, resistance distances appeared to be more closely associated with genetic structure patterns than either least-cost path lengths or geographic distances, as predicted by theory (McRae 2006; McRae and Beier 2007). The highest correlations were identified when R_{resid} as quantified from the 1977 forest fragmentation data was used as the connectivity measure (Table 3). In analyses involving F_{ST} , the observed correlation between $F_{\text{ST}}/(1 - F_{\text{ST}})$ and $R_{\text{resid}}(1977)$ was the single largest value out of the 720 possible matrix permutations that could be performed on the data. Correlations from 1993–94 and 2008 data were markedly lower than that observed from the 1977 data. Current maps derived from analyses with Circuitscape and locations of least-cost paths are illustrated in Fig. 3.

Discussion

Connection redundancy

Our analyses identified significant genetic structure among populations of Pflimer's Parakeet (Table 2). Interestingly, genetic differentiation patterns were not associated with geographic distances between populations (Table 3). Instead, our analyses revealed that genetic differences were most closely associated with a newly derived variable that quantified the extent of connectivity redundancy between locations (R_{resid} , Table 3). Correlations were substantially greater for R_{resid} based on the 1977 forest fragmentation patterns than for data that reflected more contemporary fragmentation stages (Table 3). Myriad ecological, life history, and demographic factors can influence genetic structure (Loveless and Hamrick 1984; Melnick 1987; Hamrick and Godt 1996; Bossart and Prowell 1998; Bohonak 1999). We consider it remarkable that this single variable, derived from forest fragmentation data alone, has such a clear association signal.

Our measure of connectivity redundancy (R_{resid}) effectively removes the component of resistance distances that is otherwise accounted for by geographic distance (Fig. 2).

Table 3 Correlations between habitat connectivity measures and pairwise genetic differentiation statistics

Connectivity measure	<i>D</i>			$F_{ST}/(1 - F_{ST})$		
	<i>r</i>	Rank	<i>P</i> value	<i>r</i>	Rank	<i>P</i> value
R_{resid} (1977)	0.721	1	0.006	0.741	1	0.001
R_{resid} (2008)	0.556	2	0.096	0.562	2	0.101
R_{resid} (1993–94)	0.510	3	0.074	0.482	3	0.082
Path complexity (1993–94)	0.459	4	0.118	0.448	4	0.122
Path complexity (2008)	0.414	5	0.156	0.408	5	0.169
Resistance distance (2008)	0.350	6	0.103	0.333	6	0.122
Resistance distance (1977)	0.325	7	0.121	0.310	7	0.156
Resistance distance (1993–1994)	0.247	8	0.131	0.277	8	0.172
Path complexity (1977)	0.241	9	0.199	0.211	11	0.219
R_{McRae} (1977)	−0.201	10	0.289	−0.239	9	0.242
R_{McRae} (2008)	−0.160	11	0.328	−0.212	10	0.281
R_{McRae} (1993–94)	−0.094	12	0.424	−0.127	12	0.375
Least-cost path length (2008)	0.073	13	0.39	0.046	13	0.403
Least-cost path length (1993–94)	0.036	14	0.44	0.009	14	0.456
Least-cost path length (1977)	0.018	15	0.456	−0.006	15	0.528
Geographic distance	0.003	16	0.474	−0.023	16	0.507
Log geographic distance	−0.013	17	0.522	−0.054	17	0.454

Analyses are ranked based on the magnitude of the correlation coefficients (*r*) and the support they provide for the listed connectivity measures. *P* values associated with each correlation coefficient are also provided. R_{resid} and R_{McRae} correspond to two measures of connectivity redundancy described in the “Methods” section

Small values indicate pairs of populations where resistance distances are low relative to their corresponding geographic distance, and therefore indicate location pairs where multiple pathways, possibly overlapping, can be used to traverse the intervening habitat. Sampling location pair [5,6] is representative of a pair of populations where there are highly redundant connection pathways between locations (Figs. 1, 3; Appendix IV in Electronic Supplementary Material). In contrast, low connection redundancy is indicated by pairs of populations that produce comparatively large resistance distances relative to their corresponding geographic distances (Fig. 2). In this case, few pathways exist between locations and indicate that more inhospitable habitat occurs in the vicinity. Location pair [3,4] typifies sampling locations characterized by low connection redundancy (Figs. 1, 3; Appendix IV in Electronic Supplementary Material).

Measures that quantify connection redundancy provide an alternate perspective towards the overall strength of habitat connections (Rayfield et al. 2011). Individuals rarely use a single dispersal route (Bélisle 2005; Driezen et al. 2007; Pinto and Keitt 2009). Instead, numerous alternate, but nearly equally-optimal pathways may be employed by a species when available. The existence of multiple pathways suggests that opportunities for movement and gene flow among locations should be more commonplace, especially in taxa that have comparatively

high dispersal abilities or those that can engage in stepping-stone dispersal processes. However, in our case, the measure of connection redundancy used in this study may instead reflect the relative availability of suitable habitat for a given species between locations, which in turn may be associated with population sizes or densities of individuals. In regions where sampling locations are connected by only a few redundant pathways, the efficacy of genetic drift and its tendency to cause genetic differentiation may be pronounced if local population sizes are relatively small due to the influence of inhospitable habitat. This hypothesis is particularly tenable in our case, as our data suggest that genetic drift is an important overall factor that influences genetic structure in Pfrimer’s parakeet. As evidence, the presence highly variable pairwise genetic differentiation values (Table 2), but no correlation between genetic and geographic distance (Table 3), corresponds to the low gene flow/high genetic drift scenario identified in the framework of Hutchison and Templeton (1999).

Our analyses suggested that connection redundancy as quantified by R_{resid} is more closely associated with genetic structure patterns than R_{McRae} . R_{McRae} is based on two estimated quantities: the resistance distance and the least-cost path distance for a pair of populations (McRae et al. 2008). Consequently, error in the estimation of these two values may be compounded when they are combined in ratio form and yield a quantity with a relatively large

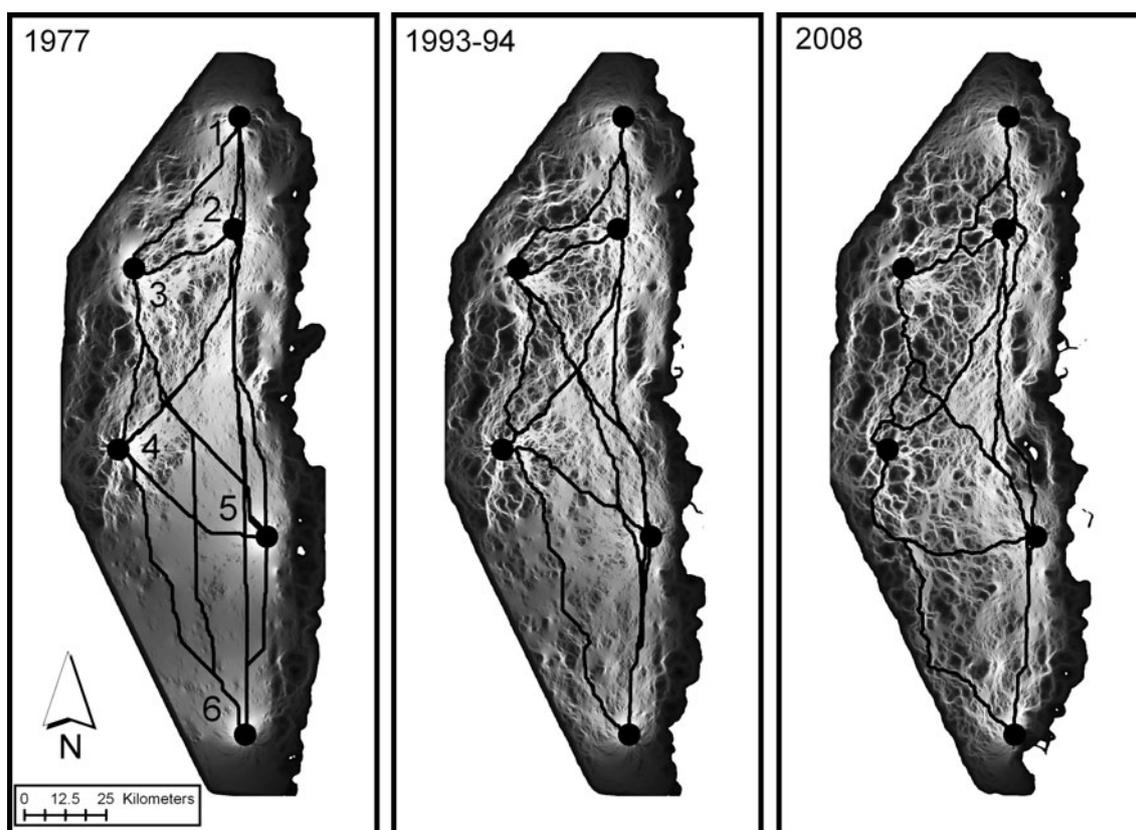


Fig. 3 Current maps of the Paranã River Basin reflecting overall connectivity of six Pfrimer's Parakeet sampling locations based on forest fragmentation data from three time points (Fig. 1). Lighter

pixels reflect greater current densities. Least cost path routes (*thick black lines*) between locations are overlaid on the current maps that were derived from Circuitscape analyses

variance. R_{resid} , in contrast, uses a single estimated quantity (the resistance distance). The second component (geographic distance) can be rigorously determined. Furthermore, R_{resid} relies on use of residuals from comparison of point values to a regression model that effectively encapsulates averages of all data points (Fig. 2). Combined, these attributes indicate that R_{resid} can provide more precise estimates of connection redundancy than R_{McRae} .

Use of empirically derived conductance surfaces

Our statistical analyses were based on empirically derived conductance surfaces that were parameterized using information about locations of dry forest fragments (Fig. 1) and the behavior of Pfrimer's Parakeet in forested and non-forested areas (Appendix II in Electronic Supplementary Material). Despite their conceptual rigor, empirically derived conductance or resistance surfaces have not been commonly used for landscape genetic analyses (Spear et al. 2010). Instead, expert opinion models have been employed in most cases, likely due to the difficulty associated with obtaining rigorous field data on many species (Spear et al. 2010). Previously, Bianchi (2010) used radio-telemetry

data to determine that Pfrimer's Parakeet rarely flies farther than 300 m from any given forest fragment. When moving between forest fragments, it was also observed that birds followed paths that provided greatest forest cover. Barring existence of extensive presence/absence information on the species, these behavioral data likely represent the most rigorous source of information to use in conjunction with our analyses at this time. The fact that our derived variable (R_{resid}) was so closely associated with genetic structure patterns (Table 3) lends further credence to the validity of both the quantitative methods employed here and the inferences that have been made.

Conservation relevance

Comparatively few landscape genetics investigations have been conducted using birds as focal species (Haig et al. 2011; but see Braunisch et al. 2010, Lindsay et al. 2008, and Pavlacky et al. 2009 as examples of exceptions). Most bird species are adapted for flight and may have the ability to rapidly circumvent or traverse inhospitable habitats that may otherwise disrupt gene flow and inter-population movements. Superficially, this attribute suggests that birds

are poor candidates for conducting meaningful landscape genetic investigations. However, latitudinal variation in flight ability exists among bird species, with taxa breeding in more equatorial locales generally demonstrating reduced vagility and greater adaptation to the forest interior (Janzen 1967; Moore et al. 2008; Newton 2003; Wallace 1889). Consequently, tropical bird species may be suitable candidates for landscape genetic investigations (Haig et al. 2011), as evidenced by the results of this study. Given pronounced deforestation rates in many equatorial regions, landscape genetic investigations will undoubtedly provide important baseline information on the current status of avian populations, and likewise help provide key insights into the habitat types and movement corridors required to maintain population connectivity in the future.

A major implication of landscape genetic investigations involves the ability to better understand how future changes across landscapes may impact genetic differentiation and organismal movement (Spear et al. 2010). However, most landscape genetics investigations are only able to use information on current landscape configuration and status. The dates of important past environmental perturbations may be difficult to unambiguously determine, especially in complex landscapes. Such limitations are important, primarily because temporal lags are expected to occur between the time point when a landscape is disturbed and the time point where the effect of the disturbance can be detected with genetic data (Landguth et al. 2010; Safner et al. 2011). If the effects of landscape alterations are not detected using genetic data, researchers may be unable to determine if (1) the disturbance actually had no effect on a given species, or (2) insufficient time has elapsed for the effects of the disturbance to become detectable (Cushman et al. 2006).

In this investigation, our analyses were aided by the availability of a 35-year time series of forest fragmentation data and deforestation trends (Fig. 1). Based on our analyses, current genetic structure patterns are most closely associated with connection redundancy patterns from 1977 (Table 3). This finding is noteworthy, as most deforestation in the Parana River Basin began in the 1970s due to government incentives to develop in the region (Espırito-Santo et al. 2009). We cannot rule out, however, the possibility that the genetic structure patterns currently observed in Pfrimer's parakeet reflect even older periods of time before intensive deforestation began. Nonetheless, if our analyses had relied only upon recent forest fragmentation data (i.e., 1993–1994 or 2008), we would have not been able to convincingly determine if forest fragmentation had no effect or if temporal lags were diminishing our ability to identify significant patterns. Given our use of reliable historical data, our analyses appear to indicate that contemporary genetic structure patterns in Pfrimer's Parakeet

are lagging behind the substantial deforestation that has occurred within the region (Fig. 1). Even if all deforestation activity ceased in the future, we would therefore still expect for genetic structure patterns to become more pronounced in the future until they become coincident with current forest fragmentation patterns. However, deforestation rates are not decreasing within the region, and at the current rate, it is predicted that the dry forests of the Parana River Basin will be eliminated save for a few extremely isolated fragments within the next few decades (Bianchi 2010). In all likelihood, this scenario will place Pfrimer's parakeet under strong extinction pressure both from (1) a landscape and habitat perspective and (2) from a genetics perspective, as the rate of genetic drift, inbreeding, and loss of diversity among population will likely tend to increase and accelerate over time.

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