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Forest fragmentation and edge effects on the genetic structure of *Clusia sphaerocarpa* and *C. lechleri* (Clusiaceae) in tropical montane forests

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Abstract: Fragmentation of tropical forests influences abiotic and biotic processes that affect the genetic structure of plant populations. In forest fragments, edge effects, i.e. changes of abiotic and biotic factors at forest edges, may be prevalent. In two forest fragments (*c.* 200 ha at *c.* 2450 m asl) of tropical montane forest in Bolivia, sympatric populations of the dioecious tree species *Clusia sphaerocarpa* and *C. lechleri* were used as case study species to compare genetic diversity and small-scale genetic structure (SGS) between edge and interior habitats. Eight microsatellite markers were employed to genotype 343 individuals including adults, juveniles and seedlings of *C. sphaerocarpa* and 196 of *C. lechleri*. Genetic differentiation was found between habitats in both species ($\Phi_{RT} = 0.071$ for *C. sphaerocarpa* and $\Phi_{RT} = 0.028$ for *C. lechleri*) and among ages in *C. sphaerocarpa* ($\Phi_{RT} = 0.016$). Overall, SGS was weak but significant with more pronounced SGS in *C. lechleri* ($Sp = 0.0128$) than in *C. sphaerocarpa* ($Sp = 0.0073$). However, positive spatial genetic autocorrelation extended only up to 10 m. For *C. sphaerocarpa*, SGS was stronger in seedling and juvenile stages than in adults and in the forest interior than at forest edges. Our results show that edge effects can extend to the genetic level by breaking-up local genetic structures, probably due to increased gene flow and enhanced pollination and seed-dispersal interactions at forest edges.

Key Words: *Clusia*, edge effects, genetic differentiation, montane forest, SGS

INTRODUCTION

Habitat fragmentation can lead to reduction in plant population size in remnant fragments (del Castillo *et al.* 2011), which in turn can affect the genetic structure of plant populations (Hamrick 2004) due to genetic drift (Ezard & Travis 2006, Young *et al.* 1996). However, whether and how the genetic structure is affected by drift depends on the level of gene flow within and among populations (Choo *et al.* 2012, Nason *et al.* 1997). Thus, potential fragmentation effects strongly depend on the mating system, pollen and seed dispersal distances and the effective population size of a species (Kettle *et al.* 2007). For tropical tree species it has been repeatedly shown that gene flow into forest fragments is larger than into comparable areas of continuous forest, through effects on pollen vectors or pollinator behaviour (Dick *et al.* 2008, Hamrick 2010, White *et al.* 2002).

Fragmentation leads to an increase of edge length relative to area in small habitat fragments (Laurance *et al.* 2007, Murcia 1995). Edge effects can have serious impacts on species diversity and composition, community dynamics, ecosystem functioning and interactions (Menke *et al.* 2012, Saunders *et al.* 1991, Vasconcelos & Luizaõ 2004). However, whether edge effects also extend to the genetic level in trees has rarely been studied. Since gene dispersal and the build-up of small-scale genetic structure (SGS) are often closely associated with seed-dispersal mutualisms (García & Grivet 2011), responses of animal seed-dispersers to edge effects may be a major determinant of genetic edge effects. In fragmented tropical forests, both reduced (Kirika *et al.* 2008, Lehouck *et al.* 2009) and increased seed removal by avian frugivores have been reported at edges of forest fragments (Farwig *et al.* 2006, Menke *et al.* 2012). Thus, genetic edge effects

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of animal-dispersed tropical plants are expected; their direction, however, is not easy to predict.

Although Kramer *et al.* (2008) reported a considerable body of literature that does not show effects of fragmentation on genetic variability in long-lived plant species, these effects may be visible only in some life stages (Ramos *et al.* 2010, van Rossum & Triest 2006, van Geert *et al.* 2008). Considering that fragmentation may have occurred after the adult plants had established, their genotype will reflect historical rather than current genetic patterns. Therefore, only recent cohorts may show consequences of fragmentation (Farwig *et al.* 2008). For instance, lower genetic diversity and higher inbreeding and genetic differentiation have been found in seedlings and juveniles than in adults in fragmented forests (Aldrich *et al.* 1998, Hensen *et al.* 2012, Kettle *et al.* 2007).

The tropical montane forests of South America are considered one of the world's main biodiversity hotspots (Kessler & Beck 2001, Myers *et al.* 2000) and have been vastly deforested in many areas, including Bolivia (Killeen *et al.* 2005). In montane cloud forests *Clusia* species form a common element (de Roca 1993). *Clusia* species depend on animal mutualists for seed dispersal (Gustafsson *et al.* 2007). Thus, changes in seed-dispersal mutualisms between forest edges and forest interior likely influence the population genetic structure of *Clusia* species. Here, we use *Clusia sphaerocarpa* and *C. lechleri* to evaluate genetic variation and SGS in edge and interior populations and we hypothesize that (1) genetic diversity differs and genetic differentiation is present between forest edge and forest interior and that (2) small-scale genetic structure (SGS) differs between forest edge and interior and among age classes.

METHODS

Study species

The species *Clusia sphaerocarpa* Planch. & Triana and *C. lechleri* Rusby (Clusiaceae) are common, medium-sized (11 m) trees in montane cloud forests of Bolivia (de Roca 1993). In the study area, they occur in sympatry in a clumped spatial distribution. They are dioecious, flower between March and July and the fruiting is from December to March. Male flowers of *C. sphaerocarpa* are *c.* 5 cm diameter with white petals while in *C. lechleri* diameter is *c.* 2.5 cm and petals are light yellow. In both species the female flowers are around *c.* 1 cm smaller than male flowers. Pollination in *Clusia* species is mainly carried out by bees collecting resin, but also by beetles, flies, lepidoptera, wasps and hummingbirds (Gustafsson *et al.* 2007). Fruits are globular capsules that dehisce to expose six or seven diaspores in *C. sphaerocarpa* and five in *C. lechleri*. The diaspores contain up 12 seeds for *C. sphaerocarpa* and up to six seeds for *C. lechleri* (Saavedra, unpubl. data). Due to their red lipid-rich aril, diaspores are primarily dispersed by small-to medium-sized birds, which mainly defecate the seeds. Some of them (e.g. *Anisognathus somptuosus*, *Diglossa cyanea*, *Myonectes striaticollis*) may move between forest fragments. Seeds are not dormant since they germinate directly after fruiting.

Study sites and sampling design

The study was conducted near Chulumani, province South Yungas, La Paz, Bolivia. As a result of the continuous action of anthropogenic fire, this region is characterized by huge deforested areas dominated by the bracken ferns *Pteridium aquilinum* var. *arachnoideum* and *Lophosoria quadripinnata* in the slopes of the valleys (so-called 'tropical savannas'; Killeen *et al.* 2005) and forest mainly remains on the montane tops and in gorges. The cover of the forest is further fragmented to grow coca, coffee and citrus fruits (Killeen *et al.* 2005). There is no exact information about the age of the fragments but information from local people indicates that the latest fires at edges can have happened no more than 5 y ago. The two *Clusia* species are quite common in the study area with densities of around 42 and 105 adult trees ha^{-1}. We sampled at two sites in fragments of *c.* 200 ha (site 1: 16°23′35.26″S, 67°33′44.26″W, 2440 m asl; site 2: 16°24′43.29″S, 67°34′03.34″W, 2450 m asl), and located at a distance of 2 km from each other. At each site, we installed a plot of 100 × 20 m in two habitat types: forest edge (3 m into the forest) and forest interior (300–400 m from the edge). Each plot was divided in 100 subplots of 2 × 10 m (Figure 1) in order to obtain accurate distances among individual for the analysis. We considered three age classes: seedlings (dbh ≤ 10 cm), juveniles (dbh > 10 cm and < 30 cm) and adults (dbh > 30 cm or flowering/fruiting). We counted, mapped and sampled all adult trees within the whole plot and we included adult trees as potential parents in a buffer area of 20 m around the plot. Juveniles were mapped and sampled in 25 regularly spaced subplots of 2 × 10 m (Figure 1). Seedlings were sampled in each of these subplots in a randomly positioned 1 × 1-m area. We collected fresh leaves of all mapped individuals and stored them separately in plastic bags with silica gel until further genetic analysis.

DNA extraction and microsatellites analysis

The extraction method followed a standard protocol (Doyle & Doyle 1987) with modifications (Hensen *et al.*

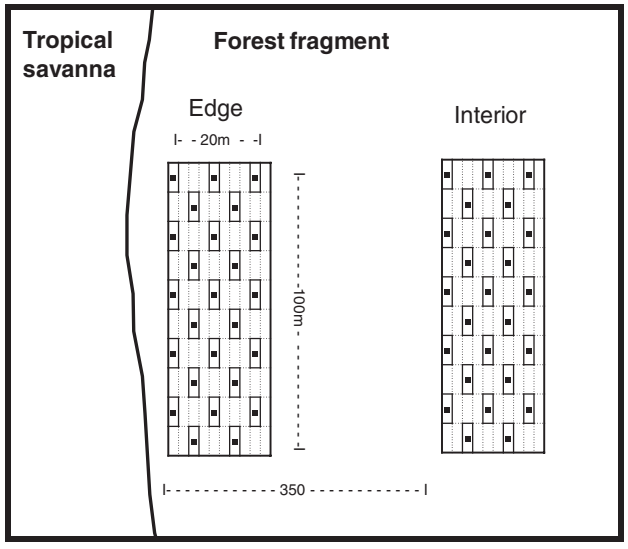

Figure 1. Schematic representation of the study design at two sites (forest fragments) of a Bolivian montane forest. At each site, we compared interior and edge plots adjacent to the deforested habitat matrix. Adults were sampled in the whole plot of 100 × 20 m and surroundings (20-m buffer). Juveniles were sampled in the smaller 25 subplots of 2 × 10 m inside of the whole plot and seedlings were sampled in subplots of 1 × 1 m randomly positioned inside of the subplots for juveniles.

2011). The individuals were genotyped using eight microsatellite markers (Clm1, Clm2, Clm5, Cln2, Cln5, Cln3, Cln7 and Cln8) previously developed from *C. minor* and *C. nemorosa* (Hale *et al.* 2002). Amplification was performed with 25 μl of reaction medium containing 1 μl of DNA (20 ng μl^{-1}), 0.8 μl (1 μl Cln8) of fluorescence labelled forward primer (5 pmol μl^{-1}) and 0.8 μl (1 μl Cln8) of reverse primer (5 pmol μl^{-1}) (metabion international, AG, Germany), 2.5 μl 2 mM dNTPs (QBiogene), 2.5 μl polymerase buffer with 2 μl (1.5 μl Clm1) $MgCl_2$ (Qbiogene), 0.2 μl (0.125 μl Cln8) Taq polymerase (Fermentas) and 13.6 μl (15.7 μl Clm1, 14.9 μl Cln8) double-distilled H_2O. For primers Clm1, Clm2 and Clm5 the PCR program was 94 °C for 3 min followed by 35 cycles with 30 s of denaturation at 94 °C, 30 s of annealing at 50 °C (55 °C for Cln2, Cln5 and Cln8), a 60-s elongation step at 72 °C, and a final elongation at 72 °C for 3 min in a Mastercycler (Eppendorf). For primers Cln3 and Cln7 the PCR program was 95 °C for 12 min followed by 10 cycles with 15 s at 94 °C, 15 s at 55 °C, 15 s at 72 °C, followed by 30 cycles with 15 s at 89 °C, 15 s at 55 °C, and 15 s at 72 °C and a final elongation at 72 °C for 10 min. PCR products were diluted 1 : 5 (1 : 10 for Clm2 and Clm5) and separated using capillary electrophoresis (MegaBace 1000, Amersham Bioscience, Uppsala, Sweden) with MegaBACE-ET ROX 400 (Amersham Bioscience) as a size standard. We used the MegaBace Fragment Profiler Software 1.2 (Amersham Bioscience) for genotyping.

Species identification

Since *Clusia sphaerocarpa* and *C. lechleri* co-occur in the plots and have similar vegetative characteristics, species identification was impossible in the field for non-flowering individuals. Therefore, we used the genotype data and applied Bayesian clustering of all individuals for the identification using the software STRUCTURE v. 2.3.3 (available at http://pritch.bsd.uchicago.edu/structure.html). STRUCTURE assigns individuals into genetically homogeneous clusters without prior knowledge of their affiliation. For all individuals, we carried out 10 independent runs per K using a burn-in period of 50 000 and collected data for 50 000 iterations for $K = 1$ to 5. We used the individual Q-values of the analysis at $K = 2$. Bayesian clustering of all 669 *Clusia* individuals identified two clusters that clearly distinguished the two species with membership coefficient of 0.937 of *C. sphaerocarpa* and *C. lechleri* for the two clusters respectively. We thus used the Q-values for $K = 2$ to assign individuals to species using 0.8/0.2 as a threshold to distinguish pure species from putative hybrids. Individuals identified in field fell in the correct resulting group by STRUCTURE. Finally, we obtained 434 individuals for *C. sphaerocarpa* (191 seedlings, 142 juveniles, 101 adults) and 196 individuals for *C. lechleri* (123 seedlings, 20 juveniles, 53 adults); 39 putative hybrid seedlings and juveniles (site 1 with 10 at the edge and 11 in the interior, site 2 with 12 and 6 respectively) were excluded from further analyses.

Population genetic analysis

We investigated genetic diversity and differentiation for both species at the habitat level (edge *vs.* interior) and for *C. sphaerocarpa* also at the level of age classes as it had sufficient sample size. Genetic diversity within populations was characterized by expected and observed heterozygosity (H_e, H_o) and fixation index (F_{IS}) using Genealex v. 6.5 (available at http://biology.anu.edu.au/GenAlEx/Welcome.html). To allow comparison between populations, that differed in sample sizes, we computed allelic richness (A_r), obtained with a rarefaction method (Hurlbert 1971) with identical sample size in FSTAT v.2.9.3.2 (available at http://www2.unil.ch/popgen/softwares/fstat.htm). Genetic differentiation was analysed by two approaches. First, as genetic differentiation among all populations estimated as G_{ST} (Nei 1987) with FSTAT v.2.9.3.2 and a non-hierarchical analysis of molecular variance (AMOVA). Second, we used hierarchical AMOVA to jointly assess differentiation among sites and habitats. For *C. sphaerocarpa*, we combined data of the two sites that

Table 1. Genetic diversity of *Clusia sphaerocarpa* and *C. lechleri* at two sites (forest fragments) in a montane forest near Chulumani, South Yungas, Bolivia. Comparison of habitats (edge vs. interior) in each site and only for *C. sphaerocarpa,* of age classes (adults, juveniles and seedlings). A_r was obtained with a rarefaction sample size of 11.

Site	Habitat	Age class	N	H_e	H_o	*Fis*	A_r
Clusia sphaerocarpa							
Site 1	Edge	All stages	51	0.38 ± 0.09	0.31 ± 0.09	0.18	3.19
		Adults	35	0.37 ± 0.09	0.30 ± 0.09	0.21	3.22
		Seedlings	15	0.37 ± 0.09	0.33 ± 0.11	0.14	2.82
	Interior	All stages	142	0.42 ± 0.11	0.33 ± 0.12	0.22	3.39
		Adults	17	0.45 ± 0.11	0.40 ± 0.13	0.12	3.44
		Juveniles	17	0.38 ± 0.12	0.29 ± 0.10	0.27	3.05
		Seedlings	108	0.42 ± 0.11	0.32 ± 0.12	0.23	3.42
Site 2	Edge	All stages	136	0.43 ± 0.10	0.31 ± 0.10	0.29	3.21
		Adults	22	0.42 ± 0.11	0.29 ± 0.10	0.33	3.33
		Juveniles	76	0.44 ± 0.09	0.32 ± 0.10	0.29	3.16
		Seedlings	38	0.39 ± 0.10	0.29 ± 0.10	0.25	3.09
	Interior	All stages	105	0.44 ± 0.11	0.34 ± 0.09	0.25	3.49
		Adults	27	0.42 ± 0.11	0.32 ± 0.10	0.25	3.53
		Juveniles	48	0.41 ± 0.11	0.29 ± 0.09	0.32	3.17
		Seedlings	30	0.48 ± 0.11	0.43 ± 0.10	0.13	3.61
Clusia lechleri							
Site 1	Edge	All stages	113	0.51 ± 0.06	0.38 ± 0.09	0.27	3.68
	Interior	All stages	11	0.46 ± 0.08	0.36 ± 0.10	0.26	3.38
Site 2	edge	All stages	38	0.47 ± 0.07	0.35 ± 0.08	0.27	3.72
	interior	All stages	34	0.44 ± 0.08	0.24 ± 0.07	0.46	3.54

were not differentiated and jointly assessed differentiation among habitats and age classes. AMOVA analyses were performed with GenAlex v. 6.5, with 999 permutations.

Small-scale genetic structure (SGS) was investigated both at the species level and for *C. sphaerocarpa* at the level of habitat (edge *vs.* interior) and age class (seedlings, juveniles and adults). These latter analyses could not be performed for *C. lechleri* due to low sample size. The SGS analyses included all age classes at species and habitat level. For all analyses, we used distance class limits of 10, 20, 30, 50, 90 and 200 m in order to assure a sufficient number of pairs of individuals per distance class. We applied two approaches to evaluate SGS. First, we used spatial genetic autocorrelation of correlation coefficients among genetic and spatial distance matrices in GenAlex v. 6.5 (Smouse *et al.* 2008). Where appropriate, 999 permutations were performed. As suggested by Banks & Peakall (2012), significance of the heterogeneity test can be declared when $P < 0.01$. Second, we used spatial genetic autocorrelation of pairwise kinship coefficients (F_{ij}) (Loiselle *et al.* 1995) in SPAGeDi v. 1.3d (available at http://ebe.ulb.ac.be/ebe/SPAGeDi.html) to quantify SGS with the *Sp* statistic. *Sp* was calculated as $Sp = -b_{log}/(1-F_{(1)})$, where b_{log} is the slope of the regression of kinship coefficients on log geographic distance and $F_{(1)}$ is the mean kinship coefficient between individuals of the first distance class. Following Fenster *et al.* (2003) and Michalski & Durka (2012), we calculated approximate confidence intervals of *Sp* using $b_{log} \pm$ twice the SE of b_{log} estimated by jack-knifing over loci.

RESULTS

Genetic diversity and population structure

Genetic diversity of *C. sphaerocarpa* and *C. lechleri* was high in all sites and habitats (Table 1). F_{IS} values did not show a consistent variation either between habitats or among class ages. F_{IS} values were positive, indicating lack of heterozygotes, most likely due to null alleles, which are commonly found when microsatellites are transferred between species.Values of allelic richness were very similar across sites, habitats and age classes. Considering all populations, genetic differentiation among populations was low but significant with overall $G_{ST} = 0.038$ and $\Phi_{RT} = 0.055$ ($P = 0.001$) for *C. sphaerocarpa* and $G_{ST} = 0.033$ and $\Phi_{RT} = 0.070$ ($P = 0.001$) for *C. lechleri*. In a hierarchical AMOVA, *C. sphaerocarpa* was not significantly differentiated among sites, but 7% of variation resided among habitats (Table 2). In contrast, *C. lechleri*, was differentiated both among sites (5% of variation) and among habitats (3%). When habitat and age were analysed in *C. sphaerocarpa*, both habitat and age were differentiated with a variation of 2% ($\Phi_{RT\_habitats} = 0.023$, $P = 0.001$; $\Phi_{RT\_ages} = 0.016$, $P = 0.001$) (Table 2).

Table 2. Analysis of molecular variance (AMOVA) for *Clusia sphaerocarpa* and *C. lechleri* among two sites (forest fragments), habitats (edge vs. interior) and only for *C. sphaerocarpa*, at age classes (adults, juveniles and seedlings) in a Bolivian montane forest (Chulumani, South Yungas).

	df	Variance	% Total	Φ Statistic	P value
Clusia sphaerocarpa					
Non-hierarchical					
Among populations	3	0.264	5	0.055	0.001
Within populations	433	4.57	95		
Hierarchical sites/habitats					
Among sites	1	0.00	0	−0.027	1.000
Among habitats	2	0.35	7	0.071	0.001
Within habitats	430	4.57	93	0.047	0.001
Hierarchical sites/habitats					
Among habitats	1	0.11	2	0.023	0.001
Among ages	4	0.08	2	0.016	0.001
Within ages	428	4.65	96	0.039	0.001
Clusia lechleri					
Non-hierarchical					
Among populations	3	0.431	7	0.070	0.001
Within populations	192	5.76	9		
Hierarchical sites/habitats					
Among sites	1	0.34	5	0.055	0.001
Among habitats	2	0.16	3	0.028	0.009
Within habitats	192	5.77	92	0.081	0.001

Table 3. Estimates of small-scale genetic structure (*Sp*) of *Clusia sphaerocarpa* and *C. lechleri* in two sites (forest fragments) of a Bolivian montane forest (Chulumani, South Yungas) comparing edge and interior forest in each site. Density was extrapolated from plots of 100 × 20 m. CI = confidence intervals.

Species	Population	Density (ha^{-1})	*Sp* (CI)
C. sphaerocarpa	Overall	105	0.0073 (0.0052–0.0091)
C. lechleri	Overall	43	0.0128 (0.0048–0.0207)
C. sphaerocarpa	Edge	130	0.0053 (0.0012–0.0094)
C. sphaerocarpa	Interior	85	0.0092 (0.0020–0.0165)

Small-scale genetic structure

Small-scale spatial genetic autocorrelation was observed at the species level for both *C. sphaerocarpa* ($\omega = 39.0$; P = 0.001) and *C. lechleri* ($\omega = 31.1$; P = 0.004). In both species, positive autocorrelation was detected only in the first distance class (10 m; Figure 2a). Separate analyses at the habitat level revealed significant spatial structure for *C. sphaerocarpa* in interior plots ($\omega = 43.2$; P = 0.001) but not so in edge populations ($\omega = 21.3$; P = 0.045). This was due to a higher autocorrelation coefficient in the first distance class in interior habitats (Figure 2b). The analysis across age classes in *C. sphaerocarpa* showed a significant spatial structure for both seedlings ($\omega = 39.5$; P = 0.002) and juveniles ($\omega = 28.9$; P = 0.009) but not so for adults ($\omega = 21.9$; P = 0.06).

The *Sp* values indicated overall weak SGS (Table 3) which tended to be lower in *C. sphaerocarpa* (*Sp* = 0.0073) than in *C. lechleri* (*Sp* = 0.0128). For C. *sphaerocarpa*, *Sp* values were slightly higher in the forest interior (0.0092) than in the forest edge (0.0053).

DISCUSSION

Edge and fragmentation effects

Neither sympatric *Clusia* species showed differences in genetic diversity between populations at the edge and in the interior of forest fragments. This finding essentially shows that the investigated populations and fragments are still large enough to maintain genetic diversity and had not yet undergone strong genetic drift. In fact, the forest fragments analysed are large and the two *Clusia* species are quite common in the study area. The temporal maintenance of genetic diversity is additionally fostered by life-history traits such as the outcrossing breeding system of the dioecious species and the longevity of the trees which allows for transgenerational gene flow (Bawa 1992, Kramer *et al.* 2008). Our study is also in line with Ramos *et al.* (2010) who reported no significant differences in genetic diversity neither for *Psychotria tenuinervis* nor for *Guarea guidonia* among fragment interior, natural and anthropogenic edge areas in an Atlantic Forest. Similarly, genetic diversity was similarly high in populations of *Prunus africana* growing in forest fragments and in continuous forests (Farwig *et al.* 2008).

Both *Clusia* species showed low but significant levels of genetic differentiation among populations. Differentiation was in the range previously observed for outcrossing tropical and subtropical tree species studied with microsatellites (Debout *et al.* 2011, Shi *et al.* 2011). The hierarchical AMOVA showed that in *C. sphaerocarpa* this differentiation does not exist between sites but between edge and interior habitats, indicating that edge effects may extend to the genetic level via effects on gene flow (Lowe *et al.* 2005). According to Dick *et al.* (2008), low population density together with density-dependent animal pollination contributes to population genetic differentiation in tropical forest trees. Thus, differences in population density of the studied *Clusia* species and composition and activity of animal pollinators between edge and interior in our study area (Kambach *et al.* 2013) could foster genetic differentiation between habitats. In *C. sphaerocarpa*, age classes were also slightly differentiated indicating that not all resident adults were similarly represented in the offspring gene pool. Other studies have also shown that fragmentation has effects on genetic structure and differ among age classes. In *Prunus africana*, differentiation was higher in seedlings than in adults

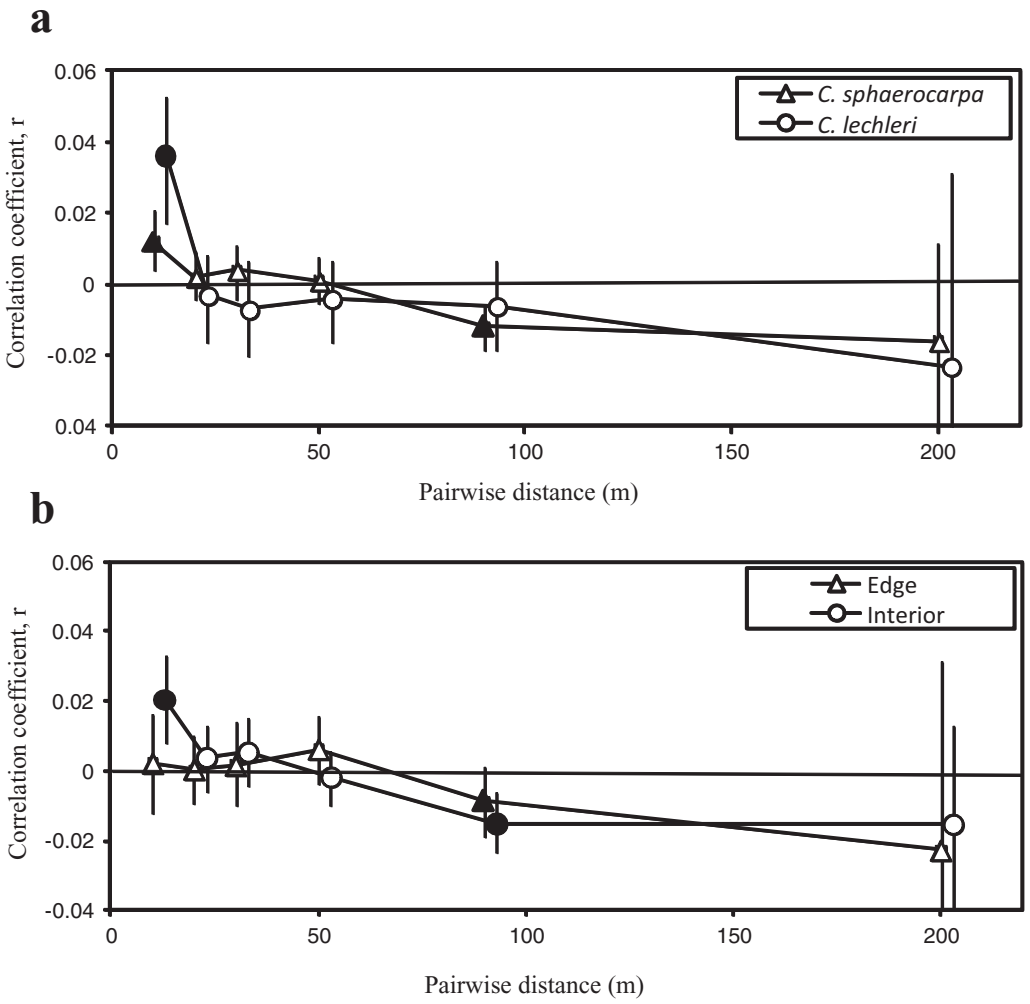

Figure 2. Significant small-scale spatial genetic autocorrelation (SGS) of *Clusia sphaerocarpa* and *C. lechleri* in edge and interior populations at two sites (forest fragments) in a Bolivian montane forest was detected in the first distance class (a) and, for *C. sphaerocarpa*, in the interior plots (b). The analysis includes individuals of all age classes. Filled symbols denote individually significant ($P < 0.05$) spatial autocorrelation, empty symbols indicate non-significant values. Error bars bound the 95% confidence interval as determined by bootstrap resampling.

(Farwig *et al.* 2008) and in *Symphonia globulifera* only populations of seedlings were genetically differentiated (Aldrich *et al.* 1998). Thus, the seedling generation may be more sensitive than adults to indicate fragmentation effects.

Small-scale genetic structure

Our results showed significant albeit weak SGS for both *Clusia* species. The *Sp* values quantifying SGS were typical for outcrossing species (Kloss *et al.* 2011, Michalski & Durka 2012) and in particular for trees (Vekemans & Hardy 2004, Shi *et al.* unpubl. data). SGS is expected to be weak in plant species with high adult densities, high pollen dispersal distances, overlapping seed shadows and homogeneous distribution of suitable recruitment sites (Dyer 2007, Hamrick & Nason 1996, Hamrick *et al.* 1993). Thus, several life-history traits contributed to the weak SGS in *Clusia.* First, the high density of adults which reduces the distance between flowering and fruiting trees and produces overlapping pollen clouds and seed shadows (Doligez *et al.* 1998, Gonzales *et al.* 2010). Second, pollination by a large guild of insects and birds which supports long-distance pollen dispersal (Gustafsson *et al.* 2007, Kettle *et al.* 2011). Finally, seed dispersal by frugivorous birds is efficient in *Clusia* and will also blur SGS (García & Grivet 2011, Hamrick & Trapnell 2011).

In both species, positive genetic autocorrelation up to a distance of 10 m was detected. In *C. sphaerocarpa*, SGS was more pronounced in the two early life stages than in adult trees. This suggests that while gene flow in general prevents the build-up of local SGS, locally, more closely related individuals have established,

likely influenced by demographic thinning between life stages. In other *Clusia* species a clumped spatial distribution was associated with the dispersal of diaspores containing multiple seeds (Bittrich & Amaral 1996). Thus, contiguous establishment of codistributed halfsibs may lead to local SGS. Seeds of *Clusia* are also displaced secondarily by ants (Passos & Oliveira 2002) which has also been observed in the study area (Gallegos, unpubl. data). Ants move seeds over short distances and may increase recruitment success by dispersing seeds to suitable establishment sites (Hanzawa *et al.* 1988, Passos & Oliveira 2002). However, it is difficult to predict whether ant-mediated secondary seed dispersal will lead to strong or weak SGS as it may increase establishment success of closely related bird-dispersed halfsibs but may also lead to small-scale mix of seeds from different bird droppings.

In *C. sphaerocarpa*, we found significant SGS and higher *Sp* values for populations in the forest interior compared with forest edges. This may be due to two non-exclusive factors. First, it is consistent with the interior population having lower adult densities. Second, an edge effect of enhanced gene flow that could be mediated by increased pollinator or seed-disperser activities. An analysis of pollinator guilds in our study area found an increase in bee species richness and abundance from forest interior to deforested habitat types (Kambach *et al.* 2013). At forest edges, bee richness and abundance tended to be higher than in the forest interior (Kambach *et al.* 2013). Higher acitivity of pollinators and increased pollen flow would be consistent with the observed edge effect on SGS in this study. This is also supported by the trend to a higher gene flow by long-distance pollen movement in disturbed and isolated trees reported for *Swietenia humilis* in tropical dry forest (White *et al.* 2002). Similarly, frugivorous birds may congregate at forest edges, leading to an increase in seed removal rates (Menke *et al.* 2012). Similar patterns of an increase in frugivore activity at forest edges have been found in the study area (Saavedra, unpubl. data). It is therefore likely that the SGS of populations of *Clusia* is blurred at forest edges because these populations receive higher gene flow than those in the forest interior, mediated by both increased pollination and seed-dispersal functions at forest edges.

In conclusion, our study provides evidence for edge effects in populations of *Clusia* species because edge and interior populations were genetically differentiated and weak patterns of SGS were wiped out at forest edges. These effects were most likely due to changes in plant–animal mutualisms at forest edges and an associated increase in pollination and seed-dispersal functions. While levels of genetic diversity were not affected in the large populations of *Clusia*, changes in the patterns of genetic structure suggest that changes in biotic interactions at forest edges extend to the genetic level in *Clusia* populations. Considering the importance of *Clusia* as a common element in montane forests, modified genetic structures at the edges of forest remnants are relevant for future conservation measures.

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