MOLECULAR ECOLOGY

Molecular Ecology (2011) **20**, 4421–4432 doi: 10.1111/j.1365-294X.2011.05293.x

Fragmentation can increase spatial genetic structure without decreasing pollen-mediated gene flow in a wind-pollinated tree

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Abstract

Fragmentation reduces population sizes, increases isolation between habitats and can result in restricted dispersal of pollen and seeds. Given that diploid seed dispersal contributes more to shaping fine-scale spatial genetic structure (SGS) than haploid pollen flow, we tested whether fine-scale SGS can be sensitive to fragmentation even if extensive pollen dispersal is maintained. *Castanopsis sclerophylla* (Lindley & Paxton) Schottky (Fagaceae), a wind-pollinated and gravity seed-dispersed tree, was studied in an area of southeast China where its populations have been fragmented to varying extents by human activity. Using different age classes of trees in areas subject to varying extents of fragmentation, we found no significant difference in genetic diversity between prefragmentation vs. postfragmentation *C. sclerophylla* subpopulations. Genetic differentiation among postfragmentation subpopulations was also only slightly lower than among prefragmentation subpopulations. In the most fragmented habitat, selfing rates were significantly higher than zero in prefragmentation, but not postfragmentation, cohorts. These results suggest that fragmentation had not decreased gene flow among these populations and that pollen flow remains extensive. However, significantly greater fine-scale SGS was found in postfragmentation subpopulations in the most fragmented habitat, but not in less fragmented habitats. This alteration in SGS reflected more restricted seed dispersal, induced by changes in the physical environments and the prevention of secondary seed dispersal by rodents. An increase in SGS can therefore result from more restricted seed dispersal, even in the face of extensive pollen flow, making it a sensitive indicator of the negative consequences of population fragmentation.

Keywords: *Castanopsis sclerophylla*, fragmentation, gene flow, isolation, spatial genetic structure

Received 12 May 2011; revision received 17 August 2011; accepted 30 August 2011

Introduction

Fragmentation decreases habitat sizes and increases spatial distances between habitats and populations. These physical changes are generally thought to decrease effective population sizes and gene flow, resulting in loss of genetic diversity and an increase in differentiation because of drift and inbreeding (Young *et al.* 1996; Chen 2000b). These expectations have been met in some studies, especially those of short-lived species (Zhao *et al.* 2006; Shapcott *et al.* 2009). However, most species of tropical trees have revealed patterns that are inconsistent with these theoretical expectations, indicating that their assumptions may be invalid or that the number of generations that had experienced fragmentation were too few for responses to be detected (Hamrick 2004; Lowe *et al.* 2005).

Dispersal of genes shapes the population genetic outcomes of fragmentation. Many plants display long-distance pollen dispersal and have seed vectors that can overcome geographical isolation. In particular, extensive

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gene flow of wind-pollinated or wind-dispersed trees can link apparently isolated habitats into a metapopulation. Oak (*Quercus*) pollen grains can be wind-transported at distances exceeding hundreds of kilometres (Stanley & Linskens 1974) and in an isolated *Pinus sylvestris* population, 4.3% of seed-siring pollen migrated at least 30 km (Robledo-Arnuncio & Gil 2005). Pollen flow can be even more extensive among trees pollinated by small insects, which can use the wind to carry them between trees more than 100 km apart (Ahmed *et al.* 2009). Consequently, in plants with extensive long-distance pollen flow, the genetic diversity of small, isolated populations may be comparable with or even higher than that of large and continuous populations, although Jump & Peñuelas (2006) did find that, given enough time, negative genetic consequences can be observed even in trees with extensive pollen flow.

Fine-scale spatial genetic structure (SGS) characterizes the spatial distribution of genetic variation within a population (Wang *et al.* 2009). Previous studies showed that Moran's *I*-values increase with decreasing dispersal distance (Epperson & Li 1996; Doligez *et al.* 1998) and clumped distributions will increase SGS (Doligez *et al.* 1998). Increases in outcrossing rate and local density, which lead to an increase in heterozygosity and spatial mixing of genotypes, will decrease SGS (Doligez *et al.* 1998), while inbreeding decreases heterozygosity and intensifies SGS (Vekemans & Hardy 2004). In general, fragmentation decreases dispersal and genetic diversity and increases inbreeding and drift, and thus stronger fine-scale SGS is expected in fragmented populations than in continuous ones (Slavov *et al.* 2010; Sebbenn *et al.* 2011). Reflecting this, Sebbenn *et al.* (2011) observed significant SGS over distances of as little as 20 m among both adults and seedlings of *Copaifera langsdorffii*, an insect-pollinated and bird-dispersed tropical tree in fragmented populations with no seed immigration and only rare pollen migration. This contrasts with long-lived wind-pollinated tree species, where fragmentation can fail to alter their genetic structure (Williams *et al.* 2007)—a discrepancy that suggests dispersal plays a critical role in determining the extent of fine-scale SGS (Doligez *et al.* 1998).

A diploid seed disperses twice the number of genes as a pollen grain. Furthermore, only seeds can germinate, grow to maturity and provide a biological base for pollen dispersal (Hu & He 2006). Fragmentation increases the distances between populations, and can also generate barriers to seed dispersal, especially for trees bearing large seeds (Wunderle 1997), leading to the clumping of progeny near mother trees (Gonzales *et al.* 2010). Given their different contributions to gene flow, we hypothesized that restricted seed dispersal generated by habitat fragmentation can increase fine-scale SGS even if there is still extensive long-distance pollen dispersal.

Castanopsis sclerophylla (Lindley & Paxton) Schottky (Fagaceae) is a monoecious tree found in the subtropical evergreen forests of southeast China. A canopy species that can grow to a height of 20 m, *C. sclerophylla* flowers in April and May. Its seeds are gravity-dispersed, so seed dispersal distances are expected to be limited, although rodents can serve as secondary seed dispersers when they carry and store seeds. Conversely, it is a wind-pollinated species, suggesting that pollen dispersal is likely to be extensive, and only low levels of genetic differentiation between populations have been recorded (Shi 2008). To determine the impacts of fragmentation on its SGS, we chose a fragmented landscape in and around Qiandao Lake, where it is one of the dominant forest species (Zhang 2006). Qiandao Lake was created by the building of Xin'anjiang Dam in 1959. There are 1078 islands with an area >2500 m^2 in the lake when water level is maximal. We sampled *C. sclerophylla* individuals from plots of the same size in habitats fragmented to varying extents, placing them into pre- or postfragmentation cohorts based on their trunk diameter (a proxy for age). We genotyped each individual using nuclear microsatellite markers, and compared genetic variation and SGS patterns of the pre- and postfragmentation cohorts. We asked the following specific questions: (i) Was there any difference in genetic diversity and differentiation among populations with different levels of fragmentation and between post- and prefragmentation cohorts? (ii) Did fragmentation alter the fine-scale SGS to different extents in isolated and continuous forest patches of the same size? If it did, we expected that more intensive SGS would be observed in seriously fragmented habitats because limited space would have restricted seed dispersal.

Materials and methods

Study sites and sampling design

Our study was conducted in the southeastern section of Qiandao Lake. We selected three study sites with different degrees of fragmentation: (i) A strongly fragmented habitat: Heyang Island (HY), an island with an area of 13 ha, containing *c.* 350 *C. sclerophylla* individuals. Because of the surrounding water, seed dispersal of *C. sclerophylla* was completely restricted to within the island. (ii) A moderately fragmented habitat: Laoshan Island (LS), the largest island (875 ha) in the study area, containing *c.* 12 000 *C. sclerophylla* individuals. (iii) A control habitat: Xianshan Peninsula (XS), a mainland area near the lakeshore that was not surrounded by water, with at least 35 000 individuals of *C. sclerophylla*,

scattered within a continuous forest of >2500 ha (Fig. 1). The vegetation in these three sites was dominated by *C. sclerophylla*, *Cyclobalanopsis glauca* and *Pinus massoniana*. A map that predates the formation of the lake shows that forest cover was originally continuous, but with a stream or small river separating LS from the other study sites. There is no historical record of events that might have led to intensive disturbance within the forest prior to formation of the lake.

In autumn 2009, 130 m × 130 m square plots were selected in each site, and each was divided into 169 subplots of 10 m × 10 m to help map the positions of individuals of *C. sclerophylla*. The plots were at least 2 km apart. All *C. sclerophylla* in the plots were mapped and their basal diameters measured to the nearest 0.1 cm. In case that sample size was too small to meet the requirements of autocorrelation analysis, we also mapped and collected tissue from additional trees surrounding the plot (<20 m). To avoid edge effects, each sampled tree was at least 20 m far from forest edges. According to annual ring samples from the study area, Zhang (2006) established a linear relationship between age and basal diameter of *C. sclerophylla*: $y = 1.1776x + 1.1552$, where x and y represent the basal diameter (cm) and the age (years), respectively. Based on this relationship, each tree was grouped into pre- (age >50 years) or postfragmentation (age <50 years) cohorts.

Several healthy mature leaves were collected from each individual and dried by silica gel for DNA extraction. Saplings with a height of <1.5 m and trees infested by termites were excluded from sampling because they had very few leaves (usually <20). Overall, we sampled 433 pre- and 351 postfragmentation individuals. Over 100 trees from each cohort were found in all three habitats, forming a total of six subpopulations (Table 1).

DNA extraction and microsatellite genotyping

Genomic DNA was extracted using the Plant Genomic DNA Kit (Tiangen, Beijing, China). Using microsatellites developed for *Castanopsis cuspidata* var. *sieboldii* Nakai (Ueno *et al.* 2000, 2003), all individuals were genotyped for eight polymorphic microsatellites (Ccu16H15, Ccu17F15, Ccu28H18, Ccu33H25, Ccu62F15, Ccu87F23, Ccu93H17 and Ccu102F36). The microsatellites were amplified with fluorescently labelled forward primers (FAM, HEX, ROX and TAMRA) on a PTC-220 DNA Dyad thermal cycler (MJ Research, Waltham, MA, USA). Fragment lengths were analysed on an ABI 3130 Genetic Analyzer (Applied Biosystem, Foster City, CA, USA) using GeneScan500(-250) Liz standard. Genotypes were scored by the software GENEMAPPER 4.0 (Applied Biosystem).

Analyses of genetic diversity, differentiation and gene flow among populations

Departure from Hardy–Weinberg equilibrium was tested using Fisher's exact test with the software TFPGA 1.3 (Miller 1997). Within each subpopulation, the number of alleles per locus (A), allelic richness (A_R) (El Mousadik & Petit 1996) and the fixation index (F_{IS}) were analysed with FSTAT version 2.9.3.2 (Goudet 1995). The number of exclusive alleles per locus (i.e. alleles that appeared in one but not the other cohort in the same population) was assessed directly from allele frequency. The observed (H_O) and expected (H_E)

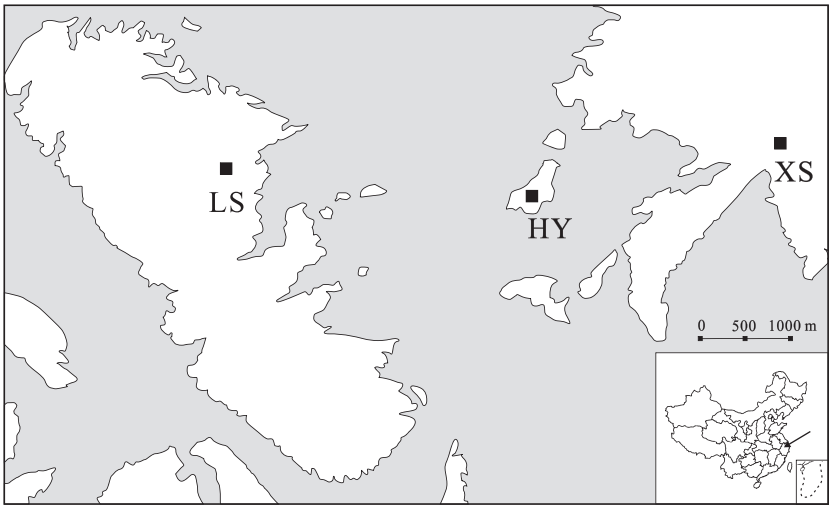

Fig. 1 Positions of the sample plots on Laoshan Island (LS), Heyang Island (HY) and Xianshan Peninsula (XS) in the southeast of Qiandao Lake.

Table 1 Genetic diversity over eight microsatellite loci and selfing rates in pre- and postfragmentation subpopulations of *Castanopsis sclerophylla* from seriously and moderately fragmented and control habitats

Habitat and coordinates of sampling plots	Subpopulation	Sample size	A	A_R	EA	H_O	H_E	F_{IS}	s_g
Seriously fragmented (N29° 32.80′, E119° 05.95′)	HY-pre	117	7.25	7.18	0.50	0.54	0.63	0.146*	**0.129** (0.010)
	HY-post	103	7.63	7.60	0.88	0.56	0.62	0.097*	0.026 (0.295)
Moderately fragmented (N29° 32.93′, E119° 04.90′)	LS-pre	151	7.25	7.00	0.63	0.55	0.60	0.087*	0.034 (0.216)
	LS-post	106	7.75	7.70	1.13	0.54	0.62	0.132*	0 (0.818)
Control (N29° 33.01′, E119° 07.31′)	XS-pre	165	8.00	7.78	0.50	0.52	0.63	0.177*	0 (0.638)
	XS-post	142	8.00	7.77	0.50	0.53	0.63	0.156*	0 (0.768)

A, average number of alleles per locus; A_R, allelic richness; H_O, observed heterozygosity; EA, average exclusive alleles number per locus for each pre- and postfragmentation subpopulation pair; H_E, expected heterozygosity; F_{IS}, fixation index and significant values are indicated by asterisks; s_g, selfing rate estimated from the microsatellite multilocus correlation structure and values in parentheses are P values that selfing rates are equal to zero (significant value is in bold).

heterozygosities (Nei 1978) were estimated by TFPGA. Because trans-specific amplification of microsatellites often results in null alleles, we checked the data for the presence of null alleles under the assumption of HWE using the software MICRO-CHECKER version 2.2.3 (Van Oosterhout *et al.* 2004).

Genetic differentiation between pre- or postfragmentation subpopulations was evaluated by F_{ST}-statistic and tested with 1500 permutations using FSTAT. G_{ST} was also implemented to evaluate genetic differentiation among subpopulations within each cohort level using FSTAT (Goudet 1995). Based on the results from FSTAT, standardized genetic differentiation was estimated by means of both F'_{ST} and G'_{ST} as described by Hedrick (2005). When calculating F'_{ST}, we implemented software RECODEDATA version 0.1 (Meirmans 2006) to assess the maximum value of F_{ST}. Historic gene flow (Nm) at the subpopulation level was then estimated using the formula proposed by Slatkin & Barton (1989). The genetic divergence index (D_{est}) proposed by Jost (2008) was also calculated by software SMOGD (Crawford 2010).

Assignment tests were conducted to detect the recent migration among different sites using STRUCTURE version 2.2 (Pritchard *et al.* 2000). To explore the number of clusters (K) with maximum likelihood, 20 runs of simulations were performed for each value of K from 1 to 10, with the following settings: admixture model, correlated allele frequencies, burn-in length of 10 000 and MCMC repetitions of 20 000. The approach described by Evanno *et al.* (2005) was adopted to define the most reasonable K by using ΔK as the criterion. Once the value of K was determined, all individuals were assigned to the K clusters probabilistically by setting a burn-in of 300 000 and 1 000 000 MCMC repetitions.

We also estimated selfing rates within subpopulations using the software RMES (David *et al.* 2007). This software calculates the selfing rate, s_g, from the multilocus correlation structure of a population sample. The estimated values are not sensitive to null alleles and scoring errors (David *et al.* 2007).

Comparisons of fine-scale SGS and estimation of gene dispersal patterns

Some historical events, such as admixture of gene pools, will produce genetic discontinuities that affect fine-scale SGS and may bias the results of analyses that assume the existence of isolation-by-distance patterns (Born *et al.* 2008). To identify whether such genetic discontinuities occurred in each plot, we utilized a Bayesian model–based clustering method (Guillot *et al.* 2005a), which was implemented in the R package GENELAND version 3.2.4 (Guillot *et al.* 2005b). This model considered the number of clusters as an unknown parameter estimated directly by Markov chain Monte Carlo (MCMC) procedures, providing estimations with high accuracy (Guillot *et al.* 2005a; Coulon *et al.* 2006). For samples from each plot, five independent runs were carried out in the spatial D-model with 100 000 MCMC iterations. Allele frequencies were assumed to be uncorrelated, as this setting is suitable for detecting the genetic consequence of ancient ecological events (Guillot *et al.* 2005a). Furthermore, the null allele model was selected in case of the disturbance of null alleles (Guillot *et al.* 2008). If more than one cluster were identified in any of the three plots, we ran GENELAND five more times to map the geographical boundary of each cluster, treating the number of clusters as a fixed parameter.

Fine-scale SGS within each subpopulation was detected by a spatial autocorrelation approach using SPAGEDI version 1.3 (Hardy & Vekemans 2002). The kinship coefficient (F_{ij}) defined by Loiselle *et al.* (1995) was adopted because this index does not take

Hardy–Weinberg equilibrium as a basic assumption (Vekemans & Hardy 2004). Within each subpopulation, we divided all pairwise comparisons into eight distance classes with 20-m intervals between consecutive classes. The significance of SGS patterns was tested by random permutation with 9999 repetitions.

The intensity of SGS in each subpopulation was quantified by SPAGeDi. As proposed by Vekemans & Hardy (2004), we used the statistic *Sp* to describe the intensity of SGS following the formula $Sp = blog/(F - 1)$, where *blog* is the slope of linear regression for kinship coefficient on the logarithm of geographical distance, and F is the average kinship coefficient between individuals in the first distance class. A total of 9999 permutations were carried out to test the significance of *blog*.

For each study site, we performed a heterogeneity test between SGS patterns in pre- and postfragmentation subpopulations using GenAlEx version 6.3 (Peakall & Smouse 2006). The autocorrelation coefficient r described by Smouse & Peakall (1999) was selected. We used the approach proposed by Smouse *et al.* (2008), which compares the real differentiation among spatial autocorrelation analyses with permutated ones from the pooled data set. Fisher's combined probabilities were computed as a gauge of departure from the null hypothesis. Criteria ω and t^2 were used to represent the divergences in pairs of spatial autocorrelation analyses as a whole and in each distance class, respectively. All eight distance classes were included in the heterogeneity tests. The number of bootstrap resamplings was set to 9999.

We used the software Spagedi to estimate the neighbourhood size (*Nb*) in each subpopulation and then inferred the effective gene dispersal range σ from *Nb*. Under Wright's isolation-by-distance model, the values of kinship coefficients in two-dimensional space are expected to decline linearly with the logarithm of geographical distance within a range from σ to 20σ at a medial mutation rate (Hardy & Vekemans 1999; Heuertz *et al.* 2003). Under this assumption, slope (*blog*) of the linear regression function can be adopted to evaluate *Nb* from the formula: $Nb = -(1 - F)/blog$ (Fenster *et al.* 2003; Heuertz *et al.* 2003). When the effective population density (D_e) is known, σ can be obtained from *Nb* by an iterative procedure (Vekemans & Hardy 2004). In tree species, the approximate value of D_e can be considered as $D/4$ (Hardy *et al.* 2006), where D is the census population density. We used the census density of *C. sclerophylla* in each sample plot to assess D_e. Linear regressions were limited within a range from σ to 20σ as the default set.

In addition to the regression-based approach, gene dispersal distances within each subpopulation was assessed directly from correlograms using GenAlEx (Peakall & Smouse 2006). The first intercept where the autocorrelation coefficient r falls from positive to negative is considered as the extent of a panmictic unit and thus becomes an estimate of small-scale gene flow (Sokal & Wartenberg 1983; Peakall *et al.* 2003).

Results

Genetic diversity, differentiation and gene flow among populations

A high level of polymorphism was present. The number of alleles of the eight microsatellite loci ranged from 4 to 17, with a mean of 10. Exclusive alleles existed in both subpopulations of each population, and postfragmentation subpopulations had the same number or more exclusive alleles than prefragmentation ones in all three populations (Table 1). The average fixation index over the eight loci differed significantly from Hardy–Weinberg expectations in all subpopulations (Table 1). Significant signs of null alleles were found in two loci (Ccu93H17 and Ccu102F36) with average frequencies of 17.6% and 18.6%, respectively. However, subsequent analyses showed the same patterns and conclusions irrespective of including the two loci or not, except for the fixation index that was not significant when excluding the two loci. Therefore, we present the results of all eight loci. All six subpopulations contained similar levels of genetic variation, with no significant difference in allelic richness (A_R), observed (H_o) and expected heterozygosity (H_e) (Table 1).

Values of all three indices (F'_{ST}, G'_{ST} and D_{est}) for postfragmentation subpopulations (0.109, 0.117 and 0.056) were similar to those for prefragmentation subpopulations (0.124, 0.137 and 0.070), and estimated gene flow among postfragmentation subpopulations (2.037) was slightly larger than that of prefragmentation ones (1.766) (see Table 2 for details).

After 20 repeated runs for K numbered from 1 to 10, ΔK reached a peak value at $K = 3$, indicating that the most likely number of clusters was three. After assignment tests using structure, each study site was dominated by a distinct cluster, which included only a few individuals from other sites (Fig. 2). The proportions of the dominant cluster in post- (64.5% and 63.9% in LS and XS, respectively) and prefragmentation (64.0% and 61.2% in LS and XS, respectively) subpopulations from the same locations were approximately at the same level except for the pair of subpopulations from the seriously fragmented area (HY), in which the proportion in the prefragmentation subpopulation (72.6%) was noticeably higher than that of the postfragmentation subpopulation (62.7%). This is indicative of an increase in migration rate after fragmentation.

Table 2 Genetic divergence (D_{est}) (upper triangle) and historic gene flow (Nm) (based on F'_{ST}) (lower triangle) in pairs of pre- and postfragmentation subpopulations from different habitats

	Prefragmentation subpopulations			Postfragmentation subpopulations		
	HY	LS	XS	HY	LS	XS
Prefragmentation subpopulations						
HY	–	0.0435	0.0812	–	0.0481	0.0684
LS	2.644	–	0.0442	0.0279	–	0.0439
XS	1.193	2.005	–	0.0677	0.0465	–
Postfragmentation subpopulations						
HY	–	4.172	1.472	–	0.0368	0.0546
LS	2.222	–	1.924	3.095	–	0.0444
XS	1.219	2.033	–	1.521	2.112	–

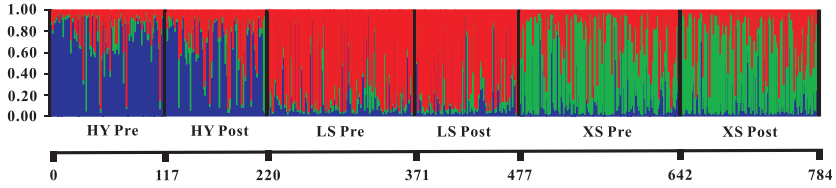

Fig. 2 Genetic structure of six subpopulations of *Castanopsis sclerophylla* as defined by STRUCTURE. Each thin vertical line represents a particular individual. The membership for each individual is showed by partitioning the corresponding line into different parts with different colours. Individuals were grouped by their original subpopulation. The ruler line shows the range of each subpopulation and cumulative sample size.

Based on a one-generation approach, selfing rates of pre- and postfragmentation subpopulations were not significantly different from 0 in the moderately fragmented and control habitats (Table 1). However, the selfing rate of the prefragmentation subpopulation of strongly fragmented population HY (0.129) was significantly larger than 0 ($P = 0.01$), whereas that of its postfragmentation subpopulation was not.

Fine-scale SGS and effective gene dispersal range

When the number of clusters was specified as unknown, results from five independent runs of GENELAND converged and inferred that only one genetic unit existed in each study site. Therefore, no evidence of genetic discontinuities was found within the three studied populations, which excludes any potential impact from historical events.

Except for the prefragmentation subpopulation on HY Island, significantly positive values of regression slope (*blog*) were found in all subpopulations (Table 3), showing significant SGS. Among the five subpopulations with significant SGS, significant positive autocorrelation existed in the first distance class (≤20 m), with the highest average value of F_{ij} (0.024) occurring in the postfragmentation HY subpopulation, and their autocorrelation coefficients declined rapidly and become nonsignificant or significantly negative in the second and third distance classes (Fig. 3). The F_{ij} values oscillated within 95% confidence intervals in the remaining distance classes except for the last distance class in the postfragmentation subpopulation of LS (Fig. 3d). In the prefragmentation subpopulation of HY, the kinship coefficient among neighbour trees within the first distance class was negative ($F_{ij} = -0.004$) but not significant, and this pattern persisted over all distance classes except for the sixth class, in which significantly positive autocorrelation was detected (Fig. 3a).

Statistic *Sp* evaluated from SPAGeDi showed differing intensities of SGS in all six subpopulations, with the lowest and the highest values found in HY pre- ($Sp = -0.0033$) and postfragmentation ($Sp = 0.0113$) subpopulations (Table 3). Note that the regression slope (*blog*) of the prefragmentation subpopulation HY was inconsistent with the results from other subpopulations, and not significant ($P = 0.98$).

Overall, correlograms of post- and prefragmentation subpopulations of population HY were significantly heterogeneous (Table 4, $P = 0.013$). Furthermore, a significant difference in the first distance class was only found between post- and prefragmentation subpopulations on HY Island ($P < 0.001$). Significant heterogeneity

Table 3 Small-scale SGS and gene flow within each of six subpopulations of *Castanopsis sclerophylla*

Habitat	Subpopulation	*Blog*	*Sp*	*Nb*	σ (m)	First intercept (m)
Seriously fragmented	HY-pre	0.0033	−0.0033	NC	NC	130.4
	HY-post	−0.0110*	0.0113	87.5	51.6	35.7
Moderately fragmented	LS-pre	−0.0086*	0.0087	113.9	69.4	38.7
	LS-post	−0.0080*	0.0081	236.5	84.6	44.9
Control	XS-pre	−0.0067*	0.0067	147.6	51.9	47.6
	XS-post	−0.0041*	0.0041	198.7	67.6	36.8

blog, slope of regression of kinship coefficients on ln(distance); *Sp*, statistic reflecting the intensity of SGS using *blog* and the mean kinship coefficient value in the first distance class; *Nb*, Wright's neighbourhood size; σ, estimate of effective gene dispersal distance inferred from *Nb*; and first intercept, the first intercept that autocorrelation coefficient *r* falls from positive to negative; SGS, spatial genetic structure.
NC, not converged; SGS, spatial genetic structure.
*$P < 0.05$.

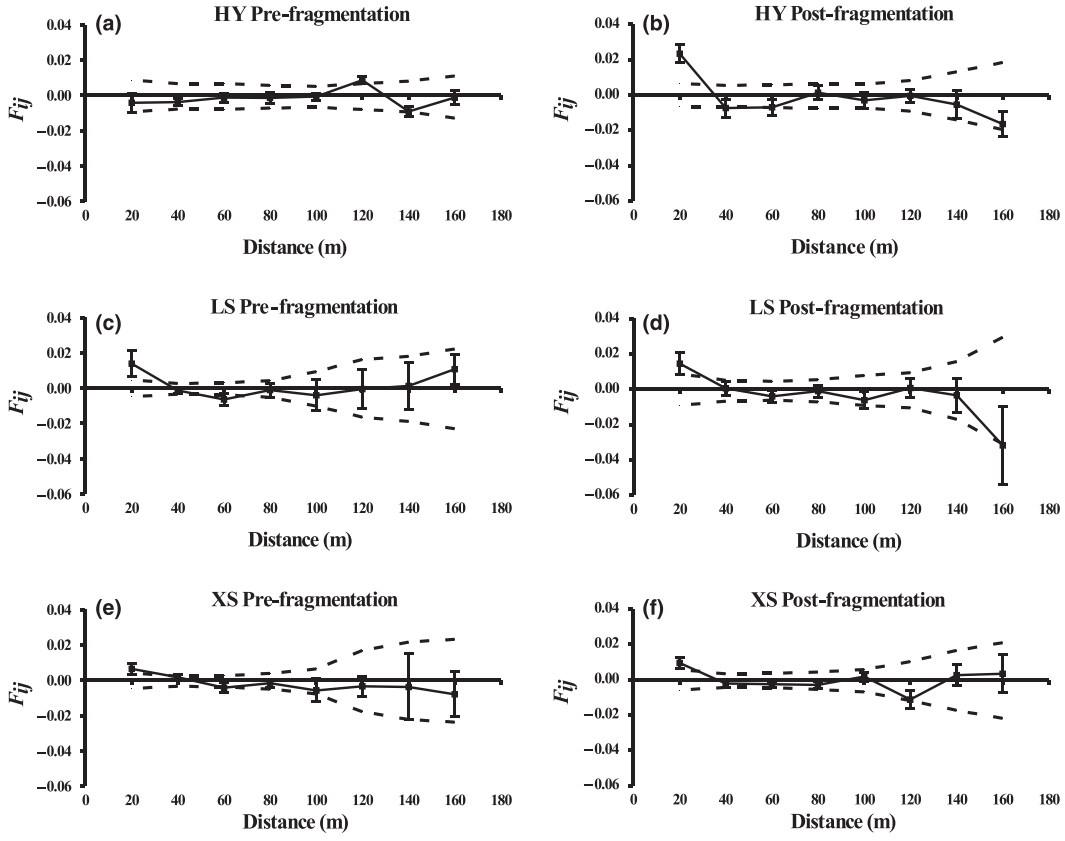

Fig. 3 Spatial autocorrelation analyses within the six subpopulations (a–f) using SPAGeDi. Dashed lines represent 95% confidence intervals under the null hypothesis that no autocorrelation exists from 9999 permutations, and error bars delineate standard errors from jackknife estimates.

also occurred between post- and prefragmentation subpopulations of population LS in the eighth distance class (140–160 m, $P = 0.038$). This departure from a homogeneous spatial pattern may result from stochastic errors, because only a few individual pairwise comparisons were involved in the farthest distance class (<80 individual pairs in each subpopulation).

After regression and iteration analyses, Wright's neighbourhood sizes (*Nb*) and effective gene dispersal distances (σ) converged in all subpopulations except for the prefragmentation subpopulation of population HY, where the initial regression slope (*blog* = 0.0033) was positive and not available for calculation of *Nb*. Estimates of *Nb* ranged from 87.5 to 236.5 individuals, with

Table 4 Heterogeneity tests of SGS between post- and prefragmentation subpopulations using GenAlEx. Statistics t^2 and ω represent the degree of differentiation of SGS between two pairwise subpopulations in each distance class and total, respectively

	Distance class (t^2)								
Pairs of subpopulations	1	2	3	4	5	6	7	8	Total (ω)
HY_{post} vs. HY_{pre}	**18.50***	0.57	1.14	0.28	0.27	1.56	0.13	1.32	**31.11***
LS_{post} vs. LS_{pre}	0.02	0.22	0.47	0	0.06	0.03	0.08	**4.24***	10.26
XS_{post} vs. XS_{pre}	0.82	1.67	0.15	0.08	2.13	0.26	0.13	0.40	13.26

*Values in bold are significant at $P < 0.05$.

the lowest value in postfragmentation subpopulation HY, and σ varied from 51.6 to 84.6 m (Table 3). The first intercept inferred from correlograms was shortest in postfragmentation subpopulation HY (35.7 m), which was only 27.4% of that in prefragmentation HY (130.4 m), while other pairs of subpopulations did not show obvious differentiation based on this index (Table 3).

Discussion

Impacts of fragmentation on genetic variation and selfing rates of Castanopsis sclerophylla

Habitat fragmentation can reduce the genetic diversity of populations, because of genetic drift and increased inbreeding (Young *et al.* 1996). However, we found similar levels of genetic diversity in strongly and moderately fragmented and control populations of *C. sclerophylla*, and postfragmentation subpopulations had high levels of genetic diversity that were comparable with prefragmentation subpopulations. High genetic diversities in fragmented plant populations have been reported frequently, particularly if fragmentation was recent or among species that are wind-pollinated or wind-dispersed (Lowe *et al.* 2005). The *C. sclerophylla* subpopulations are not very small and experienced fragmentation over 50 years ago (representing one to two generations), so drift had not had an opportunity to greatly influence genetic variation. More importantly, high rates of gene flow have apparently played a major role in maintaining genetic variation within the fragmented subpopulations, with higher estimated rates of gene flow among postfragmentation subpopulations than prefragmentation ones. This was indicated by lower differentiation among postfragmentation subpopulations, although this difference was not significant. Furthermore, exclusive alleles existed in all postfragmentation subpopulations, also indicating a strong gene flow from outside.

Castanopsis sclerophylla is a wind-pollinated and gravity plus rodent-dispersed tree. Water is a major barrier to the dispersal of its seeds and may block all movement of seeds among the three subpopulations. However, isolation distances of about 2 km clearly do not block the movement of pollen grains among the subpopulations. Although no direct data on pollen dispersal of *C. sclerophylla* is available, the long-distance dispersal of pollen grains has been observed in other Fagaceae species and other wind-pollinated species. Oak pollen grains can be wind-transported for distances exceeding hundreds of kilometres (Stanley & Linskens 1974). Given the inevitably dramatic decline in seed dispersal following the isolation of the subpopulations when the lake was created, the similar genetic differentiation among pre- and postfragmentation subpopulations suggests an increase in pollen dispersal following the raising of water levels. Such an increase in pollen dispersal is likely to be due to increased wind currents above the water once the intervening vegetation had been removed (Sork & Smouse 2006; Hanson *et al.* 2008).

Following habitat destruction, increased selfing rates are expected in response to any decrease in population densities and dispersal potential, a response that has been confirmed in several empirical studies (Doligez & Joly 1997; Aldrich & Hamrick 1998; Chen 2000a; Fuchs *et al.* 2003) and a recent meta-analysis (Aguilar *et al.* 2008). No obvious change (Hall *et al.* 1994; Dick *et al.* 2003) or decreased selfing rates (Mathiasen *et al.* 2007) have nonetheless been observed in fragmented populations of some plants. Fagaceae species are mostly wind-pollinated and outcrossing; though, some can self-pollinate (Fernández-M & Sork 2005). In *C. sclerophylla*, selfing rates of subpopulations from the moderately fragmented and control habitats were not significantly different from zero, irrespective of the age of the plants. In the strongly fragmented habitat, the selfing rate for the prefragmentation subpopulation was significantly larger than zero, possibly due to local limited pollen dispersal before fragmentation. However, the selfing rate for the postfragmentation subpopulation was not significantly larger than zero. Such a decrease in selfing rate is consistent with an increase in pollen dispersal among populations following fragmentation.

Changes in SGS between subpopulations in differentially fragmented habitats

Seed dispersal syndrome, rather than pollen flow, is the dominant factor determining fine-scale SGS, especially in outcrossing and wind-pollinated species (De-Lucas *et al.* 2009). In plants with effective long-distance seed dispersal, such as wind-dispersed trees, weak SGS is frequently observed, with no significant differences between continuous and fragmented populations (Williams *et al.* 2007; Born *et al.* 2008; Moreira *et al.* 2009). However, isolation effects on seed dispersal are more frequently detected, with stronger SGS recorded in fragmented populations (De-Lucas *et al.* 2009; Slavov *et al.* 2010; Sebbenn *et al.* 2011).

Our results revealed different SGS responses among subpopulations, despite the high genetic diversity within subpopulations and low differentiation among them that resulted from extensive pollen dispersal. There was little difference between pre- and postfragmentation subpopulations in the moderately fragmented and control habitats. By contrast, fine-scale SGS of *C. sclerophylla* (based on both *Sp* and heterogeneity tests) was significantly changed in the strongly fragmented subpopulation (island HY) following landscape alteration. The postfragmentation subpopulation on island HY had the smallest neighbourhood size and shortest effective gene dispersal distance, indicating a more limited gene dispersal range. Although we could not estimate neighbourhood size and effective gene dispersal distance for the prefragmentation subpopulation of population HY (because the iterative procedure did not converge), a lack of autocorrelation and negative *Sp* indicated that all the sampled individuals probably belonged to one panmictic unit: that is our sample scale was too small to detect the true extent of within-population gene flow (De-Lucas *et al.* 2009).

Given the extensive pollen flow, the intensified SGS and restricted dispersal inferred from SGS in the strongly fragmented habitat (HY) indicates a reduction in seed dispersal distances. As island HY is surrounded by water, it was unlikely that the heavy seeds of *C. sclerophylla* will often have arrived from other populations. Therefore, the postfragmentation subpopulation HY will have been recruited from local seeds, generating dispersal distances that were shorter than equivalent-aged subpopulations LS and XS.

The behaviour and abundance of seed dispersers can also change in fragmented habitats (Terborgh *et al.* 2001; Lopez & Terborgh 2007). Carnivorous mammals have disappeared from island HY, and this has resulted in rodents reaching unusually high densities (R. Wang, X-Y Chen and Yi-Su Shi, unpublished). This in turn has led to increased predation of *C. sclerophylla* seeds (all the monitored seeds on HY were predated whereas about 7% of seeds survived in the other two subpopulations) (R. Wang *et al.*, unpublished). Rare recruitment events on HY may be the products of seeds that were undetected by rodents and so lacked secondary dispersal. Such seeds are likely to be more concentrated near their maternal trees than seeds with additional secondary dispersal. Edge effects may also mean that seeds dispersed to the periphery of the forest may have reduced survivability, which may strengthen SGS among postfragmentation subpopulations (Laurance *et al.* 2002).

In combination with previous observations of high pollen-to-seed dispersal ratios in wind-pollinated and gravity plus rodent-dispersed species (Petit *et al.* 2005), our results show that the relative contributions of seed and pollen dispersal are scale-dependent. Pollen-to-seed dispersal distance ratios of Fagaceae species are among the highest recorded, mostly larger than 150 (Petit *et al.* 2005), and at a regional scale, the contribution of pollen dispersal to gene flow is much higher than that of seed dispersal. As spatial scale declines, the ratio becomes smaller, and at the scale of several kilometres, pollen-to-seed dispersal ratios in *Castanopsis fargesii* were found to be 1.1–2.2 (Chen *et al.* 2008). At a scale of <200 m, the role of seed dispersal might therefore be expected to be higher than that of pollen dispersal, and evidence for this is provided by the significant change in SGS in the strongly fragmented population.

Sensitive signs of genetic consequences of fragmentation

Fragmentation can lead to negative genetic consequences for remnant populations and contribute to declines in biodiversity. Identifying such negative impacts as early as possible is important for species conservation and management and efforts have been made to find the most sensitive signs of early genetic effects of fragmentation and to identify the factor(s) influencing them. Genetic diversity and differentiation are two frequently observed consequences of fragmentation. Analytical and numerical simulation studies on the temporal dynamics of genetic variation in subdivided populations has shown that genetic differentiation approaches equilibrium values much more rapidly than genetic diversity (Crow & Aoki 1984; Varvio *et al.* 1986). This pattern has been confirmed by empirical studies (Keyghobadi *et al.* 2005), which showed that loss of genetic diversity can be largely alleviated by even relatively low levels of gene flow between remnant populations (Lowe *et al.* 2005). However, it is clear that changes in genetic diversity and differentiation following habitat fragmentation take a number of

generations to become apparent and, as in *C. sclerophylla*, most studies that have examined genetic consequences of fragmentation in tropical trees have found no clear differences between fragmented and control populations. Clearly, neither genetic diversity nor differentiation is a sensitive indicator of the genetic consequences of fragmentation among forest trees.

Increased inbreeding and higher selfing rates within remnant populations are deleterious outcomes of habitat fragmentation that can be observed very rapidly, in the first subsequent generation (Lowe *et al.* 2005). However, canopy disruption can increase wind currents and facilitate wind-borne pollen movement or the movement of pollinators, and meta-analysis has indicated that fragmentation typically has nonsignificant overall effects on inbreeding coefficients, because of persistent long-distance pollen dispersal (Aguilar *et al.* 2008). This limits the value of inbreeding coefficients and selfing rates as indicators of genetic perturbations following fragmentation.

Fine-scale SGS of a plant population is mainly determined by gene flow via pollen and seeds. Except for a few plants with wind-dispersed seeds (Bacles *et al.* 2006), most have more restricted seed dispersal than pollen dispersal. Fine-scale SGS is therefore expected to be particularly sensitive to restricted seed dispersal, a common feature in fragmented habitats. In the case of *C. sclerophylla*, a strengthened SGS was observed in a fragmented habitat within a single generation (Fig. 3), without any decrease in genetic diversity or increases in differentiation and inbreeding or selfing rates. Fine-scale SGS is therefore sensitive to fragmentation, even in a long-lived tree with extensive pollen dispersal among populations (De-Lucas *et al.* 2009) and can be used as a sensitive method to examine genetic consequences of fragmentation on plants that provides an early warning of the need for conservation action.

Conclusions

Our results confirmed previous observations of a relatively weak negative effect of fragmentation on genetic diversity among long-lived, wind-pollinated trees. Because of extensive pollen dispersal, no significant change in differentiation was found among postfragmentation subpopulations compared to prefragmentation ones. Selfing rates decreased significantly in the postfragmentation subpopulation in the most seriously fragmented habitat, suggesting that fragmentation had increased wind-borne pollen dispersal. However, fine-scale SGS in the postfragmentation subpopulation from the most seriously fragmented habitat was significantly strengthened, despite its large population size ($N = \sim 350$), suggesting that restricted seed dispersal played a more important role in small-scale genetic variation arrangement. Our results showed that fine-scale SGS is sensitive to fragmentation and can be useful as an early indicator of negative consequences of fragmentation in plant populations. In populations displaying a significant change in SGS, some countermeasures to remove dispersal barriers or to increase dispersal should be undertaken to maintain seed dispersal that is comparable with that in continuous habitats, so as to avoid subsequent biparental inbreeding and drift.

Acknowledgements

We thank Gao-Fu Xu for providing kindly support during the field surveys; Bin Liu for field work; Min-Yan Cui, Min Liu, Yi-Su Shi, Xin Tong and Na-Na Xu for help in the experiments; and Li Shen for help in digitizing the sampling map. This work was supported by the National Natural Science Foundation of China (30970430), the Fundamental Research Funds for the Central Universities (78220028) and '211 Projects' of ECNU.

References

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Molecular Ecology*, **17**, 5177–5188.

Ahmed S, Compton SG, Butlin RK, Gilmartin PM (2009) Wind-borne insects mediate directional pollen transfer between desert fig trees 160 kilometers apart. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 20342–20347.

Aldrich PR, Hamrick JL (1998) Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. *Science*, **281**, 103–105.

Bacles CFE, Lowe AJ, Ennos RA (2006) Effective seed dispersal across a fragmented landscape. *Science*, **311**, 628.

Born C, Hardy OJ, Chevallier M-H *et al.* (2008) Small-scale spatial genetic structure in the Central African rainforest tree species *Aucoumea klaineana*: a stepwise approach to infer the impact of limited gene dispersal, population history and habitat fragmentation. *Molecular Ecology*, **17**, 2041–2050.

Chen X-Y (2000a) Effect of plant density and age on the mating system of *Kandelia candel* (L.) Druce (Rhizophoraceae), a viviparous mangrove species. *Hydrobiologia*, **432**, 189–193.

Chen X-Y (2000b) Effects of habitat fragmentation on genetic structure of plant populations and implications for the biodiversity conservation. *Acta Ecologica Sinica*, **20**, 884–892.

Chen X-Y, Fan X-X, Hu X-S (2008) Roles of seed and pollen dispersal in natural regeneration of *Castanopsis fargesii* (Fagaceae): implications for forest management. *Forest Ecology and Management*, **256**, 1143–1150.

Coulon A, Guillot G, Cosson JF *et al.* (2006) Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. *Molecular Ecology*, **15**, 1669–1679.

Crawford NG (2010) SMOGD: software for the measurement of genetic diversity. *Molecular Ecology Resources*, **10**, 556–557.

Crow JF, Aoki K (1984) Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. *Proceedings of the National Academy of Sciences of the United States of America*, **81**, 6073–6077.

David P, Pujol B, Viard F, Castella V, Goudet J (2007) Reliable selfing rate estimates from imperfect population genetic data. *Molecular Ecology*, **16**, 2474–2487.

De-Lucas AI, González-Martínez SC, Vendramin GG, Hidalgo E, Heuertz M (2009) Spatial genetic structure in continuous and fragmented populations of *Pinus pinaster* Aiton. *Molecular Ecology*, **18**, 4564–4576.

Dick CW, Etchelecu G, Austerlitz F (2003) Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology*, **12**, 753–764.

Doligez A, Joly HI (1997) Mating system of *Carapa procera* (Meliaceae) in the French guiana tropical forest. *American Journal of Botany*, **84**, 461–470.

Doligez A, Baril C, Joly HI (1998) Fine-scale spatial genetic structure with nonuniform distribution of individuals. *Genetics*, **148**, 905–920.

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theoretical and Applied Genetics*, **92**, 832–839.

Epperson BK, Li T-Q (1996) Measurement of genetic structure within populations using Moran's spatial autocorrelation statistics. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 10528–10532.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Fenster CB, Vekemans X, Hardy OJ (2003) Quantifying gene flow from spatial genetic structure data in a metapopulation of *Chamaecrista fasciculata* (Leguminosae). *Evolution*, **57**, 995–1007.

Fernández-M JF, Sork VL (2005) Mating patterns of a subdivided population of the Andean oak (*Quercus humboldtii* Bonpl., Fagaceae). *Journal of Heredity*, **96**, 635–643.

Fuchs EJ, Lobo JA, Quesada M (2003) Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree *Pachira quinata*. *Conservation Biology*, **17**, 149–157.

Gonzales E, Hamrick JL, Smouse PE, Trapnell DW, Peakall R (2010) The impact of landscape disturbance on spatial genetic structure in the Guanacaste tree, *Enterolobium cyclocarpum* (Fabaceae). *Journal of Heredity*, **101**, 133–143.

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity*, **86**, 485–486.

Guillot G, Estoup A, Mortier F, Cosson JF (2005a) A spatial statistical model for landscape genetics. *Genetics*, **170**, 1261–1280.

Guillot G, Mortier F, Estoup A (2005b) GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes*, **5**, 712–715.

Guillot G, Santos F, Estoup A (2008) Analysing georeferenced population genetics data with Geneland: a new algorithm to deal with null alleles and a friendly graphical user interface. *Bioinformatics*, **24**, 1406–1407.

Hall P, Orrell LC, Bawa KS (1994) Genetic diversity and mating system in a tropical tree, *Carapa guianensis* (Meliaceae). *American Journal of Botany*, **81**, 1104–1111.

Hamrick JL (2004) Response of forest trees to global environmental changes. *Forest Ecology and Management*, **197**, 323–335.

Hanson TR, Brunsfeld SJ, Finegan B, Waits LP (2008) Pollen dispersal and genetic structure of the tropical tree *Dipteryx panamensis* in a fragmented Costa Rican landscape. *Molecular Ecology*, **17**, 2060–2073.

Hardy OJ, Vekemans X (1999) Isolation by distance in a continuous population: reconciliation between spatial autocorrelation analysis and population genetics models. *Heredity*, **83**, 145–154.

Hardy OJ, Vekemans X (2002) SPAGEDI: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.

Hardy OJ, Maggia L, Bandou E *et al.* (2006) Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. *Molecular Ecology*, **15**, 559–571.

Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution*, **59**, 1633–1638.

Heuertz M, Vekemans X, Hausman J-F, Palada M, Hardy OJ (2003) Estimating seed vs. pollen dispersal from spatial genetic structure in the common ash. *Molecular Ecology*, **12**, 2483–2495.

Hu X-S, He F (2006) Seed and pollen flow in expanding a species' range. *Journal of Theoretical Biology*, **240**, 662–672.

Jost L (2008) G_{ST} and its relatives do not measure differentiation. *Molecular Ecology*, **17**, 4015–4026.

Jump AS, Peñuelas J (2006) Genetic effects of chronic habitat fragmentation in a wind-pollinated tree. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 8096–8100.

Keyghobadi N, Roland J, Matter SF, Strobeck C (2005) Among- and within-patch components of genetic diversity respond at different rates to habitat fragmentation: an empirical demonstration. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 553–560.

Laurance WF, Lovejoy TE, Vasconcelos HL *et al.* (2002) Ecosystem decay of Amazonian forest fragments: a 22-year investigation. *Conservation Biology*, **16**, 605–618.

Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany*, **82**, 1420–1425.

Lopez L, Terborgh J (2007) Seed predation and seedling herbivory as factors in tree recruitment failure on predator-free forested islands. *Journal of Tropical Ecology*, **23**, 129–137.

Lowe AJ, Boshier D, Ward M, Bacles CFE, Navarro C (2005) Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. *Heredity*, **95**, 255–273.

Mathiasen P, Rovere AE, Premoli AC (2007) Genetic structure and early effects of inbreeding in fragmented temperate forests of a self-incompatible tree, *Embothrium Coccineum*. *Conservation Biology*, **21**, 232–240.

Meirmans PG (2006) Using the AMOVA framework to estimate a standardized genetic differentiation measure. *Evolution*, **60**, 2399–2402.

Miller MP (1997) *Tools for Population Genetic Analyses (TFPGA) v1.3: A Windows Program for the Analysis of Allozyme and Molecular Genetic Data*. Department of Biological Sciences, Northern Arizona University, Flagstaff.

Moreira PA, Fernandes GW, Collevatti RG (2009) Fragmentation and spatial genetic structure in *Tabeluia*

ochracea (Bignoniaceae) a seasonally dry Neotropical tree. *Forest Ecology and Management*, **258**, 2690–2695.
Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.
Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.
Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution*, **57**, 1182–1195.
Petit RJ, Duminil J, Fineschi S *et al.* (2005) Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Molecular Ecology*, **14**, 689–701.
Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
Robledo-Arnuncio JJ, Gil L (2005) Patterns of pollen dispersal in a small population of *Pinus sylvestris* L. revealed by total-exclusion paternity analysis. *Heredity*, **94**, 13–22.
Sebbenn AM, Carvalho ACM, Freitas MLM *et al.* (2011) Low levels of realized seed and pollen gene flow and strong spatial genetic structure in a small, isolated and fragmented population of the tropical tree *Copaifera langsdorffii* Desf. *Heredity*, **106**, 134–145.
Shapcott A, Bau B, Powell M (2009) Fragmentation and genetic diversity in *Romnalda* (Dasypogonaceae), a rare rain forest herbaceous genus from New Guinea and Australia. *Biotropica*, **41**, 128–136.
Shi M-M (2008) *A Comparative Study of Genetic Structure Between the Central and Peripheral Populations of Castanopsis sclerophylla*. Master Thesis, East China Normal University.
Slatkin M, Barton NH (1989) A comparison of three indirect methods for estimating average levels of gene flow. *Evolution*, **43**, 1349–1368.
Slavov GT, Leonardi S, Adams WT, Strauss SH, DiFazio SP (2010) Population substructure in continuous and fragmented stands of *Populus trichocarpa*. *Heredity*, **105**, 348–357.
Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity*, **82**, 561–573.
Smouse PE, Peakall R, Gonzales E (2008) A heterogeneity test for fine-scale genetic structure. *Molecular Ecology*, **17**, 3389–3400.
Sokal RR, Wartenberg DE (1983) A test of spatial autocorrelation analysis using an isolation-by-distance model. *Genetics*, **105**, 219–237.
Sork VL, Smouse PE (2006) Genetic analysis of landscape connectivity in tree populations. *Landscape Ecology*, **21**, 821–836.
Stanley R, Linskens H (1974) *Pollen: Biology, Biochemistry and Management*. Springer, Berlin.
Terborgh J, Lopez L, Nunez P *et al.* (2001) Ecological meltdown in predator-free forest fragments. *Science*, **294**, 1923–1926.
Uneo S, Yoshimaru H, Kawahara T, Yamamoto S (2000) Isolation of microsatellite markers in *Castanopsis cuspidate* var. *sieboldii* Nakai from an enriched library. *Molecular Ecology*, **9**, 1188–1190.
Uneo S, Yoshimaru H, Kawahara T, Yamamoto S (2003) A further six microsatellite markers for *Castanopsis cuspidate* var. *sieboldii* Nakai. *Conservation Genetics*, **4**, 813–815.
Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.
Varvio SL, Chakraborty R, Nei M (1986) Genetic variation in subdivided populations and conservation genetics. *Heredity*, **57**, 189–198.
Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, **13**, 921–935.
Wang R, Ai B, Gao BQ *et al.* (2009) Spatial genetic structure and restricted gene flow in a functionally dioecious fig, *Ficus pumila* L. *pumila* (Moraceae). *Population Ecology*, **51**, 307–315.
Williams DA, Wang Y, Borchetta M, Gaines MS (2007) Genetic diversity and spatial structure of a keystone species in fragmented pine rockland habitat. *Biological Conservation*, **138**, 256–268.
Wunderle JMJ (1997) The role of animal seed dispersal in accelerating native forest regeneration on degraded tropical lands. *Forest Ecology and Management*, **99**, 223–235.
Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology & Evolution*, **11**, 413–418.
Zhang X (2006) *Maintenance mechanism and ecological restoration of* Castanopsis sclerophylla *populations on Islands in Qiandao Lake Region*. PhD Dissertation, East China Normal University.
Zhao A-L, Chen X-Y, Zhang X, Zhang D (2006) Effects of fragmentation of evergreen broad-leaved forests on genetic diversity of *Ardisia crenata* var. *bicolor* (Myrsinaceae). *Biodiversity and Conservation*, **15**, 1339–1351.

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Data accessibility

Sampling locations and microsatellite data for the three studied sites: Dryad entry doi:10.5061/dryad.500dm.