

Fragmentation of Atlantic Forest has not affected gene flow of a widespread seed-dispersing bat

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Abstract

Habitat loss and resultant fragmentation are major threats to biodiversity, particularly in tropical and subtropical ecosystems. It is increasingly urgent to understand fragmentation effects, which are often complex and vary across taxa, time and space. We determined whether recent fragmentation of Atlantic forest is causing population subdivision in a widespread and important Neotropical seed disperser: *Artibeus lituratus* (Chiroptera: Phyllostomidae). Genetic structure within highly fragmented forest in Paraguay was compared to that in mostly contiguous forest in neighbouring Misiones, Argentina. Further, observed genetic structure across the fragmented landscape was compared with expected levels of structure for similar time spans in realistic simulated landscapes under different degrees of reduction in gene flow. If fragmentation significantly reduced successful dispersal, greater population differentiation and stronger isolation by distance would be expected in the fragmented than in the continuous landscape, and genetic structure in the fragmented landscape should be similar to structure for simulated landscapes where dispersal had been substantially reduced. Instead, little genetic differentiation was observed, and no significant correlation was found between genetic and geographic distance in fragmented or continuous landscapes. Furthermore, comparison of empirical and simulated landscapes indicated empirical results were consistent with regular long-distance dispersal and high migration rates. Our results suggest maintenance of high gene flow for this relatively mobile and generalist species, which could be preventing or significantly delaying reduction in population connectivity in fragmented habitat. Our conclusions apply to *A. lituratus* in Interior Atlantic Forest, and do not contradict broad evidence that habitat fragmentation is contributing to extinction of populations and species, and poses a threat to biodiversity worldwide.

Keywords: bats, gene flow, habitat fragmentation, population genetics, seed dispersal, spatially explicit simulations

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Introduction

Anthropogenically driven changes to ecosystems are pervasive and pose major threats to biodiversity (Frankham

1995; Fischer & Lindenmayer 2007). In the tropics, habitat destruction and fragmentation are exerting a particularly heavy toll on some of the most diverse terrestrial ecosystems (Laurance *et al.* 2001; Hubbell *et al.* 2008). Fragmentation can have significant consequences for populations. For example, fragmented landscapes typically have more edge, fewer resources, and potentially

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poor-quality or altered habitat due to human and invasive species incursions (Harrison & Bruna 1999). Additionally, fragmentation can modify dispersal (Hanski 1999; Prugh *et al.* 2008), which is essential for preventing extinction of declining populations (rescue-effects; Brown & Kodric-Brown 1977), or can help maintain high local abundance (mass-effects; Shmida & Wilson 1985). Population connectivity can also be an important determinant of genetic diversity and local adaptation (e.g. Petren *et al.* 2005). However, consequences of fragmentation on populations depend on the environmental, temporal and spatial context in which it occurs, and the traits of species experiencing it (Fischer & Lindenmayer 2007; Struebig *et al.* 2011). A more generalized understanding of effects of habitat fragmentation may be possible (Didham *et al.* 2012), but more research is required to reveal trends in species responses that correspond in predictable ways with aspects of life history, behaviour or physiology.

For flying (volant) animals, maintenance of population connectivity in fragmented landscapes is often expected because of high dispersal potential, but species do not consistently conform to these expectations (e.g. Martínez-Cruz *et al.* 2007; Meyer *et al.* 2009). Within particular taxa such as birds or bats, species demonstrate considerable variation in their responses to habitat connectivity and resource availability, sometimes notably affected by one, both or neither (e.g. Bierregaard & Stouffer 1997; Cosson *et al.* 1999; Meyer *et al.* 2008). Studies have also found genetic structure (reflecting restricted gene flow) in nonmigratory (e.g. Worthington Wilmer *et al.* 1999; Rivers *et al.* 2005; Meyer *et al.* 2009; Rossiter *et al.* 2012), and some migratory bat species (e.g. Petit & Mayer 1999; Castella *et al.* 2000; Miller-Butterworth *et al.* 2003), illustrating potential for restricted movement even in volant animals.

Restricted movement is sometimes linked to life-history traits, particularly mobility (e.g. Meyer *et al.* 2009; Struebig *et al.* 2011). Meyer *et al.* (2009) found population differentiation and effects of small-scale fragmentation on genetic diversity in two codistributed Neotropical bats, with the weaker disperser showing stronger effects. These results require cautious interpretation due to low sample sizes and the use of a single mitochondrial gene. Nevertheless, a similar study of codistributed Old World bats (Struebig *et al.* 2011) found declines in genetic diversity in the species predicted to be most vulnerable to habitat fragmentation (lower population densities, exclusively forest-roosting, relatively low mobility). These studies indicate bats may be vulnerable to decreased habitat area and connectivity associated with fragmentation—even at small spatial scales—and suggest traits such as species mobility

may be predictive of susceptibility. However, more studies are needed to determine the generality of such findings.

Understanding responses of highly mobile pollinators and seed dispersers is especially important because they can influence plant population dynamics in fragmented landscapes (Medellin & Gaona 1999; Quesada *et al.* 2003), especially in the tropics where a large number of tree species depend on animals for reproduction (Dirzo *et al.* 2011). In the Americas, at least 549 plant species (191 genera, 62 families) have seeds dispersed by bats (family Phyllostomidae; Lobo *et al.* 2009), and Neotropical bats are also known to pollinate flowers of at least 360 plants (159 genera, 44 families: Fleming *et al.* 2009). In addition, plant-visiting bats are often the most abundant mammal species in the tropics (Patterson *et al.* 2003). Consequently, habitat perturbation effects are important not just for bat population viability, but also have implications for connectivity and maintenance of many plant populations (Muscarella & Fleming 2007; Kunz *et al.* 2011). Yet, in the field of fragmentation-research bats are far less studied than their primary volant vertebrate counterpart, birds. Some research indicates bats experience demographic fluctuations and changes in community composition in response to fragmentation (Fleming 1988; Gorresen & Willig 2004; Meyer & Kalko 2008). However, information about movement potential in fragmented landscapes is lacking, particularly for seed- and pollen-dispersing species in the Neotropics.

To study movement of small animals over large spatial scales, it is not generally feasible to use mark-recapture or radio-tracking (Burland & Wilmer 2001). By analysing genetic variation in a landscape context, insight can be gained into patterns of individuals' movements and population connectivity (Manel *et al.* 2003). One challenge for studying fragmentation with mobile species is having sufficient power to detect a population genetic signature of recent historical processes (but see Epps *et al.* 2005; Segelbacher *et al.* 2008; Pavlacky *et al.* 2009), where detection depends on factors including number and variability of genetic markers, effective population size, and degree of reduction in gene flow. Recent technological advances are helping to meet these challenges. Second-generation sequencing makes it possible to obtain large quantities of genetic information and facilitates marker development in non-model organisms, which can significantly increase power to observe even small and recent effects of population subdivision (Abdelkrim *et al.* 2009). Moreover, computational advances have made the use of computer simulations and randomization models much more widespread in ecological (e.g. Tello & Stevens 2012) and evolutionary research (e.g. Jaquiere *et al.*

2011). Simulations can illuminate the processes that drive patterns in empirical data (Epperson *et al.* 2010) and determine detection limitations (Balkenhol *et al.* 2009), including the (sometimes short) time lapse required to detect landscape effects on spatial genetic structure (e.g. Cushman & Landguth 2010).

In this study, we determine whether recent and broad-scale habitat fragmentation has caused population subdivision in a Neotropical seed-dispersing bat, *Artibeus lituratus* (Chiroptera: Phyllostomidae). This is accomplished by two means. First, population genetic structure is compared between a highly fragmented landscape and a region of minimally disturbed, continuous forest. Observed structure within the fragmented landscape is also compared with expected levels of structure in simulated landscapes under different degrees of reduction in migration. If fragmentation has had an effect on population genetic structure, then (i) higher levels of population differentiation and stronger isolation by distance (IBD) among subpopulations should be detected in the fragmented forest landscape relative to the continuous forest landscape, and (ii) genetic structure in the fragmented landscape should be

similar to structure of simulated landscapes where dispersal has been considerably reduced.

Methods

Study system and field work

Bats were sampled at 14 sites in Paraguay and five sites in the Misiones Province of Argentina (Table S1, Supporting information, Fig. 1). In the last 60 years, Paraguayan Alto Paraná Atlantic Forest (APAF) has been rapidly deforested (Facultad de Ingenieria Agronomica 1994; Hansen *et al.* 2008), and what remains is highly fragmented. With only ~7–16% of its original total expanse remaining, Atlantic Forest is among the most endangered forest types today (Ribeiro *et al.* 2009). This is of special concern considering the high levels of biodiversity and endemism found therein (Brooks *et al.* 2006). Paraguayan APAF sites are embedded in a mosaic of single-family farms, plantations (primarily soybeans: *Glycine max*) and ranchland, with forest patches ranging from <100 to 67 000 ha (Di Bitetti *et al.* 2003). In contrast, Misiones possesses over a million

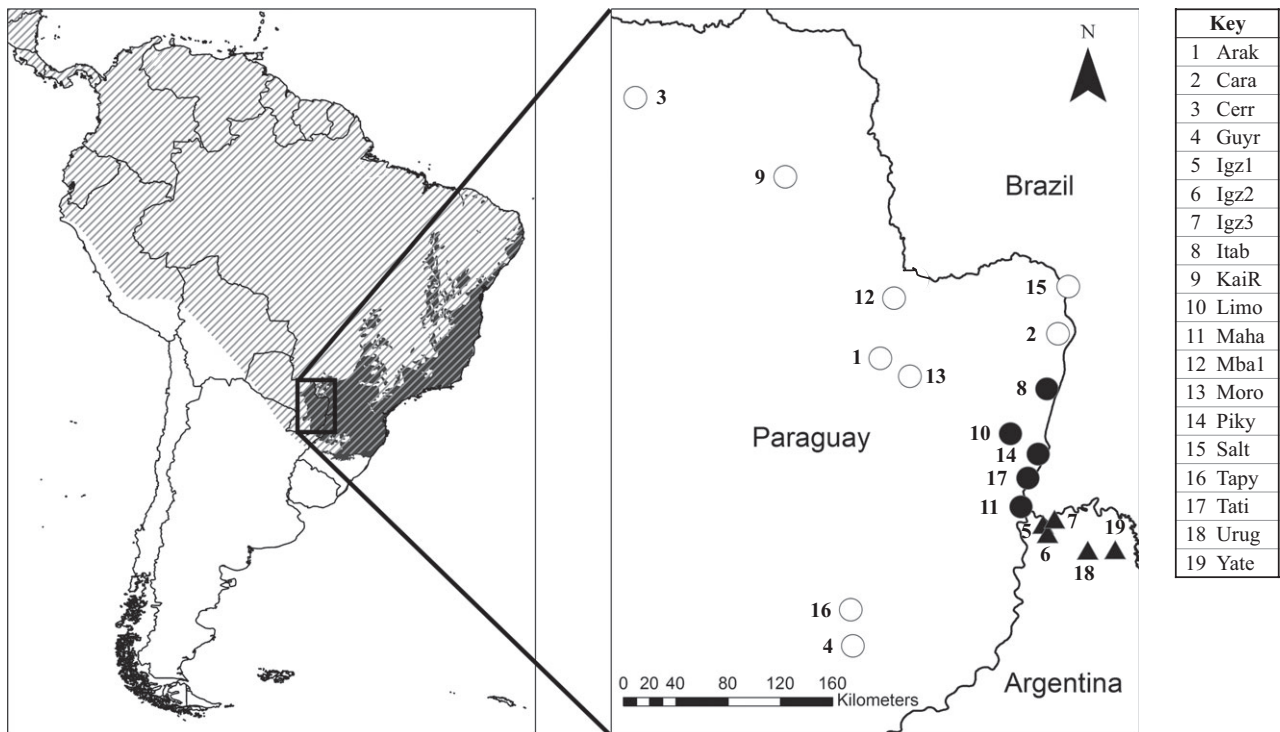


Fig. 1 *Artibeus lituratus*' distribution in South America (light grey lines) and the historical extent of Atlantic Forest (dark grey) are indicated in the left panel. Data obtained from Nature Serve (www.natureserve.org; Patterson *et al.* 2007) and WWF's Global 200 Ecoregions (www.worldwildlife.org), respectively. Sites in continuous ('all cont. '; black triangles) and fragmented ('all frag. '; circles) landscapes are shown. Black circles indicate fragmented sites ('best frag. ') that best matched geographic distances and sample size ($n = 5$) of continuous sites. Key refers to site characteristics as summarized in Table S1 (Supporting information). Maps composed in ARCMAP v9.2 (ESRI 2006).

hectares of roughly contiguous forest. Together, these landscapes provide a natural-experimental setting to understand mechanisms shaping population response in the aftermath of rapid landscape fragmentation.

On average, genetic data came from 28 (range: 19–41) adult *A. lituratus* per site. Tissue samples were obtained from each bat (liver/kidney or wing membrane preserved in 95% EtOH). Details on collection, handling and disposal of specimens are in the supplementary methods (Appendix S1, Supporting information).

DNA extraction and genotyping

Genomic DNA was extracted with a QIAGEN DNeasy Blood & Tissue Kit, following manufacturer's instructions. Five hundred and twenty-six bats were genotyped at 15 microsatellite loci: three originally developed for *Artibeus jamaicensis* (Ortega *et al.* 2002), and 12 developed specifically for *A. lituratus* (McCulloch & Stevens 2011). Thermocycler settings, polymerase chain reaction conditions, and genotyping and scoring information are given in Table S2 and in the supplementary methods (Appendix S1, Supporting information).

Significant deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were tested at each locus for each sampling site in GENEPOP v4.0.10 (Raymond & Rousset 1995) using a Markov chain Monte Carlo (MCMC) method (10 000 dememorizations, 1000 batches, 10 000 iterations-per-batch) following the algorithm of Guo & Thompson (1992). To determine significant departure from HWE, a sequential Bonferroni correction was applied to maintain an experiment-wise error rate of 0.05. Expected (H_E) and observed heterozygosity (H_O) per locus, and allelic richness per locus and per site, were calculated in the R package 'adegenet' (Jombart 2008).

Missing data

Extractions from liver/kidney typically yielded ≥ 22 μg of DNA, while wing membrane extractions [sites 'KaiR' (19 bats) and 'Moro' (13 bats)] had much lower yield (2–3 μg). Bats yielding too little DNA could not be genotyped at all loci. Genotyping was incomplete for locus AjA80 at KaiR and Moro. These missing data could result in inflated or incalculable values of genetic structure for those sites at locus AjA80. Rather than removing an entire locus due to missing data for two of 19 sites, missing data for locus AjA80 were replaced with alleles randomly chosen from the global pool of AjA80 alleles, where the probability of being chosen corresponded to the allele's global frequency (across all sites). This procedure did not generate artificial structure (Appendix S1, Fig. S2, Supporting information).

We additionally ran all analyses of empirical data without locus AjA80. Its exclusion did not generate any qualitatively different results.

Bayesian analyses

We performed Bayesian clustering analyses in STRUCTURE (Pritchard *et al.* 2000) and the R package 'Geneland' (Guillot *et al.* 2005) to assess genetic structure and estimate the most likely number of populations (clusters: 'K') without defining populations a priori. Bayesian methods tend to overestimate K when there is strong IBD (Safner *et al.* 2011), and often fail to identify populations with low genetic differentiation, such as $F_{ST} < 0.05$ (Guillot 2008). We found only one genetic cluster (Fig. S5, Supporting information), which could indicate either weak genetic structure between sites or panmixia. Details in Appendix S1 (Supporting information).

Genetic differentiation

To determine whether weak but significant genetic structure characterized the fragmented landscape, we utilized traditional population-based analyses in which individuals were grouped a priori by sampling locality (sites). Two types of measures were used to characterize population structure in our system: (i) pairwise genetic distances, using chord distance Cavalli-Sforza & Edwards (CSE: Cavalli-Sforza & Edwards 1967) and principle component analysis (PCA: Legendre & Legendre 1998) and (ii) pairwise genetic differentiation, using ' F_{ST} ' (Nei 1973) and 'Jost's-D' (Jost 2008).

Cavalli-Sforza & Edwards values were calculated in GENETIX (Belkhir *et al.* 1996–2004). Statistical significance was determined by comparing the observed value of the estimator to the estimator's distribution obtained by performing 5000 random permutations of the data set, under the null hypothesis of HWE. F_{ST} (Nei 1973) was calculated in the R package 'adegenet' (Jombart 2008), and pairwise values were standardized before all analyses as $F_{ST}/(1-F_{ST})$. Jost's-D was calculated in the R package 'DEMEtics' (Gerlach *et al.* 2010) using the sample bias correction proposed by Jost (2008). Statistical significance of Jost's-D values was determined by comparing the empirical value to a null distribution produced by randomly assigning alleles across populations 1000 times.

Principle component analysis distances were calculated in a multivariate space defined by a principal component analysis for genetic data. Before running the PCA, genotypic data were transformed into an allele frequency matrix (i.e. rows were individuals, columns were alleles) in 'adegenet' (Jombart 2008), and frequencies

were centred and standardized. Missing data for each locus were replaced with the mean global allele frequency (Jombart 2011). Each PC accounted for little covariation among alleles in the data set, suggesting that allele frequencies were largely uncorrelated. To define the genetic multivariate space, the first 125 PC axes (out of 250) were kept, reducing the dimensionality of the data by half, while maintaining the majority of genetic variation (80%). Population centroids in the multivariate space defined by the selected PCs were calculated from constituent individuals, and then, pairwise site-to-site distances were calculated between centroids using function 'dist' (with Euclidean distance) in R package 'stats' (R-project 2011).

Comparison of genetic structure in fragmented and continuous landscapes

If fragmentation were reducing gene flow between sites, then we might expect a steeper increase in genetic differentiation for a given geographic distance across a fragmented landscape than across continuous forest. This effect of fragmentation should be indicated by stronger IBD in the fragmented than in the continuous landscape, and can be evaluated by comparing the relationship between genetic and geographic distances in both landscapes using Mantel tests (Legendre & Legendre 1998). Alternatively, even if no IBD were found in either landscape or the same IBD was found in both landscapes, effects of fragmentation could be evidenced by higher average genetic distances in the fragmented landscape. In other words, fragmentation might be causing greater genetic differentiation independently of the effect of geographic distance. This expectation can be tested by comparing genetic distances between fragmented and continuous sites using the PERMDISP procedure (see below).

To compare IBD in continuous and fragmented landscapes, Mantel tests (Goslee & Urban 2007) were used to correlate pairwise genetic distances (Jost's- D , F_{ST} , CSE and PCA) with pairwise geographic distances (Table S3, Supporting information). This was first done for all sites ('all', $n = 19$) and for all fragmented sites ('all frag.', $n = 14$) to evaluate whether any evidence for IBD was detected in the data set. Because the number of sites and the geographic area of both landscapes were different, the IBD prediction was tested both by comparing continuous sites ('all cont.') to a subset of the fragmented landscape sites ('best frag.': Itab, Limo, Maha, Piky & Tati: see Table S1, Supporting information for site abbreviations), and by comparing 'all cont.' to all fragmented sites ('all frag.'). The set 'best frag.' contained five fragmented sites that best matched pairwise geographic distances among the five continuous sites, were of the same sample size

($n = 5$) and were spatially proximate to the continuous set (minimizing differences from regional environmental variation). Statistical significance of the Mantel's r (r_M) statistic was determined based on 10 000 random matrix permutations, and using an alpha of 0.05 in a two-tailed test. A significant difference in IBD between fragmented and continuous landscapes would be determined by the absence of overlap of 95% confidence limits (CL) between either of the fragmented site-sets and continuous sites. CL were created by bootstrapping data 10 000 times (Mooney & Duval 1993).

To compare the average genetic structure between fragmented and continuous landscapes, we used the procedure PERMDISP (Anderson 2006), a technique that has been used in community ecology to compare differences in species composition among sites using measures of ecological dissimilarity (e.g. Chase 2010). This technique, based on principle coordinates analysis, transformed genetic distances between sites within a group to genetic distances between each site and the group centroid (the 'average' site). It then compared whether distances to the centroid in one group were larger or smaller than the distances of sites in a different group to their corresponding centroid. In this case, the analysis compared whether distances of fragmented sites to the fragmented centroid were larger than distances of continuous sites to the continuous centroid – testing whether sites in the fragmented landscape were more genetically dissimilar from one another (i.e. larger distances) than were sites in the continuous landscape. For statistical significance, either a parametric F -test or a permutation procedure could be used. Both approaches led to identical conclusions, so only P -values based on 5000 permutations are presented. This comparison was also based on 'all cont.' and 'best frag.' or 'all frag.'. PERMDISP analyses were conducted in R using the functions 'betadisper' and 'permutest.betadisper' in 'vegan' (Oksanen *et al.* 2012).

Due to low sample size, it is possible the PERMDISP procedure would not have enough power to detect a real effect of fragmentation. We therefore analysed statistical power of the PERMDISP test given our available sample sizes. Detailed methods and results are in the Appendix S1 (Supporting information). We found low power in PERMDISP tests is primarily caused by very small differences (low effect size) in intersite genetic distances between fragmented and continuous landscapes. Furthermore, these extremely small differences are consistent with a sampling effect, rather than with an effect of reduction in migration rates due to fragmentation. We showed the PERMDISP test conducted using all fragmented sites ('all frag.') should have enough power to detect even minor reductions in gene flow among populations due to fragmentation (Appendix S1; Fig. S3B,C, Supporting information).

Comparing genetic structure between empirical and simulated landscapes

Dispersal of individuals in an artificial landscape was simulated with two main objectives: (i) to ascertain whether, given genetic sampling and time since fragmentation, it was possible to detect effects of reduced dispersal on current patterns of genetic structure (i.e. evaluate efficacy and power of our analyses) and (ii) to derive an estimate of the minimum amount of migration among populations necessary to produce observed levels of genetic structure in the fragmented forest landscape. We performed these spatially explicit simulations using 'Easypop' (Balloux 2001). Easypop models genetic exchange between populations resulting from different mating systems, and dispersal of varying frequencies and distances, while accounting for a number of other biologically meaningful parameters. Simulation parameters described below were set to mimic conditions within the study system. When in doubt about a particular parameter value, we selected a value that would produce a conservative result (i.e. minimized the potential for genetic structure to arise).

Number and location of simulated populations. For the simulations, we merged all sites that were connected by contiguous forest. This resulted in 15 populations corresponding to 14 fragmented sites and a combination of all continuous sites. Their spatial locations correspond to that of empirical sites (Table S4, Supporting information; Fig. 1).

Mating system. A polygynous mating system was simulated, which corresponds to the biology of *A. lituratus* (Sagot & Stevens 2012). Variation in male reproductive success due to polygyny tends to reduce effective population size, potentially increasing genetic structure. This can be offset by extra-harem copulations by subordinate males. Mating by subordinate males was set to 0.7 (70%) based on available information for *Artibeus* (Chaverri *et al.* 2008; Ortega *et al.* 2008) and two other species (Heckel & Von Helversen 2003; Kerth & Morf 2004).

Mutation rate and model. A high mutation rate ($\mu = 0.001$ per allele per generation) was chosen to minimize diversity loss and genetic differentiation. This is within the documented range of microsatellite mutation rates (Ellegren 2004). A single-step mutation model was simulated.

Initial number of loci, alleles and population diversity. We simulated 14 loci, equal to the empirical data, and permitted 25 allelic states per locus—higher than the empirical average but within the empirical range (to

account for potential downward bias in the empirical data set due to sampling). Initial population diversity was set to maximal to simulate panmixia: alleles drawn from all possible allelic states were randomly assigned to genotypes of the first generation.

Initial fragmented patch area and effective population size. Population sizes for reserves in Paraguay and Argentina are unknown. Nevertheless, rough yet realistic values can be estimated. This was done by estimating historic site areas, and then calculating initial population sizes based on these forest-area estimates, and estimates of bat density. The deforestation rate for Paraguayan APAF has been $\sim 1486 \text{ km}^2/\text{year}$ since the 1940s (data points span 1945–1997; Cartes 2003). We conservatively focused on deforestation only over the last 50 years. There were approximately 1 700 000 ha of Atlantic Forest remaining in Paraguay in 2010 ('present day'), while in 1960, there were approximately 7 430 000 ha. The proportion of Paraguayan Atlantic Forest that a given site presently represents was determined using the 2010 area of each site and the total (2010) area of Atlantic Forest in Paraguay. The approximate area for each site in 1960 was then estimated by applying that same (2010) site proportion to the total forest acreage in the past (1960), assuming an equal deforestation rate across Paraguay.

Estimated population densities of *Artibeus* species range from 2 to 7 bats/ha (Handley *et al.* 1991b; Leigh & Handley 1991; Chaverri *et al.* 2008; Gallo *et al.* 2010). While 2 bats/ha is the best-documented population density estimate within the genus *Artibeus* (Leigh & Handley 1991), we conservatively used 4 bats/ha because *A. lituratus* is relatively abundant in the study region. Thus, initial population sizes (N) per site were calculated by multiplying historic patch area (in hectares) by 4. In turn, effective population sizes (N_e) per site were estimated by multiplying historic population sizes by 0.42 (calculated N_e/N ratio for a bat, Storz *et al.* 2001). Estimated (1960) area and corresponding N_e can be found in Table S4 (Supporting information).

Number of generations. Approximately 25 generations are estimated to have passed since fragmentation began, based on an average 2-year generation time. On Barro Colorado Island, Panama, Leigh & Handley (1991) calculated an average female lifespan of 1.6 years and 1.18 offspring per lifetime for *A. jamaicensis*. While not a sister species, *A. jamaicensis* is similar in many aspects of behaviour, body-size and development to *A. lituratus*, so these estimates are expected to be applicable.

Migration rates and distances. Multiple sets of simulations were run using different migration/dispersal

rates: 0, 0.1, 0.3, 0.5, 0.7 and 0.9. Migration rates were intended to capture scenarios from total isolation ($m = 0$) to essentially continuous forest ($m = 0.9$). Simulations were also run using two contrasting dispersal distances selected to capture realistic upper and lower bounds for movement: 5 km (home range, *A. jamaicensis*, Handley *et al.* 1991a) and 30 km (longest recorded dispersal distance for *A. lituratus*, Menezes *et al.* 2008).

Migrating sex. In polygynous mammals, males tend to disperse more and females tend to be more philopatric (Greenwood 1980; McCracken & Wilkinson 2000), although exceptions are not unusual (e.g. Dechmann *et al.* 2007). However, modelling the dispersal of only one sex could unrealistically inflate genetic structure. To avoid this and because patterns of dispersal are not well documented for *A. lituratus*, simulations were run for male-only dispersal, and for which both sexes dispersed with equal distances and probabilities.

Other settings. Each simulation was run for 50 generations, and a particular simulation scenario was replicated 100 times. At each generation, F_{ST} values were calculated, and the 100 replicates were used to construct confidence intervals. The 2.5% and 97.5% quantiles of the distribution of 100 F_{ST} values produced per generation were used as limits for confidence intervals. Empirical average F_{ST} was compared to simulated F_{ST} at generation 25.

Effects of underestimating bat density. Main simulations were based on a density of 4 bats/ha (see above). Additional simulations were run using larger population densities (20 bats/ha) to determine whether these yielded substantively different levels of genetic structure than those found using 4 bats/ha. The effect of increasing bat density is minimal and unlikely to change conclusions from our main set of simulations (Fig. S4, Supporting information). Information on parameterization and results of these simulations is in Appendix S1 methods and Table S4 (Supporting information).

Results

Genetic diversity

No loci were linked. After Bonferroni correction, departure from HWE was significant at site Mba1 (Bonferroni-adjusted $\alpha = 0.003$, P -value = 0, $F_{IS} = 0.10$), and locus N29507 (test of HWE across all populations: adj. $\alpha = 0.0031$, P -value = 0, $F_{IS} = 0.16$; analyses per population: out of HWE at Igz3) and at locus F05378 (adj. $\alpha = 0.0033$, P -value = 0.0003, $F_{IS} = 0.08$, out of HWE at Mba1), possibly due to higher frequency of null alleles.

Locus N29507 was excluded from subsequent analyses due to high F_{IS} . F05378 was kept because of weaker F_{IS} , and because results of analyses run without it (using 13 loci) were not qualitatively different from analyses that included it (14 loci). Number of alleles per locus ranged from 10 to 28 (mean: 18), and expected heterozygosity (H_E) was high (mean: 0.80, range: 0.60–0.91). Summary data for sites and loci are in Tables S1 and S2 (Supporting information).

Genetic structure

Mantel analyses indicated correlations between F_{ST} , PCA and CSE distances were positive and strong (Fig. S1, Supporting information). Jost's D values were only moderately correlated with F_{ST} and not significantly associated with either PCA or CSE distances (Fig. S1, Supporting information). This suggests that F_{ST} , PCA and CSE distances are partially redundant, and that Jost's D values could potentially provide complementary insights. Thus, results are presented primarily for F_{ST} and Jost's D . Analyses repeated for PCA or CSE distances produce identical conclusions and are not shown.

Genetic structure was consistently low, and often not statistically significant, regardless of the metric used (Tables S5 and S6, Supporting information): F_{ST} [mean/average across all pairs (range): 0.012 (–0.006–0.022)], Jost's- D [0.017 (–0.042–0.115)], CSE [0.022 (0.013–0.032)] and PCA [2.728 (1.944–3.312)]. Sites lacked obvious or consistent differences in genetic structure between continuous and fragmented landscapes.

Comparison of genetic structure in fragmented and continuous landscapes

Isolation by distance. Genetic and geographic distances were not significantly correlated ($r_M \approx$ zero) regardless of site-set or genetic distance measure used (Table 1, Fig. 2). Confidence limits of the r_M broadly overlapped between 'best frag.' and 'all cont.' site-sets, as well as for the most liberal comparison, between 'all frag.' and 'all cont.' site-sets (Table 1). IBD was also nonsignificant for all sites ($n = 19$).

PERMDISP procedure. Geographic distances among all sites in the fragmented landscape were significantly higher than in the continuous landscape (Fig. 3A). When the comparison was limited to only 'best frag.', however, differences disappeared (Fig. 3D). Genetic structure among sites was not significantly different between continuous and fragmented landscapes using any measure of genetic distance, even when using all fragmented sites (Fig. 3B,C). These conclusions do not

Table 1 Isolation by distance was not significant for any genetic distance metric or site-set. Mantel tests correlated pairwise (log) geographic distance with genetic distances for different site-sets. Significance was determined at a *P*-value of 0.05 (two-tailed test) and calculated based on 10 000 permutations in ‘ecodist’ (Goslee & Urban 2007). Ten thousand iterations were used to determine bootstrapped confidence limits (CL). Pairwise distances can be found in Tables S3, S5 and S6 (Supporting information)

Site-set (no. sites)	Genetic distance	r_M	Lower CL (2.5%)	Upper CL (97.5%)
all (19)	F_{ST}	-0.102	-0.256	0.053
	Jost’s- <i>D</i>	-0.122	-0.251	-0.009
all frag. (14)	F_{ST}	-0.092	-0.246	0.079
	Jost’s- <i>D</i>	-0.240	-0.373	-0.119
best frag. (5)	F_{ST}	0.159	-0.558	0.758
	Jost’s- <i>D</i>	0.066	-0.758	0.905
all cont. (5)	F_{ST}	-0.342	-0.556	-0.085
	Jost’s- <i>D</i>	-0.041	-0.478	0.200

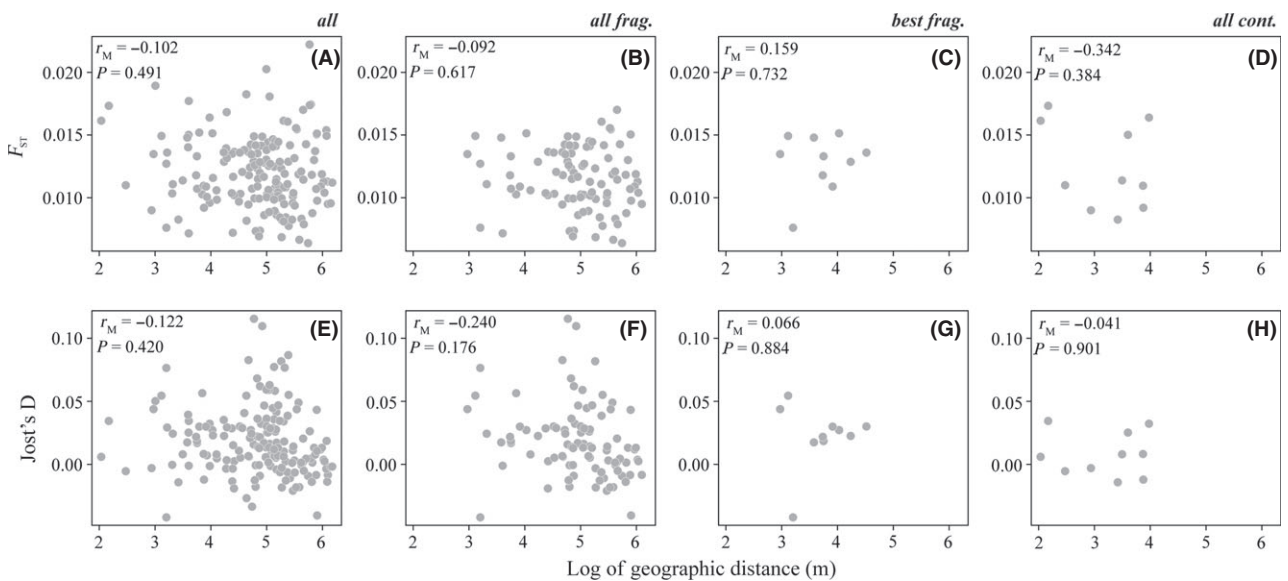


Fig. 2 Mantel test results correlating pairwise log geographic distance (metres) with genetic structure metrics F_{ST} (A–D) and Jost’s *D* (E–H) for all sites ($n = 19$; A, E), fragmented sites (‘all frag.’: $n = 14$; B, F), the most comparable fragmented sites (‘best frag.’: $n = 5$; C, G) and continuous forest sites (‘all cont.’: $n = 5$; D, H). There was no statistically significant IBD. Significance was determined at a *P*-value of 0.05 (H_0 : $r_M = 0$) and calculated based on 10 000 permutations (R package ‘ecodist’).

result from lack of power; at the least, the more liberal comparison between ‘all frag.’ and ‘all cont.’ should have enough statistical power to detect even small reductions in migration, even when dispersal distances are large (Fig. S3B,C, Supporting information).

Comparing empirical and simulated landscapes

Simulated outcomes of fragmentation for 15 subpopulations were qualitatively similar for male-only dispersing vs. both sexes. Results presented are for both sexes dispersing. Simulated population genetic structure was strongly influenced by movement: F_{ST} values increased with decreases in dispersal distance or migration rate (Fig. 4A,B). Under the most extreme case of complete

isolation since fragmentation ($m = 0$), F_{ST} increased immediately and rapidly, and by 25 generations had already reached close to an order of magnitude greater than empirical differentiation. Moreover, regardless of simulated migration rate (0.1–0.9), short dispersal distances (5 km) resulted in F_{ST} values that, while low, were consistently greater than empirical differentiation (Fig. 4A). Similarly, decreased migration rates produced clear genetic structure among simulated populations even when using a large dispersal distance (30 km: Fig. 4B). This indicates that reduced migration among sites should be detectable in empirical genetic structure given the genetic sampling we performed and our expected time since fragmentation. In fact, simulated F_{ST} values approaching empirical genetic differentiation

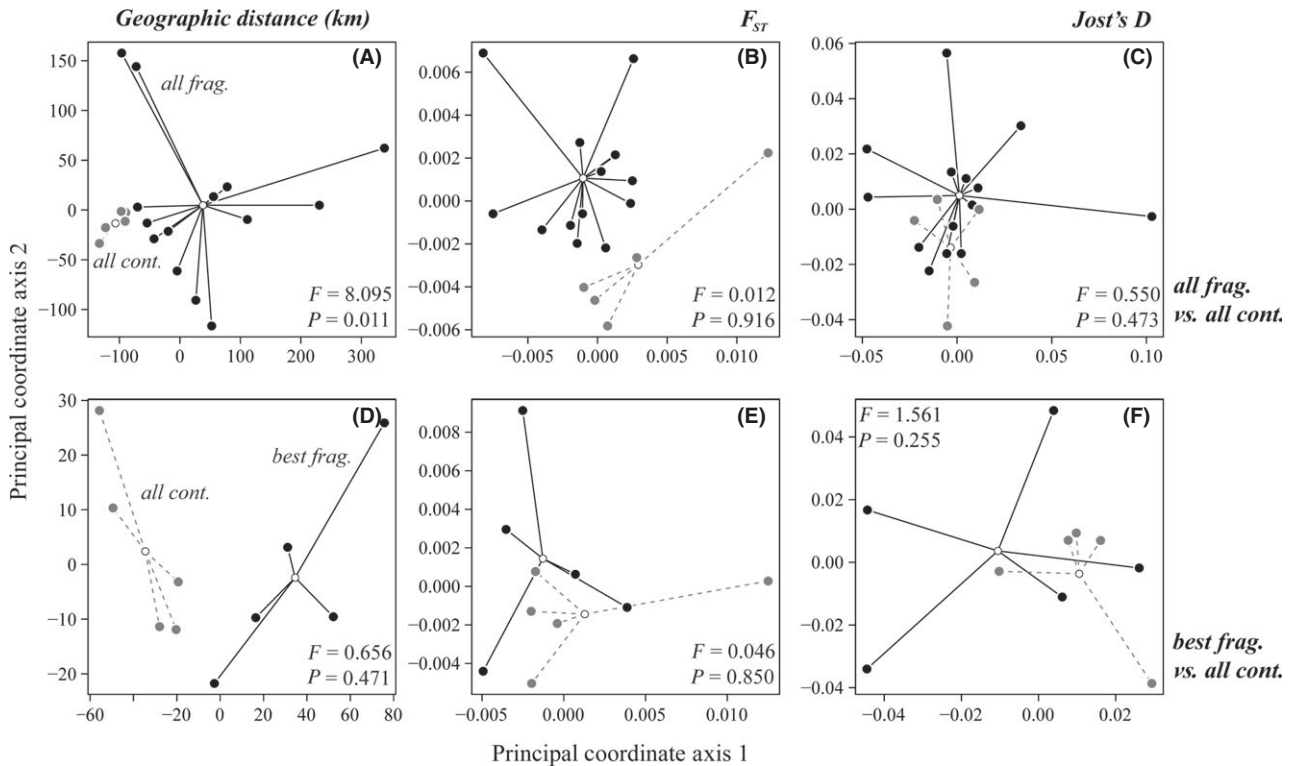


Fig. 3 Comparison of magnitude of geographic and genetic (F_{ST} and Jost's D) dissimilarities between sites in continuous ('all cont.': grey) and fragmented (black) landscapes using the PERMDISP approach; comparisons were made for both the full set of fragmented sites ('all frag.', $n = 14$; A–C) and the subset 'best frag.' ($n = 5$; D–F). Geographic (A, D) and genetic distances (B, C, E and F) were decomposed into independent axes using a principal coordinate analysis. Then, distances from each site to the group centroid (hollow circles) were calculated, and compared between groups. Only the first two principal coordinates axes are shown; but all axes were used for analysis. There was no significant difference in level of genetic structure among sites in continuous vs. fragmented landscapes, even for comparisons between 'all frag.' and 'all cont.' (B, C)—despite 'all frag.' showing significantly larger intersite geographic distances than did sites in the continuous landscape (A).

levels were only found when simulated dispersal distance was large and migration rate was high (Fig. 4B).

Similarly, simulated data showed that fragmentation should be expected to produce strong IBD, yet empirical IBD was not statistically significant, and in fact trended in the opposite direction from expected (Fig. 4C,D). Only when total absence of migration ($m = 0$) was simulated did we find IBD by generation 25 that included the observed level of IBD found in the fragmented landscape. However, this apparent fit was caused by the broad confidence interval, and the fact that $m = 0$ caused decoupling of spatial distance from genetic distance. A similar level of IBD would be expected if $m = 1$ were simulated. Moreover, the magnitude of simulated F_{ST} values when $m = 0$ is inconsistent with empirical data (Fig. 4E,F).

Discussion

Anthropogenically driven habitat fragmentation is pervasive. Understanding effects of fragmentation on

population connectivity is critical to predict and mitigate effects that large-scale modifications to ecosystems might have on species. We determined whether recent fragmentation of Atlantic Forest is causing subdivision in populations of *A. lituratus*, a widespread Neotropical seed disperser. We found genetic structure of *A. lituratus* in Alto Paraná Atlantic Forest is weak, and our results are consistent with intact, or nearly intact, contemporary migration between sites and long-distance dispersal ability.

Low genetic structure in a highly mobile animal

Simulations indicate that major reductions in gene flow in this species should be detectable in a very short time period—even if dispersal distances are typically large. This conclusion remains even though a number of parameter estimates were conservative (i.e. minimized the potential for genetic structure to arise). For example, models treated populations as initially panmictic (maximum genetic diversity), used a high mutation rate, and

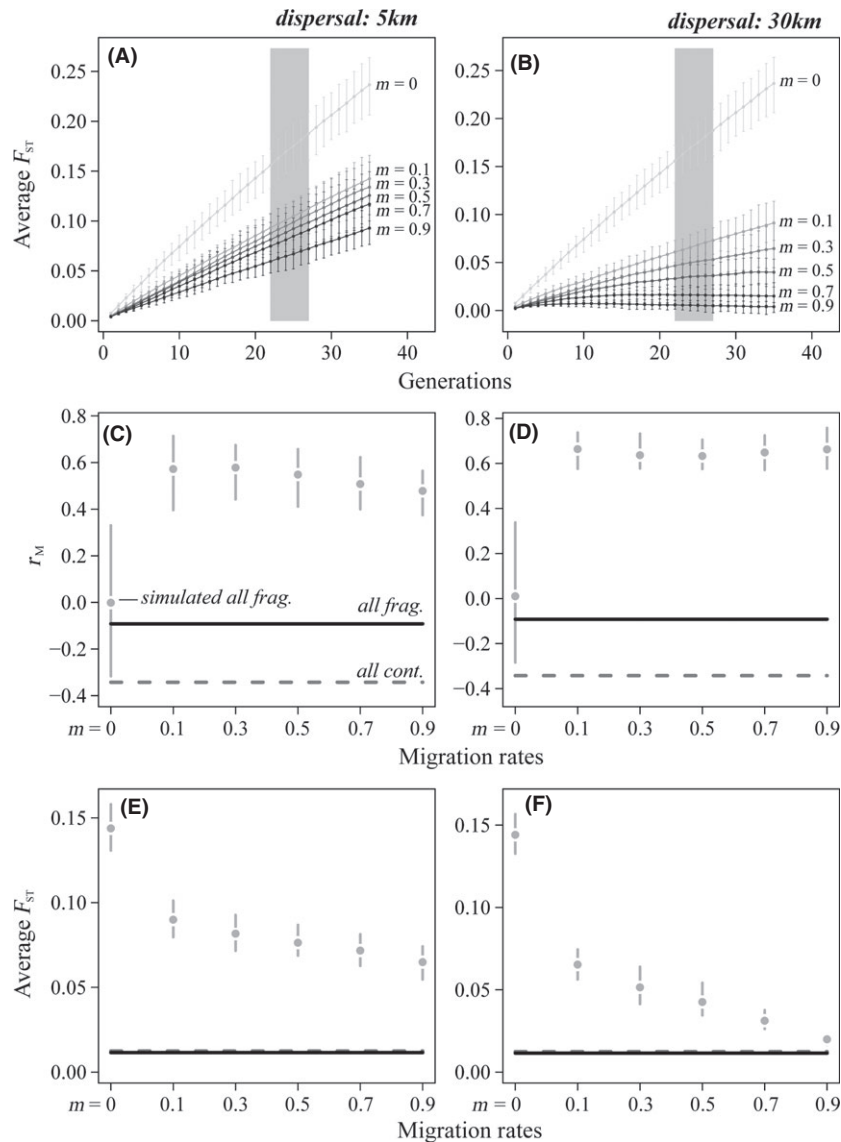


Fig. 4 (A, B) genetic structure results from simulating 15 sites with varied intersite average dispersal distance (30 or 5 km) and migration rate (0–0.9). Detailed parameterization information is found in the Methods and Table S4 (Supporting information). Vertical lines indicate the range of F_{ST} values over 100 replicate simulations for a given generation. Grey bar highlights the time span under consideration, ~25 generations since fragmentation. (C, D) Mantel statistic (IBD tests) for empirical data, and for simulated data at generation 25. (E, F) magnitude of (average) F_{ST} values between pairs of sites for simulated (generation 25) and empirical data. In (C–F), horizontal lines represent empirical Mantel r and average empirical F_{ST} (black: ‘all frag.’, grey-dashed: ‘all cont.’; both nonsignificant). Grey points show the median of simulated values based on fragmented sites at generation 25 for each simulated migration rate. Grey lines around points represent confidence intervals estimated using 2.5% and 97.5% quantiles, distribution based on 100 simulated values. Simulations show that, given almost any reduction in migration, clear and significant structure should be observable in the form of IBD and high F_{ST} . In almost all cases, structure in simulations is inconsistent with empirical data. The only exception is for IBD when simulated migration is zero. In this situation, IBD is so low because lack of dispersal results in complete decoupling of geographic and genetic distances. However, the magnitude of pairwise F_{ST} values at $m = 0$ are inconsistent with this scenario being likely in the empirical data.

included a case where both sexes had equal propensity for dispersal—all of which would tend to minimize genetic structure. Additionally, simulations were parameterized based on only 50 years of deforestation. If gene flow had been reduced for more than a few generations,

it should be detectable based on our sampling. This remains true even if population densities are much higher than estimates used for our main analyses (Fig. S4, Supporting information). Despite adequate power to detect genetic differentiation resulting from

reduction in gene flow, there are few indications of genetic structure for *A. lituratus* in the study region.

Even though observed genetic diversity falls in a range typical for noninbred mammals (Frankham *et al.* 2002), that so little spatial genetic structure is detected across such a large area is somewhat unexpected for an ostensibly nonmigratory bat species. There are multiple factors that might facilitate intersite connectivity for *A. lituratus* in APAF. While the landscape of eastern Paraguay has undergone extensive deforestation, there remain forest patches that may function as stepping stones connecting large reserves. Furthermore, in agricultural or ranching areas, lone trees could expedite movement by providing temporary roosting sites or food (Kelm *et al.* 2008). In addition, *A. lituratus* do not appear to disfavour moderately disturbed forest (Gorresen & Willig 2004) and are known to forage on pioneer species often found in disturbed areas or secondary growth, such as those of the genus *Piper* and *Cecropia* (Garcia *et al.* 2000; da Silva *et al.* 2008). The ability to exploit multiple food resources could enable population resilience to changing landscape dynamics, if generalist animals are likely to perceive habitat as having more resources and being less fragmented (Bascompte *et al.* 2006). Lack of genetic structure might also be in part a consequence of feeding strategy. *Artibeus* species feed on *Ficus* (Moraceae; Morrison 1978; de Moraes Weber *et al.* 2011). *Ficus* species, like many tropical trees, often exhibit low population densities, which combined with fruiting asynchronicity would encourage long-distance foraging movements between fruiting trees (Morrison 1978; Nazareno & de Carvalho 2009).

Plant–animal interactions in fragmented landscapes

Results from seed-dispersal studies suggest habitat fragmentation can affect plants both by reducing the number of seeds reaching patches (e.g. Benitez-Malvido 1998) and the number of individual plants contributing to the colonizing seed pool (reviewed in Sork & Smouse 2006). Furthermore, plant species are often primarily pollinated or dispersed by only one or a few taxa (e.g. Morrison 1978; Muchhala & Thomson 2009). Examples of this include many groups of plants adapted for bat seed dispersal (e.g. species in the families Araceae, Urticaceae, Clusiaceae, Piperaceae and Solanaceae) and pollination (e.g. various genera in the families Campanulaceae and Marcgraviaceae; Fleming *et al.* 2009; Lobova *et al.* 2009). Thus, the potential for numerous plant species, especially in the tropics, to adapt to anthropogenic changes to habitat is certainly influenced by their animal counterparts, but this link is largely unexplored. Our results indicate that *A. lituratus*, a relatively generalist and numerically dominant (in the study region; Stevens *et al.* 2004) seed disperser, exhibits little genetic structure across a

fragmented landscape in eastern Paraguay. As a consequence, plant species that depend on *A. lituratus* for dispersal, like *Ficus* species, might also be expected to be panmictic across the same region, thereby buffering the community from some impacts of deforestation. Identifying taxa with potential to be keystone species in fragmented landscapes may prove increasingly important as habitat loss threatens tropical environments worldwide.

Fragmentation effects and traits of species

It is unclear if results from our study can be generalized to other Neotropical seed dispersers and pollinators. Relatively few studies have attempted to predict bat responses to habitat fragmentation based on life-history traits, or behaviour, but Meyer *et al.* (2008) found edge sensitivity was a dominant predictor of changes in Neotropical bat abundance due to fragmentation and that dispersal propensity across a hostile (water) matrix was predictive of genetic diversity and differentiation in two species (Meyer *et al.* 2009). Among Old World species, Rossiter *et al.* (2012) found that foliage-roosting bats had lower genetic connectivity than cave-roosting species, and Struebig *et al.* (2011) found a bat with lower mobility and population density exhibited declines in genetic diversity associated with fragment area. Burney & Brumfield (2009) found that Neotropical canopy birds show weaker genetic structure across potential barriers (the Andes, the Amazon River, the Madiera River) than understory species, suggesting dispersal propensity is affected by ecological differences among species. Specialization in habitat preference, for example, has been shown to predict responses to habitat fragmentation in a number of taxa. An explicit comparison of genetic structure for two closely related snakes across a fragmented landscape found strong structure for the specialist species, but no structure for the generalist (DiLeo *et al.* 2010). Interestingly, this finding may hold true for specialists with higher potential mobility: Coulon *et al.* (2008) found relatively strong population structure in a naturally fragmented landscape for the Florida Scrub Jay, a habitat specialist and cooperative breeder, and that gap size between patches drove reductions in gene flow rather than geographic distances *per se* (Coulon *et al.* 2012).

Artibeus lituratus' broad distribution, potential high-mobility foraging strategy, and status as a relatively generalist frugivore could all reasonably explain weak structure in the study region. Other bats in this region share subsets of those traits (López-González 2005) and might be expected to respond in predictable ways to habitat fragmentation. If generalist species are relatively robust to habitat fragmentation, this could have positive implications for conservation in the study region and

elsewhere in the Neotropics. However, more research is necessary to determine differential effects of fragmentation on gene flow in generalist and specialist species, particularly for seed-dispersing taxa in the tropics. It is also possible that reductions in (still high) migration across the fragmented landscape of Paraguay may be occurring, but are insufficient to have been detected. Minor reductions in overall high levels of migration would not produce detectable changes in genetic structure within the time frame of analysis, assuming dispersal distances were typically large. Given enough time, or continued deforestation, effects could emerge.

Conclusion

Highly mobile pollinating and seed-dispersing taxa can play an important role in mediating plant population responses to habitat disturbances, particularly in the tropics where many plants depend on animals for reproduction, and habitat loss is a growing threat. Nevertheless, research on the genetic structure of tropical and subtropical taxa is limited (Storfer *et al.* 2010), particularly for plant-visiting species. Our study demonstrates high levels of genetic connectivity for an abundant and widespread seed-dispersing bat across a region of ~140 000 km², much of which is heavily deforested. We conclude that population connectivity of *A. lituratus* is relatively resilient to forest fragmentation that has occurred to date. It is nevertheless important to note that genetic connectivity is but one of several factors that could be affected by habitat fragmentation. Other avenues by which fragmentation could influence populations of this and other species include reductions in reproductive output due to factors such as decreased quantity or quality of food, and increased competition or predation. Furthermore, while this study strongly supports high levels of present genetic connectivity consistent with frequent intersite movements, it is possible further fragmentation could result in isolation. More studies are needed to determine conditions governing resilience in potentially keystone, highly mobile taxa like *A. lituratus*, and to understand whether resilience of seed dispersers translates into resilience of plant species that interact with them.

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Data accessibility

Previously published *A. lituratus* microsatellite primer sequences and associated data: Dryad doi:10.5061/dryad.9089.

Empirical microsatellite genotypes used for analyses: Dryad doi:10.5061/dryad.bj57t.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Contains supplementary methods, tables, and figures.