Agriculture, Ecosystems and Environment 185 (2014) 9–15

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Research Paper

Resisting habitat fragmentation: High genetic connectivity among populations of the frugivorous bat *Carollia castanea* in an agricultural landscape

Simon P. Ripperger[a,b,*], Marco Tschapka[b,c], Elisabeth K.V. Kalko[b,c], Bernal Rodríguez-Herrera[d], Frieder Mayer[a,e]

[a] *Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Invalidenstraße 43, 10115 Berlin, Germany*
[b] *Institute of Experimental Ecology, University of Ulm, Albert-Einstein-Allee 11, 89069 Ulm, Germany*
[c] *Smithsonian Tropical Research Institute, PO Box 0843-03092 Balboa, Panama*
[d] *Escuela de Biología, Universidad de Costa Rica, PO Box 11501-2060 San Pedro, Costa Rica*
[e] *Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Altensteinstraße 6, 14195 Berlin, Germany*

ARTICLE INFO

Article history:
Received 3 June 2013
Received in revised form
27 November 2013
Accepted 2 December 2013

Keywords:
Carollia castanea
Habitat fragmentation
Genetic population structure
Isolation by distance
Mitochondrial D-loop

ABSTRACT

Anthropogenically dominated landscapes are frequently a patchwork composed of manmade structures and natural habitat remnants. Especially tropical landscapes are progressively turning into such heterogeneous mosaics leading to simplification of animal communities and (partial) isolation of the scattered survivors. Modern molecular approaches provide powerful tools to detect discontinuities in gene flow among local populations. The aim of this study was to evaluate genetic connectivity on a small geographic scale among local bat populations that inhabit forest fragments in an agricultural matrix in north-east Costa Rica. We focused on *Carollia castanea* (Phyllostomidae), a small frugivorous bat that mainly feeds on pepper plants. We analyzed DNA sequences of the mitochondrial D-loop of 173 adult individuals. There was no significant global population differentiation detectable ($F_{ST} = 0.008$, $p = 0.17$) and regular gene flow among populations was indicated by low pairwise F_{ST}-values, even in highly fragmented areas. Solely with increasing geographic distance gene flow was weakened, indicated by a significant isolation by distance pattern ($R^2 = 0.55$, $p < 0.05$). Our study shows that *C. castanea* can cope better with small-scale habitat fragmentation than other phyllostomid bat species, at least in an agriculturally dominated landscape. This is probably because of its tolerance toward disturbed habitats for foraging that enables it to maintain genetic exchange among populations that are separated by areas under human influence.

1. Introduction

During the past decades, humans have progressively shaped the appearance of landscapes leading some to declare this era the Anthropocene (Steffen, 2010). Anthropogenic changes in land-use convert once continuous habitats into mosaic landscapes and are doing so at an alarming pace, posing a significant threat to biodiversity (Gardner et al., 2009; Sala et al., 2000). It is unclear to what degree species can cope with these rapidly changing environments. While rapid adaptations have been observed in several species, many other species are decreasing in abundance or going extinct (reviewed in Stockwell et al., 2003).

Animal populations inhabiting anthropogenic mosaic landscapes are faced with a number of challenges. Habitat loss, the reduction of the amount of available habitat, directly leads to decreasing population sizes (Bender et al., 1998). Fragmentation-associated decreases in food abundance may additionally affect population densities (Zanette et al., 2000). Habitat fragmentation creates edge and matrix habitats that favor more tolerant species over those more sensitive, and hence frequently leads to alterations in community structure (Henle et al., 2004). Typically, habitat generalists are less vulnerable than specialists to decreases in habitat availability and quality (Bender et al., 1998). Generalist species might persist in smaller fragments as the neighboring matrix might offer additional resources (Andrén, 1994). Accordingly, movement patterns should be highly species-specific, depending on the species' risks and benefits from different land cover types (Fahrig, 2007).

The effects of habitat loss and fragmentation on animal behavior might result in discontinuities in gene flow among potentially isolated populations. In the long term, this will lead to sub-population differentiation and affected populations will face the risk of loss of genetic diversity by elevated rates of inbreeding and genetic drift (Holderegger and Di Giulio, 2010). Numerous studies on genetic

* Corresponding author. Tel.: +49 30 2093 8468; fax: +49 30 2093 8565.
E-mail address: simon.ripperger@mfn-berlin.de (S.P. Ripperger).

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http://dx.doi.org/10.1016/j.agee.2013.12.006

structure and genetic diversity of animal populations in the context of habitat degradation and fragmentation highlight negative effects. For example, various amphibians were shown to experience population differentiation and loss of genetic diversity on small geographic scales after habitat degradation (Dixo et al., 2009; Richardson, 2012; Sarasola-Puente et al., 2012). Human encroachment has also led to decreased genetic connectivity in flightless insects (Keller and Largiadèr, 2003; Vandergast et al., 2007) and even among populations of large mammalian species (Epps et al., 2005; Riley et al., 2006).

Mobility is frequently cited as an attribute that may make animals less prone to these changes (Henle et al., 2004). However, empirical evidence on the effect of habitat fragmentation in flying organisms is mixed, even on small spatial scales. Connectivity among butterfly populations decreased in the presence of a natural barrier, resulting in elevated genetic differentiation (Keyghobadi et al., 2005), whereas bees were resistant to population differentiation in fragmented habitats (Exeler et al., 2010; Zimmermann et al., 2011). Contrasting genetic effects were also shown for birds ranging from partial absence of genetic exchange (Méndez et al., 2011; Woltmann et al., 2012) to high gene flow among fragmented populations (Canales-Delgadillo et al., 2012). This variability in species-specific responses suggests that predictions on species' sensitivity to habitat fragmentation seem to be more complex than a simple function of mobility. Species related life-history traits, ecological plasticity or the evolutionary context, e.g., the kind of habitat in which a species evolved, might also substantially influence species' vulnerability (Fahrig, 2007; Rossiter et al., 2012).

Population genetic studies on bats remain scarce, especially in tropical ecosystems that experienced anthropogenic habitat alterations. Only few studies evaluated such effects on small geographic scales (Asher, 2009; Meyer et al., 2009; Ripperger et al., 2013; Struebig et al., 2011). Meyer et al. (2009) and Struebig et al. (2011) found species-specific declines of genetic diversity of populations from forest fragments compared to continuous forest that were linked to species' vagility. Ripperger et al. (2013) detected small-scale population differentiation in bats inhabiting forest remnants in an anthropogenic matrix. Rossiter et al. (2012) showed that even in unmodified habitats, gene flow may be naturally limited on a small geographic scale depending on ecological and behavioral traits. Due to the low number of studies on only few bat species, the knowledge on small-scale genetic effects is scarce, especially in complex agricultural landscapes.

In order to assess the consequences of persisting in an agricultural habitat mosaic on genetic diversity and population differentiation we worked on populations of *Carollia castanea* (Phyllostomidae) from forest remnants and continuous forest. We analyzed DNA sequences of the mitochondrial D-loop. Our study was conducted in a mosaic landscape in the northern Caribbean lowlands of Costa Rica. Specifically, we tested the hypotheses that (1) the population of *C. castanea* inhabiting a fragmented, agriculturally dominated landscape will show signs of genetic population differentiation on a small geographic scale and (2) molecular diversity of local populations will be linked to habitat variables that may act as proxies for population size (fragment area and food availability) and genetic exchange with neighboring populations (landscape connectivity).

C. castanea is an excellent study species for gaining more insight into the parameters that affect the susceptibility of bats to habitat fragmentation. On the one hand it is a relatively small bat species (11–16 g body weight; Reid, 2009) that forages within rather small home ranges of only a few hectares (Bonaccorso et al., 2007). This limited mobility might cause an increased risk for genetic erosion in degraded habitats in consequence of limited gene flow. On the other hand *C. castanea* should at least in part be used to edge and matrix habitats as it is strongly specialized on *Piper* plants as food resource and forages in both mature and successional forest types (Thies and Kalko, 2004; Voigt et al., 2012).

2. Methods

2.1. Study area and focus species

Our study was conducted in the northern Caribbean lowlands of Costa Rica (10°25′N, 84°05′ W). Wide parts of this region are characterized by a mosaic of forest remnants, cattle pastures, fruit plantations (mainly banana and pineapple) and urban structures such as roads and villages. The main anthropogenic fragmentation impact lasted up until the late 1980s (Sánchez-Azofeifa et al., 2007). From this mosaic we selected nine forest fragments of different size and moderate to high degree of isolation (see Fig. 1). We also chose a control site within the protected area of the Braulio Carillo National Park, a site with very low human impact. Fragment area varied from 38 to 412 ha. The sampling sites were located at low altitudes ranging from ca. 50 to 300 m asl. The vegetation was dominated by primary forest and late secondary succession stages.

In our study we focused on *C. castanea*, a small phyllostomid bat (11–16 g) that is common to evergreen forests and second growth (Reid, 2009). It usually roosts in tree holes and overhanging banks and is specialized on pepper plants, feeding on both understorey and gap *Piper* (Thies and Kalko, 2004). Mean home range area is approximately 12 ha (100% minimum convex polygon; Bonaccorso et al., 2007).

2.2. Sample collection and molecular analyses

The bats were captured using mist-nets set at ground level during two field seasons (March to August 2010, January to August 2011). Three to five netting sites were homogenously distributed over the fragments. Identification of bats in the field was based on morphological traits following Timm and LaVal (1998). We collected tissue samples from the wing membrane with a biopsy punch (Ø 4 mm, Stiefel®) at a standardized position between the 4th and 5th digit and stored samples in 80% ethanol. When the hole in the membrane cicatrized, a spot remained visible which lacked pigmentation. The standardized sampling procedure ensured avoiding resampling of bats during the entire sampling period. Only adult individuals were considered for further molecular analyses. Bats were considered adult when the epiphyseal gaps were closed and the phalangeal–metacarpal joints were knobby (Brunet-Rossinni and Wilkinson, 2009). The tissue samples were processed following the salt-chloroform method (Müllenbach et al., 1989) to extract the DNA. Polymerase chain reaction was used to amplify the highly polymorphic mitochondrial D-loop with the primers E (Wilkinson and Chapman, 1991) and P* (Wilkinson et al., 1997), following the protocol given in Wilkinson et al. (1997). The resulting fragment was sequenced from one end with Primer P* and BigDye Terminator Cycle Sequencing Kit version 1.1. Sequencing was performed on an Abi Prism Genetic Analyzer 3130 XL. Sequences were aligned and edited using CodonCode Aligner 3.5. We uploaded the sequences of 49 individual haplotypes to GenBank (GenBank Accession numbers KF964497–KF964545).

2.3. Population genetic analyses

We used the software Arlequin 3.5 for standard population genetic analyses (Excoffier et al., 2005). We tested for population differentiation using Analysis of Molecular Variance (Amova) and generated pairwise F_{ST}-values in order to assess differences between local populations. We adjusted significance levels using a sequential Bonferroni correction based on $\alpha = 0.05$. We calculated gene diversity (h) and theta k (θ_k) as a measure of the molecular

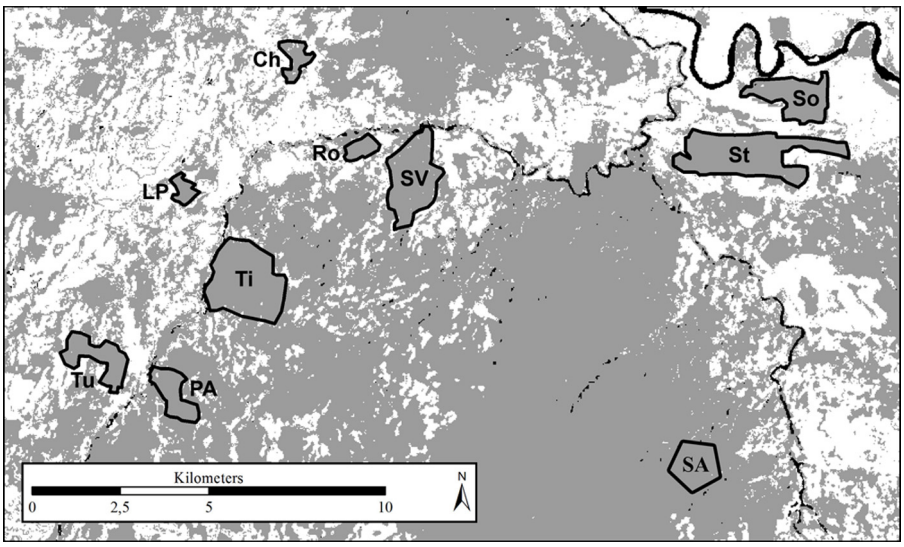

Fig. 1. The 10 sampling sites in the study area in north-east Costa Rica; gray indicates forest cover, white indicates non-forest cover, black indicates water (except the framings of the study sites); Tu = El Tucan; PA = Hacienda Pozo Azul; Ti = La Tirimbina; LP = Las Palmitas; SV = Selva Verde Lodge; Ro = El Roble; Ch = Rancho Chilamate; St = Finca Starke; So = Finca Sofia; SA = Sueño Azul Resort (control site).

diversity of individual populations. Linear geographical distances and genetic distances (pairwise F_{ST}) were used to perform Mantel tests to detect a possible pattern of isolation by distance (IBD; Mantel, 1967). We applied a Spatial Analysis of Molecular Variance to define reasonable groups of populations including genetic characteristics and geographic location (Samova 1.0; Dupanloup et al., 2002). The Samova allocates populations to k groups. To obtain a reasonable grouping the user selects k in such a way that the intergroup variation F_{CT} is maximized or the change between two F_{CT} values starts to decline with a further increase of k. The grouping structure generated by Samova was then tested by an Amova in Arlequin 3.5.

2.4. Determination of habitat variables

We measured patch size of forest fragments from land cover maps from 2001 in ArcGIS 10 (raster data; cell size 28.5 × 28.5 m; the map included 32 land cover categories; Sesnie et al., 2008). To estimate landscape connectivity we quantified several primary and secondary vegetation elements in 400 m buffers around the fragments. The choice of the buffer width was guided by the average long axis across home range areas in *C. castanea* (438 ± 106 m; Bonaccorso et al., 2007). As potentially connecting land cover between foraging sites we chose all kinds of primary and secondary natural vegetation (primary vegetation, early and late secondary succession, swamps and riparian forest). As *C. castanea* includes both *Piper* from closed forest and from disturbed sites into its diet (Thies and Kalko, 2004) those land cover types should be recognized by *C. castanea* as possible foraging areas. We tested for correlations between genetic diversity (θ_k and h) and habitat variables (patch size, connectivity) by linear regression analyses in R 2.15.1 (R Core Team, 2012).

2.5. Estimation of food availability

We established 10 plots within each forest fragment and the control site to estimate occurrence and abundance of *Piper* plants, as they comprise a large portion of *C. castanea*'s diet. To randomly pick the plot locations we superimposed a grid (grid lines were spaced 100 m apart) on the land cover map. We selected the coordinates of 10 nodes ensuring a homogenous distribution over the individual forest fragment or continuous forest site. We fitted 7 × 7 m plots around the node location, recorded the occurrence of *Piper* (presence/absence of *Piper* plants or seedlings) and the abundance of potential food resources (number of *Piper* plants exceeding 1 m in height which represent potential food resources; smaller plants do not produce flowers and fruits; Letourneau, 2004). In order to test whether plots containing *Piper* plants were equally common in the sampled fragments we performed a Fisher Exact Test on *Piper* occurrence. To account for a possible influence of food resources on genetic diversity we ran linear regression analyses between genetic diversity indices and *Piper* abundance. All tests were performed in R 2.15.1.

3. Results

3.1. Genetic diversity

We collected tissue samples from a total of 173 adult individuals of *C. castanea* (113 males and 60 females). The analysis of 396 base pairs of the mitochondrial D-loop resulted in 33 polymorphic sites leading to 49 unique haplotypes. The number of haplotypes detected in a single local population varied between six and 17 (Table 1). The distribution of individual haplotypes over sampling sites is shown in Appendix A. Gene diversity (h) varied in forest fragments from 0.857 in Ch to 0.971 in Tu and molecular diversity θ_k within local populations ranged from 4.5 in Ch to 24.61 in Tu. The control site in continuous forest (SA) revealed the highest values for both h (0.985) and θ_k (58.45) compared to the fragmented sites (Table 1).

3.2. Population structure

We detected slight but significant global population differentiation (Amova, $F_{ST} = 0.02$, $p < 0.05$, 10,000 permutations). After sequential Bonferroni correction pairwise F_{ST}-values only remained significant between St and LP (Table 2). However, in general, comparisons with the fragment St tended to result in elevated F_{ST}-values. This might be a bias generated by the low sample size in St of only nine individuals. Repeating the Amova excluding St showed no significant global population differentiation ($F_{ST} = 0.008$, $p = 0.17$, 10,000 permutations). We therefore omitted St from all further analyses. Analyzing sexes

Table 1
Habitat parameters and molecular diversity of local bat populations.

Site	Patch size [ha]	Suitable matrix		No. bats		No. haplotypes	θ_k	h
		[ha]	[%]	♂	♀			
SA	Control	Control	Control	7	5	11	58.45	0.985 ± 0.040
Ti	412	298	66.2	11	10	14	17.19	0.895 ± 0.061
St	408	183	30.6	7	2	6	6.69	0.917 ± 0.073
SV	280	185	51.9	11	6	11	12.40	0.934 ± 0.043
So	187	57	16.1	8	5	7	5.43	0.872 ± 0.067
Tu	153	144	38.9	17	7	17	24.61	0.971 ± 0.019
PA	99	209	79.1	12	5	11	12.40	0.927 ± 0.045
Ch	68	60	25.3	11	4	7	4.50	0.857 ± 0.065
Ro	47	73	42.3	16	9	14	12.33	0.883 ± 0.052
LP	38	63	37.4	15	5	12	11.75	0.947 ± 0.028

Table 2
Pairwise F_{ST}-values for local populations of *C. castanea*.

	SA	Ti	St	SV	So	Tu	PA	Ch	Ro	LP
SA	–									
Ti	0.050	–								
St	0.044	0.080	–							
SV	−0.010	0.005	0.056	–						
So	0.001	0.064	0.082	0.015	–					
Tu	0.004	−0.020	0.059	−0.007	0.028	–				
PA	0.000	−0.010	0.087	−0.019	0.027	−0.025	–			
Ch	0.069	−0.014	0.162	0.024	0.082	−0.013	−0.013	–		
Ro	0.015	0.004	0.078	−0.003	−0.008	−0.013	−0.019	−0.011	–	
LP	0.070	0.010	0.152*	0.031	0.075	0.021	0.001	0.002	0.016	–

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

separately showed no differentiation for females ($F_{ST} = -0.011$, $p = 0.63$, 10,000 permutations) but a significant global population differentiation for males ($F_{ST} = 0.036$, $p < 0.01$, 10,000 permutations).

To explain the slight variation in pairwise F_{ST}-values in the sample including both males and females, we tested for isolation by distance (IBD). We detected a significant IBD pattern over the remaining nine sites ($R^2 = 0.55$, $p < 0.05$). Samova was also indicative of an influence of geography. The best fitting group number k for a maximization of F_{CT} was 2 as F_{CT} decreased with $k = 3$. An Amova on the population structure proposed by Samova with $k = 2$ groups showed a slight but significant population differentiation ($F_{ST} = 0.031$, $p < 0.05$). The first group comprised all eight forest fragments in the western part of the study area that are located relatively close to each other (Ch, LP, SV, Ro, Ti, PA, Tu; see Fig. 1). A second group contained the fragment So and the control site SA, which are both farther apart from the fragments in group one.

3.3. Influence of habitat parameters and food resources on genetic diversity in forest fragments

Linear regression analyses did not detect a significant correlation between patch size and molecular diversity indices of local populations inhabiting forest fragments (θ_k: $R^2 = 0.09$, $p = 0.47$; h: $R^2 = 3 \times 10^{-7}$, $p = 0.99$). We found no significant relationship between landscape connectivity and genetic diversity indices (θ_k: $R^2 = 0.28$, $p = 0.18$; h: $R^2 = 0.07$, $p = 0.53$).

The occurrence of *Piper* plants differed significantly among the sampled forest fragments (Table 3; Fisher Exact Test, $p < 0.05$). However, regression analyses revealed no significant relationship between abundance of *Piper* plants and gene diversity (h: $R^2 = 0.07$, $p = 0.53$) and only a trend for θ_k ($R^2 = 0.50$, $p = 0.05$).

Table 3
Number of plots per site containing *Piper* and number of plants >1 m.

Site	*Piper* plots	*Piper* plants
SA	3	7
Ti	7	16
St	6	22
SV	2	2
So	5	9
Tu	4	15
PA	6	9
Ch	0	0
Ro	2	12
LP	4	7

4. Discussion

In the first instance we detected minimal but significant levels of global population differentiation ($F_{ST} = 0.02$, $p < 0.05$) indicating discontinuous gene flow among the local populations of *C. castanea*. However, the pairwise F_{ST} table indicated that most genetic variation within the sample was contributed by one single sampling site, St, that was probably undersampled ($n = 9$). As an undersized sample might affect the accuracy of the analysis on population differentiation (Morin et al., 2009), we excluded this site from all further analyses. The global differentiation, that was in line with our expectations given our first hypothesis, vanished after omitting St ($F_{ST} = 0.008$, $p = 0.17$) and none of the pairwise F_{ST}-values among local populations remained significant, indicating regular genetic exchange.

The lack of population differentiation in *C. castanea* is in contrast to a study conducted in the same forest fragments on the similar sized stenodermatine bat *Dermanura watsoni*, for which significant population differentiation was found (Ripperger et al., 2013). In terms of body size (*C. castanea*: 11–16 g, *D. watsoni*: 9–15 g; Reid, 2009) and mobility while foraging (100% minimum convex polygon: *C. castanea*: 11.9 ± 3.7 ha, *D. watsoni*: 3.6 ± 4.06; Chaverri et al., 2007; Bonaccorso et al., 2007), characteristics that are frequently used to predict fragmentation sensitivity (Henle et al., 2004), *C. castanea* is rather similar to *D. watsoni*. A potential sensitivity to habitat fragmentation of *C. castanea* was also demonstrated by a study in an island system in Panama which revealed a strong decrease in abundance on islands with increasing distance to the mainland

and absence on more than half of the islands (Meyer and Kalko, 2008; Meyer et al., 2008). Within this island system the larger and more mobile phyllostomids *Carollia perspicillata* and *Uroderma bilobatum* showed significant levels of population differentiation (F_{ST} = 0.06, $p < 0.02$ and F_{ST} = 0.01, $p < 0.05$, respectively) while geographic distances of only a few kilometers among local populations were comparable to our study (Meyer et al., 2009). Those studies suggest that *C. castanea* could likewise be prone to population differentiation after habitat fragmentation due to limited vagility. Our data, however, shows that *C. castanea* is able to maintain gene flow among local populations that are separated by an agricultural matrix. Separate tests for each sex showed a similar pattern of non-differentiation in females, while males showed a significant global population differentiation. These findings are in line with sex-biased dispersal in terms of natal dispersal of females before first conception followed by long-term site fidelity and male philopatry. Such dispersal patterns are rare among mammals but were shown already in two species of Neotropical emballonurid bats (Nagy et al., 2012, 2013), resulting in local occurrence of maternally related males.

The differences between *C. castanea* in our study and *C. perspicillata* in Meyer et al. (2009) are probably accounted for by distinct matrix types in the two studies. Whereas open water represents a strong contrast to the forest habitat on the islands, the fragments in our study were surrounded by various matrix structures such as fruit plantations, cattle pastures with scattered trees, or early regrowth, that probably were more permeable for the bats. Corridor structures such as riparian forests along rivers were occasionally present and further improved connectivity. Across taxa, resistance to movement and mortality is higher, when the matrix contrasts more strongly with the natural habitat (Eycott et al., 2012). Hence we assume that movement, and thus gene flow, for frugivorous bats may be facilitated through a structurally diverse landscape matrix compared to open water bodies.

However, not all frugivorous bats seem to be equally capable to cope with an agricultural landscape. Significant levels of population differentiation among local populations of *D. watsoni* (Ripperger et al., 2013) but no evidence for population differentiation in *C. castanea* (this study) inhabiting the same forest fragments might result from different foraging strategies. While *D. watsoni* includes a wide range of large-seeded fruits from mature forest into its diet (de Melo et al., 2009), *C. castanea* feeds primarily on fruits of *Piper* spp. from forest interior as well as from early successional stages (Thies and Kalko, 2004). Besides within mature forest *Piper* spp. can establish in several successional stages from completely open habitats to secondary forest (Greig, 1993; Zahawi and Augspurger, 1999). As movement probability into matrix habitats is usually higher when the matrix offers benefits to animals (reviewed in Fahrig, 2007), *C. castanea* can be encountered in both mature forest and successional sites (Voigt et al., 2012) and may occasionally even occupy roosting sites in open areas (Kelm et al., 2008). This tolerance toward disturbed habitats might facilitate movements and hence gene flow among forest patches.

Beyond population differentiation, populations of fragmentation-sensitive bat species may suffer a loss of genetic diversity that is correlated with habitat parameters such as patch size and/or habitat connectivity (Ripperger et al., 2013; Struebig et al., 2011). This is because population size and connectivity between local populations are important factors driving genetic diversity (Potter et al., 2012; Traill et al., 2010). Contrary to our expectations given our second hypothesis, we found no significant correlation between habitat parameters and molecular diversity. This result also suggests that *C. castanea* is relatively resistant to habitat fragmentation and does not rely on high landscape connectivity for inter-patch movements. Patch size is a common predictor for the population size of animals a forest fragment might sustain, but it might be less predictive for habitat generalists (Bender et al., 1998). Hence, in *C. castanea*, which is rather flexible in its habitat demands and may forage within and outside forest, but is a strong feeding specialist, food availability rather than patch size should limit population size. Indeed, occurrence of *Piper* plants varied significantly among forest fragments, but there was no significant correlation between *h* and *Piper* abundance, and only a trend for θ_k. As *C. castanea* has the option to include fruits from successional areas into its diet (Thies and Kalko, 2004), food scarcity inside the forest fragments might be compensated and might not directly affect population size. In general, *C. castanea* was one of the most abundant species in our study area (unpublished data). Since loss of genetic diversity, caused by genetic drift, proceeds faster within small populations (Johansson et al., 2007), genetic diversity of fragmented *C. castanea* populations might respond slower than in other species and might additionally be balanced by migrants.

Despite the absence of a global population differentiation, gene flow decreased with increasing geographic distance. A Mantel test detected a significant isolation by distance (IBD) pattern among the sampled populations. Samova proposed a population structure consisting of two groups with the first group containing the seven populations from the western part of the sampling area that are located more closely to one another. So and SA lie farther to the east and formed a second group. Testing this structure in Arlequin yielded low but significant levels of differentiation. However, the structure inferred by Samova suggests that fragmentation per se does not necessarily interrupt genetic exchange. The populations of the western group partly inhabit forest patches in strongly fragmented areas (e.g., LP and Ch, see Fig. 1) but seem to be genetically homogenous. Furthermore, ongoing gene flow rather than recent historical separation of local populations is expected to cause correlation between genetic and geographical distances (Beebee and Rowe, 2008). As gene flow among local bat populations can be limited even in undisturbed habitats (Rossiter et al., 2012) we assume that the IBD pattern is not attributable to fragmentation.

5. Conclusions

Small-scale genetic effects on phyllostomid bats following the conversion of continuous habitat into patchy landscape mosaics are still only poorly understood. Our current knowledge is based on a handful of studies from different systems with varying fragment-matrix contrast. Our paper demonstrates that an agricultural matrix does not necessarily restrict gene flow among local populations of frugivorous bats living in fragments, as reported in the case of a water matrix for even larger, more mobile species (Meyer et al., 2009). However, resistance toward genetic erosion can be species-specific as indicated by analyses of different species from the same study area (Ripperger et al., 2013; this study). These factors underline the difficulties in making predictions on species' responses to habitat fragmentation, even for closely related species. High mobility of flying vertebrates is not necessarily a guarantee to cope well with mosaic landscapes; consideration of landscape composition and life history traits of the observed species is urgently necessary.

Acknowledgments

We thank Marcelo Lopez of Sueño Azul Resort, Alberto Quintana of Hacienda Pozo Azul, Giovanna Holbrook of Selva Verde Lodge and all private land owners for the permission to conduct fieldwork. For assistance in the field we are grateful to Emanuel Rojas, Elder Miranda, and Katrin Heer, and to Martina Nagy for advising in the laboratory. We thank Steven Sesnie for providing the land cover map and Ann Cespedes & Emma Berdan for their

help to improve this manuscript. Logistical support was provided by Chiquita Brands International. This work was approved by Javier Guevara (Resolutions: 047-2010-SINAC, 004-2011-SINAC, 128-2011-SINAC). Funding for field work was provided by a grant of the "Deutscher Akademischer Austauschdienst" (DAAD) to SPR.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2013.12.006.

References

Andrén, H., 1994. Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat—a review. Oikos 71, 355–366.
Asher, C., 2009. Patterns of genetic diversity in populations of two bat species (*Sturnira ludovici* and *Artibeus toltecus*) in Cusuco National Park, Honduras. Bioscience Horizons 2, 147–154.
Beebee, T., Rowe, G., 2008. An Introduction to Molecular Ecology, second ed. Oxford University Press, New York, NY.
Bender, D.J., Contreras, T.A., Fahrig, L., 1998. Habitat loss and population decline: a meta-analysis of the patch size effect. Ecology 79, 517–533.
Bonaccorso, F.J., Winkelmann, J.R., Shin, D., Agrawal, C.I., Aslami, N., Bonney, C., Hsu, A., Jekielek, P.E., Knox, A.K., Kopach, S.J., Jennings, T.D., Lasky, J.R., Menesale, S.A., Richards, J.H., Rutland, J.A., Sessa, A.K., Zhaurova, L., Kunz, T.H., 2007. Evidence for exploitative competition: comparative foraging behavior and roosting ecology of short-tailed fruit bats (Phyllostomidae). Biotropica 39, 249–256.
Brunet-Rossinni, A.K., Wilkinson, G.S., 2009. Methods for age estimation and the study of senescence in bats. In: Kunz, T.H., Parsons, S. (Eds.), Ecological and Behavioral Methods for the Study of Bats. , second ed. Johns Hopkins University Press, Baltimore, MD, pp. 315–325.
Canales-Delgadillo, J.C., Scott-Morales, L., Korb, J., 2012. The influence of habitat fragmentation on genetic diversity of a rare bird species that commonly faces environmental fluctuations. J. Avian Biol. 43, 168–176.
Chaverri, G., Quirós, O.E., Kunz, T.H., 2007. Ecological correlates of range size in the tent-making bat *Artibeus watsoni*. J. Mammal. 88, 477–486.
de Melo, F.P.L., Rodríguez-Herrera, B., Chazdon, R.L., Medellin, R.A., Ceballos, G.G., 2009. Small tent-roosting bats promote dispersal of large-seeded plants in a neotropical forest. Biotropica 41, 737–743.
Dixo, M., Metzger, J.P., Morgante, J.S., Zamudio, K.R., 2009. Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. Biol. Conserv. 142, 1560–1569.
Dupanloup, I., Schneider, S., Excoffier, L., 2002. A simulated annealing approach to define the genetic structure of populations. Mol. Ecol. 11, 2571–2581.
Epps, C.W., Palsbøll, P.J., Wehausen, J.D., Roderick, G.K., Ramey II, R.R., McCullough, D.R., 2005. Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. Ecol. Lett. 8, 1029–1038.
Eycott, A., Stewart, G., Buyung-Ali, L., Bowler, D., Watts, K., Pullin, A., 2012. A meta-analysis on the impact of different matrix structures on species movement rates. Landscape Ecol. 27, 1263–1278.
Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinf. 1, 47–50.
Exeler, N., Kratochwil, A., Hochkirch, A., 2010. Does recent habitat fragmentation affect the population genetics of a heathland specialist, *Andrena fuscipes* (Hymenoptera: *Andrenidae*)? Conserv. Genet. 11, 1679–1687.
Fahrig, L., 2007. Non-optimal animal movement in human-altered landscapes. Funct. Ecol. 21, 1003–1015.
Gardner, T.A., Barlow, J., Chazdon, R., Ewers, R.M., Harvey, C.A., Peres, C.A., Sodhi, N.S., 2009. Prospects for tropical forest biodiversity in a human-modified world. Ecol. Lett. 12, 561–582.
Greig, N., 1993. Regeneration mode in neotropical *Piper*: habitat and species comparisons. Ecology 74, 2125–2135.
Holderegger, R., Di Giulio, M., 2010. The genetic effects of roads: a review of empirical evidence. Basic Appl. Ecol. 11, 522–531.
Henle, K., Davies, K.F., Kleyer, M., Margules, C., Settele, J., 2004. Predictors of species sensitivity to fragmentation. Biodivers. Conserv. 13, 207–251.
Johansson, M., Primmer, C.R., Merilä, J., 2007. Does habitat fragmentation reduce fitness and adaptability? A case study of the common frog (*Rana temporaria*). Mol. Ecol. 16, 2693–2700.
Kelm, D.H., Wiesner, K.R., Helversen, O.V., 2008. Effects of artificial roosts for frugivorous bats on seed dispersal in a neotropical forest pasture mosaic. Conserv. Biol. 22, 733–741.
Keller, I., Largiadèr, C.R., 2003. Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. Proc. R. Soc. B 270, 417–423.
Keyghobadi, N., Roland, J., Strobeck, C., 2005. Genetic differentiation and gene flow among populations of the alpine butterfly *Parnassius smintheus*, vary with landscape connectivity. Mol. Ecol. 14, 1897–1909.
Letourneau, D.K., 2004. Mutualism, antiherbivore defense, and trophic cascades: Piper ant—plants as a mesocosm for experimentation. In: Dyer, L.A., Palmer, A. (Eds.), Piper: A Model Genus for Studies of Phytochemistry, Ecology, and Evolution. Kluwer Academic/Plenum Publishers.
Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27, 209–220.
Méndez, M., Tella, J.L., Godoy, J.A., 2011. Restricted gene flow and genetic drift in recently fragmented populations of an endangered steppe bird. Biol. Conserv. 144, 2615–2622.
Meyer, C.F.J., Kalko, E.K.V., 2008. Assemblage-level responses of phyllostomid bats to tropical forest fragmentation: Land-bridge islands as a model system. J. Biogeogr. 35, 1711–1726.
Meyer, C.F.J., Fründ, J., Lizano, W.P., Kalko, E.K.V., 2008. Ecological correlates of vulnerability to fragmentation in neotropical bats. J. Appl. Ecol. 45, 381–391.
Meyer, C.F.J., Kalko, E.K.V., Kerth, G., 2009. Small-scale fragmentation effects on local genetic diversity in two phyllostomid bats with different dispersal abilities in Panama. Biotropica 41, 95–102.
Morin, P.A., Martien, K.K., Taylor, B.L., 2009. Assessing statistical power of SNPs for population structure and conservation studies. Mol. Ecol. Res. 9, 66–73.
Müllenbach, R., Lagoda, P.L.J., Welter, C., 1989. An efficient salt-chloroform extraction of DNA from blood and tissues. Trends Genet. 5, 391.
Nagy, M., Knörnschild, M., Voigt, C.C., Mayer, F., 2012. Male greater sac-winged bats gain direct fitness benefits when roosting in multimale colonies. Behav. Ecol. 23, 597–606.
Nagy, M., Günther, L., Knörnschild, M., Mayer, F., 2013. Female-biased dispersal in a bat with a female-defence mating strategy. Mol. Ecol. 22, 1733–1745.
Potter, S., Eldridge, M., Cooper, S., Paplinska, J., Taggart, D., 2012. Habitat connectivity, more than species' biology, influences genetic differentiation in a habitat specialist, the short-eared rock-wallaby (*Petrogale brachyotis*). Conserv. Genet. 13, 937–952.
R Core Team, 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Richardson, J.L., 2012. Divergent landscape effects on population connectivity in two co-occurring amphibian species. Mol. Ecol. 21, 4437–4451.
Riley, S.P.D., Pollinger, J.P., Sauvajot, R.M., York, E.C., Bromley, C., Fuller, T.K., Wayne, R.K., 2006. A southern California freeway is a physical and social barrier to gene flow in carnivores. Mol. Ecol. 15, 1733–1741.
Ripperger, S.P., Tschapka, M., Kalko, E.K.V., Rodriguez-Herrera, B., Mayer, F., 2013. Life in a mosaic landscape: anthropogenic habitat fragmentation affects genetic population structure in a frugivorous bat species. Conserv. Genet. 14, 925–934.
Rossiter, S.J., Zubaid, A., Mohd-Adnan, A., Struebig, M.J., Kunz, T.H., Gopal, S., Petit, E.J., Kingston, T., 2012. Social organization and genetic structure: insights from codistributed bat populations. Mol. Ecol. 21, 647–661.
Reid, F.A., 2009. A Field Guide to the Mammals of Central America & Southeast Mexico. Oxford University Press, New York, NY.
Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Global biodiversity scenarios for the year 2100. Science 287, 1770–1774.
Sánchez-Azofeifa, G.A., Pfaff, A., Robalino, J.A., Boomhower, J.P., 2007. Costa Rica's payment for environmental services program: intention, implementation, and impact. Conserv. Biol. 21, 1165–1173.
Sarasola-Puente, V., Madeira, M., Gosá, A., Lizana, M., Gómez-Moliner, B., 2012. Population structure and genetic diversity of *Rana dalmatina* in the Iberian Peninsula. Conserv. Genet. 13, 197–209.
Sesnie, S.E., Gessler, P.E., Finegan, B., Thessler, S., 2008. Integrating Landsat TM and SRTM-DEM derived variables with decision trees for habitat classification and change detection in complex neotropical environments. Remote Sens. Environ. 112, 2145–2159.
Steffen, W., 2010. Observed trends in Earth System behavior. WIREs: Clim. Change 1, 428–449.
Stockwell, C.A., Hendry, A.P., Kinnison, M.T., 2003. Contemporary evolution meets conservation biology. Trends Ecol. Evol. 18, 94–101.
Struebig, M.J., Kingston, T., Petit, E.J., Le Comber, S.C., Zubaid, A., Mohd-Adnan, A., Rossiter, S.J., 2011. Parallel declines in species and genetic diversity in tropical forest fragments. Ecol. Lett. 14, 582–590.
Timm, R.M., LaVal, R.K., 1998. A field key to the bats of Costa Rica. Occup. Publ. Ser. Univ. Kansas 22, 1–30.
Thies, W., Kalko, E.K.V., 2004. Phenology of neotropical pepper plants (Piperaceae) and their association with their main dispersers, two short-tailed fruit bats, *Carollia perspicillata* and *C. castanea* (Phyllostomidae). Oikos 104, 362–376.
Traill, L.W., Brook, B.W., Frankham, R.R., Bradshaw, C.J.A., 2010. Pragmatic population viability targets in a rapidly changing world. Biol. Conserv. 143, 28–34.
Vandergast, A.G., Bohonak, A.J., Weissman, D.B., Fisher, R.N., 2007. Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history: phylogeography and landscape genetics of a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: *Stenopelmatus*). Mol. Ecol. 16, 977–992.
Voigt, C.C., Voigt-Heucke, S.L., Kretzschmar, A.S., 2012. Isotopic evidence for seed transfer from successional areas into forests by short-tailed fruit bats (*Carollia* sp.: Phyllostomidae). J. Trop. Ecol. 28, 181–186.
Wilkinson, G.S., Chapman, A.M., 1991. Length and sequence variation in evening bat D-loop mtDNA. Genetics 128, 607–617.

Wilkinson, G.S., Mayer, F., Kerth, G., Petri, B., 1997. Evolution of repeated sequence arrays in the D-loop region of bat mitochondrial DNA. Genetics 146, 1035–1048.
Woltmann, S., Kreiser, B., Sherry, T., 2012. Fine-scale genetic population structure of an understory rainforest bird in Costa Rica. Conserv. Genet. 13, 925–935.
Zahawi, R.A., Augspurger, C.K., 1999. Early plant succession in abandoned pastures in ecuador. Biotropica 31, 540–552.
Zanette, L., Doyle, P., Trémont, S.M., 2000. Food shortage in small fragments: evidence from an area-sensitive passerine. Ecology 81, 1654–1666.
Zimmermann, Y., Schorkopf, D., Moritz, R., Pemberton, R., Quezada-Euan, J., Eltz, T., 2011. Population genetic structure of orchid bees (Euglossini) in anthropogenically altered landscapes. Conserv. Genet. 12, 1183–1194.