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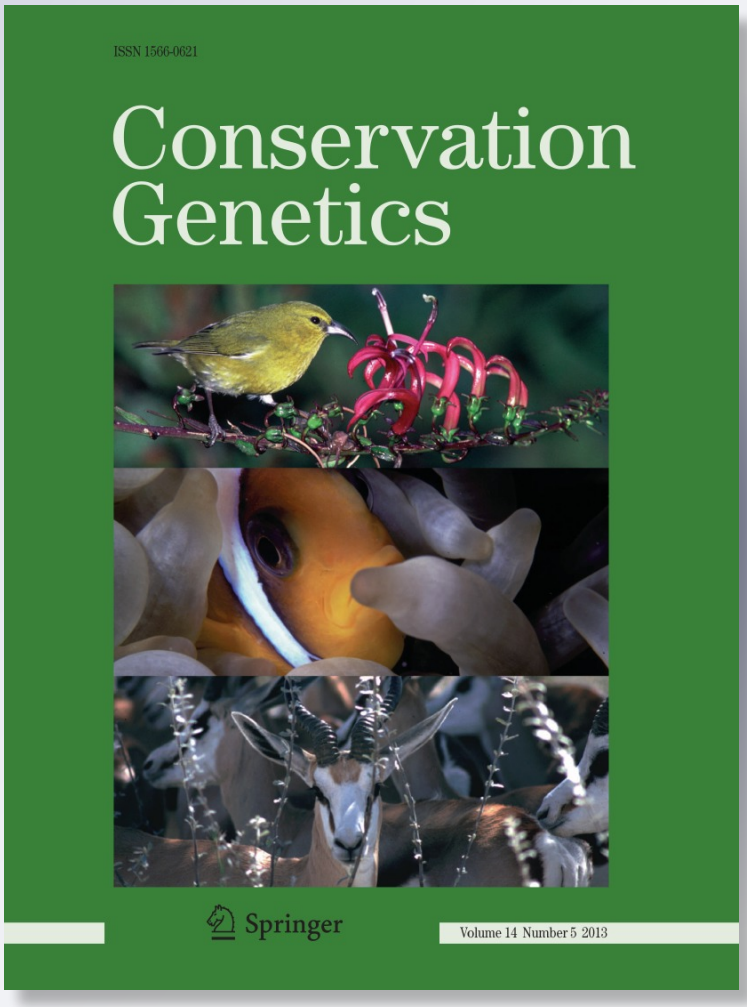

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RESEARCH ARTICLE

Life in a mosaic landscape: anthropogenic habitat fragmentation affects genetic population structure in a frugivorous bat species

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Abstract Bats are often considered highly mobile and hence less susceptible to habitat fragmentation than other animals. We tested this basic assumption by studying populations of *Dermanura watsoni*, a frugivorous phyllostomid bat, inhabiting forest fragments in an agriculturally dominated landscape in northeastern Costa Rica. We used the mitochondrial D-loop DNA-sequence data to examine genetic diversity and population structure. A significant population differentiation ($F_{ST} = 0.05$, $p < 0.001$) over a geographical scale of approximately 20 km was detected. Genetic diversity within fragments correlated with patch size and the amount of suitable habitat in the surrounding matrix. The composition of the matrix in close proximity to the fragments explained variation in genetic diversity best. However, only habitat parameters measured from 1986 land cover conditions can explain current genetic diversity, and not those from 2001. Our study demonstrates that bats, despite their high mobility, are not secure from genetic erosion in anthropogenically modified landscapes. Population differentiation can occur on a surprisingly small geographic scale and after short time periods. Our findings illustrate the importance of considering several points in time when testing for an influence of habitat parameters as it might be decades until they are reflected by genetic diversity.

Keywords *Dermanura watsoni* · Habitat fragmentation · Genetic diversity · Mitochondrial D-loop

Introduction

During the last decades habitat degradation and fragmentation caused by changes in land use have reached critical levels from local to global scales, especially in the tropics (Wade et al. 2003; Hansen et al. 2010). In many tropical countries, expanding human settlements and an increased need for agricultural areas accelerate deforestation rates and fragmentation of once continuous habitats at an alarming pace, thus endangering biodiversity on multiple scales (Sala et al. 2000; Laurance et al. 2001; Foley et al. 2005). Declining species diversity in animal and plant communities and decreases in species abundance are consequences of anthropogenic activities such as logging and hunting (Cosson et al. 1999; Daily et al. 2003; Fahrig 2003; Craul et al. 2009). This is why forest remnants play a crucial role for conservation in areas under strong human influence as they may constitute the last retreat for many endangered tropical species (Turner and Corlett 1996; Dotta and Verdade 2011).

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In the long run, habitat disruption by distinct dispersal barriers such as streets or large agricultural areas may cause discontinuities in gene flow that gradually endanger populations' fitness, adaptability and thus long-term survival, through loss of genetic diversity (Johansson et al. 2007; Dixo et al. 2009; Roberts et al. 2011). The potential to adapt by micro-evolutionary processes, which is based on genetic variability, is essential to persist in a changing environment (Visser 2008). Once habitat has been fragmented there might be a time lag after which the genetic impact becomes visible (Keyghobadi et al. 2005; Vandergast et al. 2007). Hence landscape parameters should be analyzed at different temporal points, to get an impression of the timescale at which landscape parameters influence genetic conditions of populations.

Different responses to matrix habitats found even in closely related species (Ricketts 2001) indicate that fragmentation sensitivity might be highly species-specific. Sensitivity to fragmentation is linked to a range of specific characteristics, including mobility and body size (Henle et al. 2004). Hence, a wide range of studies focusing on species with restricted mobility documented negative genetic effects of fragmentation and habitat loss in various animal groups, including invertebrates (Dhuyvetter et al. 2005; Vandergast et al. 2007), amphibians (Makeeva et al. 2006; Dixo et al. 2009), reptiles (Cunningham and Moritz 1998; Stow and Briscoe 2005) and small terrestrial mammals (Hirota et al. 2004; Neuwald 2010). Even large populations of highly mobile mammalian species, e.g. sheep, mountain lions, coyotes, and bobcats, may suffer genetic erosion after human habitat degradation (Ernest et al. 2003; Epps et al. 2005; Riley et al. 2006).

As bats are considered mobile compared to other animal groups (Kalcounis et al. 1999), they appear at first glance to be relatively insensitive to habitat fragmentation. This is perhaps why during the initial emergence of genetic methods bats were largely underrepresented, despite their high diversity (Burland and Wilmer 2001). In particular, tropical bat species failed to capture the attention of ecologists applying molecular methods, and the few studies assessing landscape genetics in bat populations focused on rather large geographic scales (Wilkinson and Fleming 1996; Ditchfield 2000; Newton et al. 2003; Carstens et al. 2004; Roberts 2006). Ecological studies demonstrated that bat species may be sensitive to fragmentation, despite their relatively high mobility, depending on their ecological characteristics (Cosson et al. 1999; Gorresen and Willig 2004; Meyer et al. 2008). Subsequent work focused on bat population structure in the context of habitat fragmentation on a small geographic scale (Asher 2009; Meyer et al. 2009; Struebig et al. 2011). Meyer et al. (2009) detected significant population differentiation of fragmented bat populations in a water matrix over a geographic scale of only a few kilometers. Struebig et al. (2011) found genetic diversity of populations inhabiting forest fragments in an agricultural landscape to decline as a function of habitat patch size.

The study of tropical bat species in anthropogenically fragmented landscapes is especially interesting as they fulfill crucial roles in tropical ecosystem functioning as pollinators and seed dispersers (Ghanem and Voigt 2012). Frugivorous neotropical bats disperse seeds of a huge array of plant species from early to late succession stages (Giannini and Kalko 2004; Muscarella and Fleming 2007; Lobova et al. 2009; Mello et al. 2011). As they are not under strong hunting pressure (Wright et al. 2007), they are especially valuable for forest regeneration and re-establishment of vegetation in degraded areas (Muscarella and Fleming 2007; Silveira et al. 2011) once larger mammalian and avian seed dispersers have already disappeared (Melo et al. 2009). Some species even hold the potential to maintain gene flow of plants between forest fragments by dispersing seeds (Galindo-González et al. 2000). Therefore, the persistence of bat populations in such anthropogenically dominated landscapes might be essential for the preservation of the remaining plant-, and in turn also animal diversity (Melo et al. 2009). Genetic diversity in fragmented populations is usually influenced by population size and connectivity (Traill et al. 2010; Potter et al. 2012). In this way, studying small-scale genetic structure of frugivorous bat populations that inhabit degraded areas allows insight into the dynamics of population differentiation, and may reveal the influence of habitat and matrix parameters on genetic diversity of populations. Molecular analysis on population connectivity with the inclusion of information on landscape characteristics might lay the groundwork for future conservation strategies.

In order to illustrate the genetic response of bat populations to habitat fragmentation, we exemplarily assessed genetic diversity of the small frugivorous bat, *Dermanura* (*Artibeus*) *watsoni* that is abundant in primary and secondary forests, and contributes to seed rain in degraded areas (Melo et al. 2009). We analyzed mitochondrial DNA sequences of populations inhabiting forest remnants in an agriculturally dominated landscape in the Caribbean lowlands of Costa Rica. Our goals were (1) to define the small-scale population structure of *D. watsoni* in an anthropogenically fragmented landscape (2) to identify discontinuities in gene flow among populations, and (3) to link genetic diversity within populations to characteristics of the habitat and the surrounding matrix.

Materials and methods

Study area and focus species

The study was conducted in the canton Sarapiquí in the northern Caribbean lowlands of Costa Rica (10°25′N,

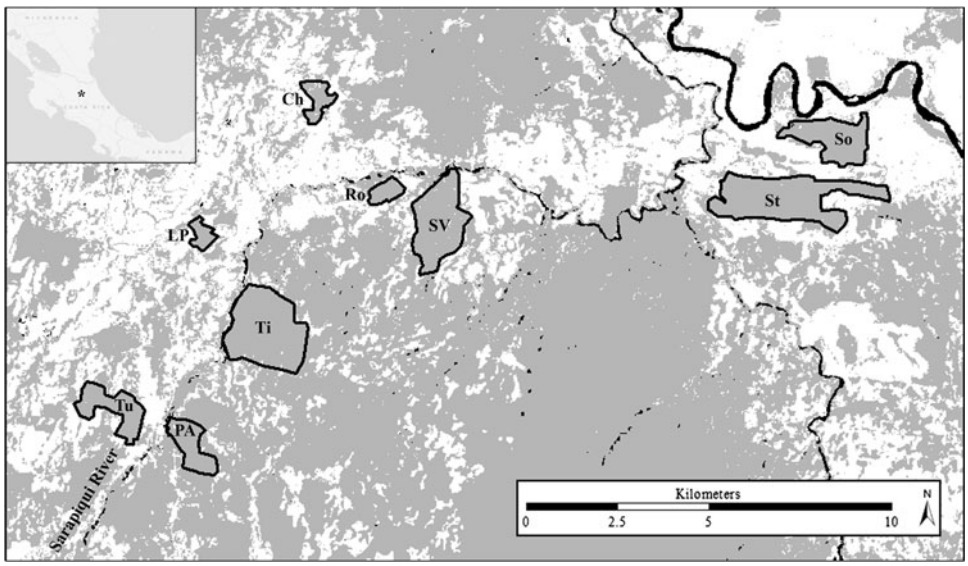

Fig. 1 Land cover map of the study area in the northern Caribbean lowlands of Costa Rica showing the nine sampling sites; *grey* indicates forest cover, *white* indicates non-forest cover, *black* indicates water (except the fragments' framing); *Tu* El Tucan, *PA* Hacienda Pozo Azul, *Ti* La Tirimbina, *LP* Las Palmitas, *SV* Selva Verde Lodge, *Ro* El Roble, *Ch* Rancho Chilamate, *St* Finca Starke, *So* Finca Sofia

Table 1 Genetic diversity at the mitochondrial D-loop in nine fragmented bat populations

Population	Area 1986	Area 2001	n	No. haplotypes	h	θ_k
Ti	367	412	27	21	0.977 ± 0.017	41.54
St	526	408	15	11	0.952 ± 0.04	17.06
SV	280	280	13	10	0.949 ± 0.051	18.02
So	436	187	22	14	0.926 ± 0.039	15.53
Tu	165	153	15	11	0.952 ± 0.04	17.06
PA	115	99	18	12	0.954 ± 0.03	14.56
Ch	1218	68	15	14	0.991 ± 0.028	95.45
Ro	48	47	26	13	0.902 ± 0.04	9.67
LP	34	38	21	14	0.957 ± 0.026	17.19

Fragment area measured in ha; n number of sampled bats, h gene diversity, θ_k theta estimator based on the number of different haplotypes

84°05′W). The region experienced extensive anthropogenic deforestation up until the 1980 s (Sánchez-Azofeifa et al. 2007), and has since been characterized by a mosaic structure, composed of forest remnants embedded into a matrix of cattle pastures, plantations and urban structures. A high-traffic road following the path of the Sarapiquí River traverses the landscape. We worked in nine forest fragments of different size and with moderate to high degree of isolation (Fig. 1). Tu, LP, and Ch are located to the north-west of the road, PA, Ti, Ro, and SV to the south or south-east, and So and St to the north-east. Patch size varied from 38 to 412 ha (Table 1; based on land cover maps from the year 2001, in ArcGIS 10. Maps were provided by Sesnie 2008). The fragments are situated at elevations ranging from ca. 50 to 300 m asl and are covered by mainly primary and late secondary succession vegetation. In our study we focused on *D. watsoni*, a small bat (ca. 12 g) which feeds mainly on fruits. It is common to abundant in primary and secondary growth and occurs usually below 800 m (Reid 2009). As a tent-making bat, *D. watsoni* constructs its roosts from leaves and forms small female-biased, mixed-sex social groups with frequent changes of individuals between roosts (Rodríguez-Herrera et al. 2007; Chaverri et al. 2008).

Sample collection and molecular analyses

We mist-netted bats during two field seasons using ground nets (March to August 2010, January to August 2011) and we occasionally captured entire social groups from leaf tent roosts during the day. A bias that may arise from sampling social groups due to the possibility of high relatedness can be neglected in *D. watsoni* as roosting associations are low (Chaverri et al. 2008). For mist-netting we established three to five homogenously distributed capture sites per fragment, depending on fragment size. Identification of bats was based on the Costa Rican field key (Timm and LaVal 1998). For distinguishing in the field between

D. watsoni and the very similar congener *D. phaeotis,* we used the presence of a third lower molar, a rather unreliable but the only available field-suitable method (Timm and LaVal 1998). However, after genotyping, the DNA sequences of the two species were easily distinguishable. We documented sex, age and reproductive state. Tissue samples were collected from the wing membrane at a standardized position between the 4th and 5th finger (biopsy punch, Ø 4 mm, Stiefel®) and stored in ca. 80 % ethanol. When the wounds healed up, a distinct scar which lacked normal pigmentation was visible during the entire sampling period and ensured the exclusion of recaptured bats from the sampling procedure. Only samples of adult individuals were included in the molecular analyses. Extraction of DNA from skin samples was performed following the salt-chloroform method (Müllenbach et al. 1989). The mitochondrial D-loop was amplified by using the polymerase chain reaction (PCR) with the primers E (Wilkinson and Chapman 1991) and P* (Wilkinson et al. 1997) following the protocol given in Wilkinson et al. (1997). The fragment was sequenced from one end using Primer P* and BigDye Terminator Cycle Sequencing Kit version 1.1 on an ABI Prism Genetic Analyzer 3130 XL. Sequences were aligned and edited in CodonCode Aligner 3.5.The sequences of 60 different haplotypes were deposited in Genbank (accession numbers: KC164694-KC164753).

Population genetic analyses and genetic diversity

We used Arlequin 3.5 for all standard calculations if not indicated otherwise (Excoffier et al. 2005). We estimated the total number of haplotypes. Gene diversity (h) and theta k (θ_k) were calculated as molecular diversity indices for individual populations. To test for global population differentiation we performed Analyses of Molecular Variance (Amova, based on Wright's F-statistic F_{ST} Wright 1951). Differences between the nine populations were assessed by pairwise F_{ST}-values (Wright 1951) and 10,000 permutations for significance tests. α-levels were adjusted by a sequential Bonferroni correction, based on $\alpha = 0.05$. To identify possible genetic barriers between groups of populations we carried out a Spatial Analysis of Molecular Variance (Samova 1.0; Dupanloup et al. 2002). The Samova combines populations into k groups wherein intergroup variation (F_{CT}) is maximized. As F_{CT} usually increases with a higher k, we defined the most likely number of groups k for our dataset when the change between two F_{CT}-values (ΔF_{CT}) started to decline with increasing k. The inferred structure was then tested by an Amova in Arlequin. We also tested for an Isolation-by-distance (IBD) pattern between Euclidean geographical distances and genetic distances (pairwise F_{ST}) with 10,000 permutations (Mantel 1967).

Determination of habitat parameters

In order to obtain variables referring to habitat quality, we implemented fine-grained land cover maps into ArcGIS 10, which were generated by the use of decision tree classifiers from aerial photographs for the years 1986 and 2001, respectively (Sesnie et al. 2008). Sesnie et al. (2008) classified land cover into 32 categories from natural forest (10 categories), wetlands (3), reforestation/forest regeneration (4), agriculture (12) and others (3). The grid size of the used raster was 28.5 × 28.5 m. We measured fragment sizes for 1986 and 2001, respectively, using ArcGIS 10. In order to assess connectivity of a fragment's surrounding matrix, we defined buffer zones of different widths (200, 400, 750, 1,000, and 2,000 m) according to the fragment's shape in 1986 and 2001, respectively. We then extracted the absolute amount of potentially suitable habitat for *D. watsoni* from the respective buffer zones (ArcGIS 10) by discounting matrix elements which strongly contrast the natural habitat. Matrix elements that were more similar to the natural habitat should be less hostile and enhance the probability of genetic exchange. Such suitable habitat included all kinds of natural forests, old forest regrowth, palm swamps, reforested areas and riparian forests. We chose these land cover categories as we frequently observed and captured *D. watsoni* in such habitat types and the present vegetation accorded to its known feeding and roosting requirements (Rodríguez-Herrera et al. 2007; Melo et al. 2009; Reid 2009). Unsuitable matrix habitat encompassed all remaining land cover types such as farmland (e.g. pineapple plantations), cattle pastures, or human infrastructure (e.g. streets or urban areas).

In order to test for dependence of molecular diversity indices (h and θ_k) on the habitat parameters mentioned above, we used linear regression models. All variables were tested for normality (Shapiro–Wilk test; $\alpha = 0.1$) and log-transformed if necessary to minimize residual standard errors of linear models (R 2.14.1).

Results

Genetic diversity

We sampled a total of 172 adult individuals of *D. watsoni* (73 males, 99 females) from the nine forest fragments. Analysis of 371 base pairs (bp) of the mitochondrial D-loop revealed 33 variable nucleotide sites leading to 60 unique haplotypes. Ten to 21 haplotypes were found per population (Table 1). The frequencies of each haplotype per fragment are shown in "Online Resource 1". Genetic diversity (h) ranged from 0.902 in Ro to 0.991 in Ch, both relatively small fragments at the present day. Those

sampling sites also showed the two extreme values for θ_k with 9.67 and 95.45, respectively.

Population structure and gene flow

Amova detected a highly significant global population differentiation ($F_{ST} = 0.052$, $p < 0.00001$, 10,000 permutations; Table 2). Pairwise F_{ST}-values ranged from very low around zero, indicating low genetic distance, to high between 0.1 and 0.2 (Table 3). However, after sequential Bonferroni correction, only four of initially 15 pairwise comparisons remained significant ($\alpha < 0.05$). Ro had three out of four significant differences, but when excluding Ro from the Amova, the global population differentiation still remained significant ($F_{ST} = 0.033$, $p < 0.01$, 10,000 permutations).

The most reasonable group number k for maximization of the F_{CT}-value by Samova was five groups ($F_{CT} = 0.08$, $p < 0.01$), as ΔF_{CT} increased until $k = 5$, but declined with a further increase of k. Ti, Tu, and So formed one group and PA, Ch, and LP a second one. Ro, St, and SV were assigned to groups with only a single population, respectively. An Amova based on the grouping structure proposed by the Samova revealed a global F_{ST}-value of 0.065 ($p < 0.01$). However, the distribution of fragments over groups as inferred from Samova did not allow the identification of geographical barriers to gene flow, as the grouping seemed to be rather random over the geographic scale of our study area. Samova grouped populations from either side of the road together (Ti with Tu and So; PA with LP and Ch) and Tu and So, the fragments furthest from each other were assigned to one group. Mantel tests could not detect isolation-by-distance (IBD) either when testing for correlation between pairwise F_{ST}-values and linear geographic distance ($R^2 = 0.09$, $p = 0.33$) nor when distance was log-transformed ($R^2 = 0.03$, $p = 0.42$).

Influence of habitat parameters on population genetic diversity

We used linear regression models to test for the influence of habitat parameters on genetic diversity. To better fit our linear models, we first tested for normality distribution of all variables mentioned in this section. All variables, with the exception of "θ_k" and "patch size 1986" passed the Shapiro–Wilk normality test ($p > 0.1$). Hence we log-transformed "θ_k", "patch size 1986" and additionally "patch size 2001" to maintain comparability between patch size 1986 and 2001.

We tested for possible influence of 2001 and 1986 patch sizes on genetic diversity of the current bat populations (θ_k and h) by linear regression models. The relationship

Table 2 Results of the Amova analysis comparing genetic variation among and within nine populations of *D. watsoni*

Source of variation	df	Sum of squares	Variance components	Percentage of variation	*P* value
Among population	8	38.215	0.1286	5.21 *Va*	<0.00001
Within population	163	381.244	2.33892	94.79 *Vb*	
Total	171	419.459	2.46752		
Fixation Index	0.052				

Significance testing after 10,000 permutations

Table 3 Pairwise F_{ST}-values for *D. watsoni* populations

	Ti	St	SV	So	Tu	PA	Ch	Ro	LP
Ti	–								
St	0.022	–							
SV	0.012	0.039	–						
So	0.013	0.068	0.043	–					
Tu	−0.006	0.072	0.031	−0.026	–				
PA	0.011	0.068	0.009	0.012	−0.007	–			
Ch	0.081	0.125	0.062	0.041	0.027	−0.021	–		
Ro	0.126*	0.212*	0.061	0.126*	0.096	0.052	0.039	–	
LP	0.066	0.137*	0.045	0.051	0.020	−0.022	−0.033	0.031	–

Sampling locations are ranked after contemporary fragment size

* Indicates significance after 10,000 permutations and a sequential Bonferroni correction at the $\alpha < 0.5$ level

Table 4 Results of linear regression models between the amounts of suitable habitat in buffers of different sizes surrounding the fragments from the years 1986 and 2001 and genetic diversity (θ_k and h)

Buffer width (m)	1986				2001			
	log(θ_k)		h		log(θ_k)		h	
	R^2	P	R^2	P	R^2	P	R^2	P
200	0.64	0.009**	0.53	0.026*	7e-6	0.99	0.06	0.52
400	0.68	0.006**	0.57	0.019*	0.004	0.88	0.11	0.38
750	0.55	0.022*	0.45	0.049*	0.002	0.90	0.09	0.42
1,000	0.44	0.049*	0.37	0.085	0.001	0.95	0.08	0.47
2,000	0.19	0.243	0.11	0.382	0.001	0.94	0.06	0.51

Significance levels: *$P < 0.05$; **$P < 0.01$

between 2001 patch size and diversity indices was not significant (θ_k: $R^2 = 0.01$, $p = 0.82$; h: $R^2 = 0.04$, $p = 0.61$). However, regression with 1986 patch size as an independent variable was significant for θ_k ($R^2 = 0.47$, $p < 0.05$) and not significant for h ($R^2 = 0.28$, $p = 0.15$).

We also assessed the influence of the surrounding matrix on genetic diversity within fragments by linear regressions between genetic diversity indices (θ_k and h) and the absolute amount of suitable habitat in five different buffer zones with increasing width separately for matrix configurations in 1986 and 2001. Suitable habitat area within 200 and 400 m buffer zones in 1986 was best suited to explain variation in current genetic diversity with high R-square values above 0.6 for θ_k and above 0.5 for h (Table 4). These relationships remained significant for θ_k up to a buffer zone width of 1,000 m and until 750 m for h. However, exceeding a width of 400 m, the explanatory power of the models decreased constantly with increasing width of the buffer zone. For the 2001 matrix data we found no significant relationship between genetic diversity and suitable habitat in any of the different buffer zones.

Discussion

Genetic diversity

The population of *D. watsoni* showed overall a high level of gene diversity (0.974 ± 0.004) despite a strong fragmentation impact during several decades. In disturbed areas a decrease in effective population size may be followed by a loss of genetic diversity (Otto and Whitlock 1997). We suppose that *D. watsoni* managed to maintain a relatively large population in the study area. This assumption was supported by our numbers of netted bats where *D. watsoni* represented together with *Carollia castanea* the most abundant species (pers. obs. Ripperger). As population size depends mainly on the amount of available resources (e.g. food, roosts) in a given habitat, specialists struggle with maintaining large populations in fragmented habitats (Henle et al. 2004). However, *D. watsoni* might have an advantage over specialist species as it feeds on a wide range of fruits from primary and secondary forest plants (Melo et al. 2009). Furthermore, *D. watsoni* does not rely on specific roosts such as caves or hollow trees, but instead constructs its roosts by modifying leaves. Here *D. watsoni* represents the most flexible species of all neotropical tent-roosting bats, using more than 40 different plant species of various families including several common epiphytes and palms (e.g. Araceae, Arecaceae, and Cyclanthaceae; Rodriguez-Herrera et al. Rodríguez-Herrera et al. 2007). Therefore, even in disturbed habitats neither food nor roost requirements should limit abundance of *D. watsoni*.

Despite its generalist and flexible lifestyle, some of the sampled populations were less genetically diverse than others. Loss of genetic diversity in fragmented populations may be a consequence of either reduced gene flow between populations by a decline of habitat connectivity or of genetic drift, which accelerates when effective population size decreases (Johansson et al. 2007). We detected a significant, positive relationship between 1986 fragment size and genetic diversity in terms of θ_k but not for h. Especially populations inhabiting small fragments, which can harbor lower numbers of individuals (Ewers and Didham 2007), are prone to suffer loss of genetic diversity by random effects such as genetic drift as shown in previous studies (Vandergast et al. 2007; Dixo et al. 2009). Patch size had no significant effect on h, but the explanatory power increased when going back in time from 2001 conditions ($R^2 = 0.04$, $p = 0.61$) to 1986 ($R^2 = 0.28$, $p = 0.15$). This indicates that patch size from slightly earlier than 1986 might have revealed a significant dependence for h as well.

Furthermore, we found significant effects of 1986 landscape connectivity on molecular diversity (θ_k and h),

which were strongest within a maximum distance of 400 m from the fragments. Landscape connectivity, measured as the amount of suitable habitat contributing to the matrix around a fragment, is likely to influence populations' genetic diversity. Matrix elements which are similar to the original habitat facilitate inter-patch movements and hence dispersal (Cronin 2003; Ewers and Didham 2007). While a decrease in population size should affect populations rather nonspecifically, matrix effects seem to be highly species-specific even among closely related species (Ricketts 2001). Herein, bats are often considered to be highly mobile and capable to readily move through fragmented landscapes (Meyer et al. 2008), but there is increasing evidence to suggest that some species are more sensitive to habitat fragmentation than others (Swihart et al. 2006; Meyer et al. 2008; de la Peña-Cuéllar et al. 2012; Rossiter et al. 2012). Especially frugivorous understory bats, such as *D. watsoni*, seem to be reluctant to conduct longer commuting flights, e.g. to cross open areas (Henry et al. 2007). A telemetry study on *D. watsoni* conducted on an island system in Panama by Albrecht et al. (2007) documented a maximum commuting distance over open water of 180 m. This limited readiness to cover distances to farther situated habitat patches might explain why habitat connectivity exerted the strongest influence on genetic variability in the fragments' close proximity (200/400 m buffer zones), where habitat should still be accessible for *D. watsoni*. On the contrary this influence becomes continuously weaker with increasing distance to the fragment (750/1,000/2,000 m buffer zones) as farther habitats might not be within reach.

Interestingly, habitat parameters measured from 1986 land cover conditions were consistently better suited to explain genetic diversity of populations than the 2001 data. Altered habitat conditions measured in 2001 seem not to be manifested in the genetic signature of some populations yet. This becomes especially apparent in Ch, which was the largest fragment in 1986 but was drastically reduced by 2001. Its current genetic diversity was still the highest for all fragments. However, as few as 25 years (1986–2011) were enough time that habitat quality is mirrored by genetic diversity. This time span corresponds to 25 generations in *D. watsoni*. Similar fast processes were reported before in bank voles (Gerlach and Musolf 2000) and in toads (Dixo et al. 2009) where negative genetic effects became detectable 25 generations after fragmentation.

Genetic population structure

Our results show that *D. watsoni* displays moderate but significant levels of genetic structuring over a maximal distance of only approximately 20 km ($F_{ST} = 0.05$, $p < 0.00001$), indicating a discontinuous gene flow between the populations. Interestingly, Meyer et al. (2009) showed similar levels of global differentiation (mitochondrial D-loop; $F_{ST} = 0.06$) for populations of the slightly larger phyllostomid bat, *Carollia perspicillata*, inhabiting mainland and islands separated by a matrix of water over a comparable geographic scale. This result suggests that a matrix dominated by agricultural flats, pasture sites and other anthropogenic structures might exhibit similar deterring effects on certain bat species as open water surfaces. While water presents a strong structural contrast to the naturally habitat, the mosaic matrix in our sampling area might be dangerous as predators feeding on bats, such as nocturnal owls, are frequently associated with forest edges or matrix habitats (Stiles and Skutch 1989; Watson et al. 2004). Furthermore, understorey frugivores such as *D. watsoni* feature a limited ability to conduct longer commutes (Henry et al. 2007) and low vagility was assumed for bat species roosting in small groups (Rossiter et al. 2012). Both limited commuting ability and low vagility are supported by telemetry studies on *D. watsoni* (Albrecht et al. 2007; Chaverri et al. 2007). Hence ecological characteristics of *D. watsoni* might lead to reduced dispersal in fragmented habitats and make them vulnerable for genetic erosion. It is currently unclear to what extent *D. watsoni*'s ecology might lead to population differentiation in continuous habitats as it was shown for example for Malaysian bat species where gene flow had natural limits across an intact forest (Rossiter et al. 2012). However, our Samova analysis separated some of the sampled populations into distinct groups despite close proximity (e.g. St-So, SV-Ro). The distances between these fragments are of the order of home range sizes of individual *D. watsoni* as observed in continuous habitat (Chaverri et al. 2007) Therefore genetic exchange can be expected in the absence of disturbance. Consequently, we assume that in our study habitat alterations have a major effect on the observed population differentiation.

Isolation by distance (IBD) was found in different taxa, including bats (Burland et al. 1999; Campbell et al. 2009). However, our sample did not reveal a pattern of IBD. Furthermore, the map (Fig. 1) indicates that genetic distances between populations might not be a simple function of geographical distance. The population which tends to have the highest and most significant pairwise F_{ST}-values (R_0) has a central geographical position in our sampling area.

Potential strong geographical barriers to gene flow, such as a heavy-traffic road crossing our sampling area might restrict habitat accessibility for bats (Kerth and Melber 2009) through the combined effects of excessive noise and light (Schaub et al. 2008; Zurcher et al. 2010). Therefore we expected bat populations to be more closely related when they are located on the same side of the road. However, the Samova approach could not confirm this

assumption as fragments from either side of the street were grouped together (e.g. PA with LP/Ch or Ti with Tu/So). Furthermore Ti, Tu, and So are distributed over the entire sampling area from south-west to north-east, indicating that no strong barrier to gene flow caused the population structure. This is also supported by Ro and St which each representing a separate group, despite their close proximity to SV and So, respectively, and the presence of linking vegetation between them.

The lack of IBD and identifiable anthropogenic barriers to gene flow suggest that the observed population differentiation is in the first instance driven by habitat parameters such as fragment size and matrix configuration leading to different levels of genetic drift and gene flow, thus shaping individual populations' genetic diversity.

Conservation implications

Habitat fragmentation is seen as a major threat to biodiversity. However, species differ in levels of sensitivity depending on ecological factors such as mobility or edge sensitivity (Henle et al. 2004; Meyer et al. 2008; Leidner et al. 2010). Even flying vertebrates such as neotropical bats were shown to lose genetic variability and suffer subdivision of populations on island systems with water as matrix (Newton et al. 2003; Meyer et al. 2009). Our study indicates that the frugivorous understorey bat *D. watsoni* is even prone to negative genetic effects on a rather small geographic scale when fragmented populations are separated by a less drastically modified matrix. Genetic diversity of populations was linked to patch size and to the configuration of the surrounding matrix in close proximity. Especially in anthropogenically degraded areas, frugivorous bats are of particular relevance to conservation issues as they disperse a large range of seeds in early to late succession stages (Cosson et al. 1999; Melo et al. 2009; Mello et al. 2011) and do not experience a strong hunting pressure by man (Wright et al. 2007).

However, our results also suggest that present genetic diversity reflects past habitat conditions. This should be considered when assessing the need for conservation measures. Populations inhabiting recently degraded habitats might still be genetically diverse and seem to maintain a considerable evolutionary potential, but they will probably suffer genetic pauperization during the next decades. We therefore suggest directly adopting conservation measures, such as reconnecting patches to foster gene flow, in order to prevent a loss of genetic diversity, rather than trying to restore genetic diversity after decades of genetic depletion.

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