

Limited genetic impacts of habitat fragmentation in an “old rare” relict tree, *Euptelea pleiospermum* (Eupteleaceae)

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Abstract *Euptelea pleiospermum* is an “old rare” tree species distributed along the high-elevation streamsides in Burma, China, and India. Deforestation and construction of roads for timber transport have highly fragmented the natural habitats of this species in the Shennongjia Forestry District. In this study, we used this fragmentation to test the hypothesis that “old rare” tree species are insusceptible to the genetic consequences of habitat fragmentation. Using eight microsatellite loci, we estimated allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), Wright’s inbreeding coefficient (F_{IS}), and genetic differentiation (F_{ST} and D_{EST}) between pre- and post-fragmentation cohorts. We found no significant differences in either genetic diversity or genetic differentiation between the two cohorts. The limited genetic effects of fragmentation may result from too few fragmented generations, because the time between the start of fragmentation (year 1970) and our study (year 2008) was less than one generation of this tree species. It should be mentioned that clonal reproduction by sprouting, a common phenomenon in many “old rare” tree species, can help *E. pleiospermum* buffer the genetic impacts of fragmentation by delaying the time between generations. Therefore, we

conclude that this “old rare” tree species show limited genetic impacts from recent habitat fragmentation. However, the elimination of rare alleles and increase of inbreeding coefficient in the post-fragmentation cohort are early warnings of deleterious genetic consequences of fragmentation. Our results provide valuable information to formulate conservation and restoration guidelines for *E. pleiospermum*.

Keywords *Euptelea pleiospermum* · Habitat fragmentation · Genetic diversity · Population structure · Old rare species · Nuclear microsatellite

Introduction

The genetic consequences of habitat fragmentation in plant populations have been intensively researched and controversial for over two decades (Young et al. 1996; Kramer et al. 2008). It has been reported that habitat fragmentation decreases the genetic diversity of plant populations (Aguilar et al. 2008; DiBattista 2008), whereas this is not always the case for long-live tree species (Kramer et al. 2008; Bacles and Jump 2011). Despite the lack of a clear response pattern in the literature (Aguilar et al. 2008), it is certain that genetic erosion can reduce individual fitness and population viability in the short term and can increase species extinction risk in the long term (Booy et al. 2000; Reed and Frankham 2003; Peterson et al. 2008). Therefore, knowledge about whether human disturbances have

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negative genetic impacts on rare tree species will benefit their future management and conservation.

Two recent reviews suggested that naturally rare species are less susceptible than common species to habitat fragmentation (Honnay and Jacquemyn 2007; Aguilar et al. 2008). Indeed, a large number of studies have reported that rare plant species on remnant patches still hold high levels of genetic diversity (Kang et al. 2005; Torres-Díaz et al. 2007; Yao et al. 2007; Šmídová et al. 2011). However, high levels of genetic diversity do not mean that no genetic erosion and/or increased genetic divergence among populations have occurred since fragmentation. Many of these studies were only concerned with genetic diversity and structure of adult cohorts, which were determined by historical rather than recent fragmentation processes (Collevatti et al. 2001; Torres-Díaz et al. 2007). Therefore, investigation of temporal population genetic changes would increase accuracy of information (Aldrich et al. 1998; Aguilar et al. 2008).

One promising approach for detecting genetic effects of fragmentation is to compare the genetic diversity and structure of pre-fragmentation old cohorts with post-fragmentation young cohorts (Kettle et al. 2007). This method has been extensively used in common timber species (Aldrich et al. 1998; Dayanandan et al. 1999; Jump and Peñuelas 2006; Fernández-M and Sork 2007; André et al. 2008; Farwig et al. 2008). However, few studies have adopted this approach in naturally rare tree species recently isolated by habitat fragmentation, and their findings suggest reduced genetic diversity and elevated levels of inbreeding in young cohorts (Kettle et al. 2007). This is not consistent with conclusion of the mentioned review (Honnay and Jacquemyn 2007). Therefore, whether naturally rare tree species are less sensitive to recent habitat fragmentation needs further evidence.

South-central China has an impressive diversity of endemic flora (Myers et al. 2000), among which many are naturally rare Tertiary-relict tree species (López-Pujo et al. 2011). Naturally rare species or “old rare” species are defined by endemics and relicts with narrow geographical range, restricted habitat specificity or small local population size (Aguilar et al. 2008; Šmídová et al. 2011). In this area, extensive deforestation occurred during the late 1950s to early 1990s (Zhang et al. 2000; López-Pujo

et al. 2006). We took advantage of this to test the genetic effects of recent human disturbance in “old rare” tree species and the results are of importance in formulating reasonable conservation plans. As it is impossible to study all “old rare” tree species, an alternative is to use surrogate species which can represent this group.

In this study, we chose *Euptelea pleiospermum* as representative species. Deforestation during 1970s to early 1980s (Bartholomew et al. 1983; Ying et al. 1999) had seriously fragmented the distribution of this plant in the study area. Although we have reported that there was no significant differences in genetic diversity of *E. pleiospermum* between natural and disturbed habitats (Wei and Jiang 2012), we have also found that its recruitment is poorer in disturbed habitats as compared to natural ones (Wei et al. 2008). Therefore, it is necessary to compare population genetic variation and structure between young and old cohorts of *E. pleiospermum* in the Shennongjia Mountains, central China. Our specific objective was to detect whether this “old rare” tree species is experiencing genetic erosion and increased genetic divergence in recently fragmented habitat.

Materials and methods

Study species

Euptelea pleiospermum Hook. f. et Thoms (Eupteleaceae) is a diploid ($2n = 28$, Pan et al. 1991), deciduous, broad-leaved tree species native to China, India, and Burma. Populations of *E. pleiospermum* are usually scattered in river valleys of remote mountainous areas (ca. 720–3600 m a.s.l., Fu and Jin 1992). The relict tree grows up to 12 m in height and 20 cm in diameter. The tree produces wind-pollinated flowers and abundant light samaras, which are dispersed by wind and/or water. Seed vigor decreases rapidly after more than one year (Wei et al. 2010a), indicating that this species can not form persistent soil seed bank.

This Tertiary-relict tree species was classed as rare species in the China Plant Red Data Book (Fu and Jin 1992). Habitat loss and fragmentation have reduced the size of many populations and increased the spatial isolation almost across its distribution range (Fu and Jin 1992).

Study sites and sampling collection

Field research was performed in the Shennongjia Forestry District (31°21'20"–31°36'20" N, 110°03'05"–110°33'50" E), central China (Fig. 1). This area is an important part of south-central China biodiversity hot-spot and is rich in Tertiary-relict and endemic plants (López-Pujó et al. 2006, 2011). In this area, *E. pleiospermum* is unevenly scattered in the riparian forests at elevation between ~1200 and 1900 m (Wei et al. 2010b).

During 1970 to 1983, the Shennongjia Forestry District was the biggest timber source in central China. Deforestation and construction of roads for lumber transport destroyed and/or fragmented much of the natural habitat (Bartholomew et al. 1983; Ying et al. 1999).

Before sampling, we established two functions for *E. pleiospermum* (He and Jiang, unpublished data) to estimate ages of individuals. First, ages of individuals with DBH of >2.5 cm were estimated by a power function correlating age and diameter at breast height (DBH) ($y = 3.3087x^{0.8832}$, $R^2 = 0.831$, $P < 0.001$, $n = 107$) developed for *E. pleiospermum* in the Shennongjia Mountains. Second, ages of individuals with DBH of ≤ 2.5 cm were evaluated through a linear equation correlating age and ground diameter ($y = 3.0173x - 0.6028$, $R^2 = 0.932$, $P < 0.001$, $n = 30$).

Since extensive deforestation was started in 1970 and we sampled in 2008, individuals older than 40 years (DBH >17 cm) can be clustered into a pre-fragmentation cohort. To avoid overlapping of pre- and post-fragmentation cohorts, individuals established in the last 10 years (DBH or ground diameter <3.5 cm) were clustered into post-fragmentation cohort.

Sampling was carried out between April and May in 2008. We chose five population fragments (A–E) covering most of the disturbed forests associated with *E. pleiospermum* in the Shennongjia Forestry District (Fig. 1). According to the criteria established above, young leaves were collected randomly from suitable individuals (at least 30 m apart) in each population and immediately stored in silica gel. A total of 120 young and 134 old individuals were sampled from the five fragments (Table 1). All samples were stored at 4 °C pending DNA extraction in laboratory.

Microsatellite analysis

Total DNA was extracted from ~0.5 g dried leaves using a modified CTAB method (Doyle and Doyle 1987) and dissolved in 1× TE buffer. The DNA concentration was diluted to 5–10 ng μL^{-1} with ultra-pure distilled water.

Fourteen published primers (Zhang et al. 2008) were tested using 30 individuals (randomly chosen

Fig. 1 Location of the five populations of *Euptelea pleiospermum* (filled circle) sampled in the Shennongjia Mountains (filled square), central China. A Pingqian, B Wenshuihe, C Yazikou, D Yangjuanhe, and E Guanmenshan

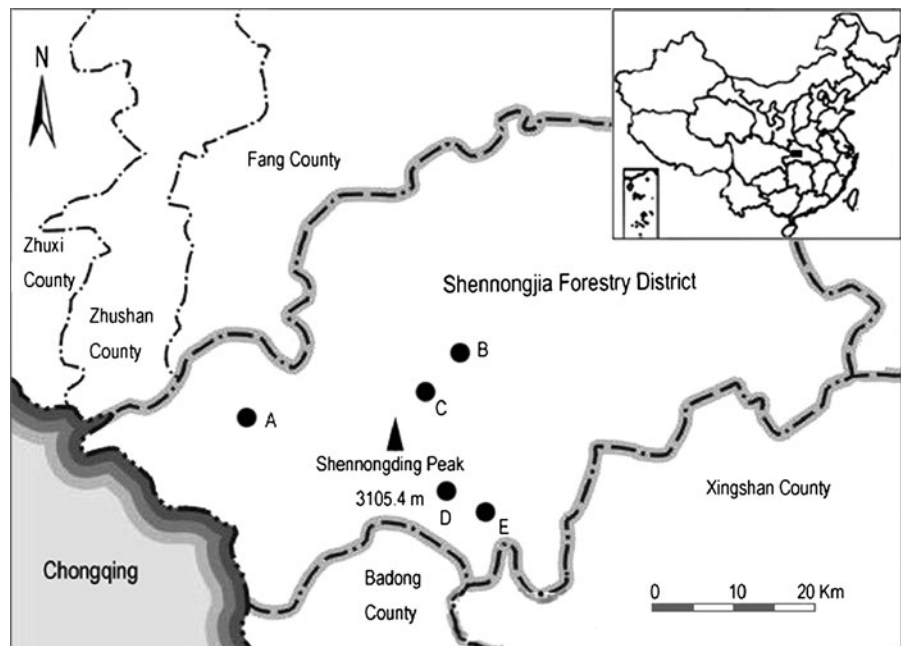


Table 1 Estimates of within-population genetic variation (Standard errors are shown in parentheses) and mutation-drift test (Under two phases model, TPM) for young and old cohortsin the five populations of *Euptelea pleiospermum* in the Shennongjia Forestry District, central China

Population	N	A_R	H_E	H_O	F_{IS}	Mutation-drift test
Young cohort						
A	28	5.81 (0.77)	0.605 (0.234)	0.429 (0.171)	0.304 (0.086)*	0.039*
B	20	6.13 (0.81)	0.662 (0.184)	0.525 (0.185)	0.226 (0.081)*	0.383
C	28	5.86 (0.82)	0.668 (0.173)	0.509 (0.080)	0.252 (0.053)*	0.461
D	17	5.25 (0.65)	0.627 (0.185)	0.544 (0.193)	0.157 (0.068)	0.039*
E	27	6.60 (0.77)	0.678 (0.187)	0.491 (0.169)	0.290 (0.084)*	0.074
	120	5.93 (0.22)	0.640 (0.014)	0.500 (0.020)	0.246 (0.026)	
Old cohort						
A	22	5.13 (0.77)	0.542 (0.276)	0.432 (0.251)	0.220 (0.079)*	0.074
B	25	6.22 (0.83)	0.681 (0.163)	0.540 (0.115)	0.223 (0.076)*	0.742
C	19	5.91 (0.75)	0.672 (0.184)	0.612 (0.176)	0.113 (0.102)	0.742
D	34	5.50 (0.80)	0.628 (0.174)	0.496 (0.154)	0.221 (0.084)*	0.742
E	34	6.57 (0.86)	0.664 (0.186)	0.529 (0.142)	0.214 (0.065)*	0.383
	134	5.87 (0.25)	0.637 (0.025)	0.522 (0.029)	0.198 (0.021)	

Parameters: N number of individuals sampled, A_R allelic richness, H_E expected heterozygosity, H_O observed heterozygosity, F_{IS} Wright's inbreeding coefficient

* Significance after Bonferroni correction for F_{IS} or $P < 0.05$ for mutation-drift test

from collected samples) and eight nuclear microsatellite loci (EP021, EP036, EP059, EP081, EP087, EP091, EP278, and EP294) that detected a suitable level of polymorphism were identified to determine genotypes of all samples.

PCR amplifications were carried out in a total volume of 10 μ L consisting of 10–50 ng of template DNA, 1 μ L of 10 \times reaction buffers, 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.25 μ M of each primer, and 0.5 U of *Taq* polymerase. PCR reactions were performed with the following cycle profile: an initial denaturation at 94 $^{\circ}C$ for 5 min; then 35 cycles of denaturation at 94 $^{\circ}C$ for 50 s, annealing at 56 $^{\circ}C$ for 50 s and extension at 72 $^{\circ}C$ for 1 min; and final extension at 72 $^{\circ}C$ for 10 min. After amplification, identification of the alleles was based on the position of the fragments in relation to a 25-bp marker ladder on a polyacrylamide gel of high resolution with silver stain.

Data analysis

The genetic diversity within-population was calculated for both pre- and post-fragmentation cohorts. Observed (H_O) and expected (H_E) heterozygosity, allele frequency, and Wright's inbreeding coefficient

(F_{IS}) were estimated by using GenAEx 6.41 (Peakall and Smouse 2006). Allelic richness (A_R) was calculated using FSTAT 2.9.3.2 (Goudet 2001). Departure from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were tested using FSTAT 2.9.3.2 (Goudet 2001). Sequential Bonferroni adjustments were used to determine significance levels for all tests with an initial α level of 0.05 (Rice 1989).

Statistical tests were executed in SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). The differences in genetic measures (A_R , H_O , H_E , and F_{IS}) between the two cohorts were tested for each parameter averaged over populations by performing pairwise nonparametric Wilcoxon matched pairs test (Sokal and Rohlf 2000). The difference in the number of rare alleles present in the two cohorts was tested by using a χ^2 contingency table test. To determine the impacts of sampling size on genetic diversity, the correlation between genetic diversity parameter (e.g., A_R and H_E) and sampling size was explored by regression analysis for both cohorts.

To detect the patterns of genetic differentiation between populations at different life stages, pairwise F_{ST} (Weir and Cockerham 1984) and D_{EST} (Jost 2008) values between populations were computed separately

for both cohorts. The two parameters were also calculated between the two cohorts at every sampling site. Jost's D_{EST} , an estimator of actual differentiation, is calculated based on allele identities rather than ratios of heterozygosity (Jost 2008). The overall value of D_{EST} for each pairwise comparison between populations was estimated as the arithmetic mean across loci (Crawford 2010). We used FSTAT 2.9.3.2 (Goudet 2001) and SMOGD 1.2.5 (Crawford 2010) to compute F_{ST} and D_{EST} , respectively. Significance tests for F_{ST} were based on 1,000 randomizations.

Pairwise nonparametric Wilcoxon matched pairs test was also used to test for differences in between-population pairwise F_{ST} and D_{EST} values between the two cohorts, respectively.

The program BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) was used to test for deviation from mutation-drift equilibrium, which is expected if populations have experienced a recent reduction in effective population size. Wilcoxon test is the most powerful and robust of the three available statistical tests when used with few polymorphic loci (Piry et al. 1999). Most microsatellite data sets better fit the two phase models (TPM) than the infinite allele model (IAM) or the stepwise mutation model (SMM) (Di Rienzo et al. 1994). Therefore, we applied Wilcoxon test to determine the significance under the TPM; 30 % of the mutations were assumed to occur under the IAM and 70 % assumed to occur under the SMM (Cornuet and Luikart 1996). The estimation was based on 1,000 randomizations.

To analyze the level and partitioning of genetic variation within and among populations, analysis of molecular variance (AMOVA) was performed for both cohorts with 1,000 permutations in ARLEQUIN 3.1 (Excoffier et al. 2005).

Results

No locus displayed consistent departures from HWE across all populations, with seven (or 17.5 %) and nine (or 22.5 %) of 40 locus–population combinations significant after corrections for multiple comparisons in pre- and post-fragmentation cohorts, respectively. Significant linkage disequilibrium between loci across all populations was detected for two of 140 comparisons after corrections for multiple comparisons in both cohorts.

A total of 84 alleles were detected in over all samples, of which 46 were rare alleles (those with a frequency of ≤ 0.05). The rare alleles were less likely to occur in the post-fragmentation cohort: 16 were absent from the young cohort, whereas only six were absent from the old cohort ($\chi^2 = 5.97$, $df = 1$, $P = 0.015$).

Moderately high levels of genetic diversity were consistently observed for both the pre- and post-fragmentation cohorts in all five populations (Table 1). All populations showed significantly ($P < 0.05$) positive F_{IS} value for both cohorts except young cohort of population D and old cohort of population C ($P > 0.001$ after Bonferroni correction) (Table 1). Relatively high F_{IS} values for both cohorts in most populations revealed a significant deficit of heterozygosity compared to Hardy–Weinberg expectations.

None of the four genetic diversity indices (A_R , H_O , H_E , and F_{IS}) demonstrated significant differences ($Z = -1.776$ to -0.104 , $P > 0.05$ for all) between the two cohorts. However, the young cohort showed a marginally higher value of F_{IS} compared to the old cohort ($Z = -1.776$, $P = 0.076$). There was no positive relationship between genetic diversity and sampling size ($R^2 = 0.020$ – 0.264 , $P > 0.05$ for all).

Pairwise F_{ST} and D_{EST} values between populations for each size cohort and between cohorts at each sampling site are shown in Table 2. The two cohorts showed similar values of F_{ST} (young = 0.037 ± 0.009 ; old = 0.038 ± 0.010 ; mean \pm SE), whereas values of D_{EST} (young = 0.054 ± 0.015 ; old = 0.039 ± 0.009) were higher in the recently established individuals. Neither parameter was significantly different between pre- and post-fragmentation cohorts (F_{ST} , $Z = -0.076$, $P = 0.940$; D_{EST} , $Z = -0.832$, $P = 0.406$). All pairwise F_{ST} values between cohorts were not significantly different from zero (Table 2).

Recent bottlenecks were detected in two of the five populations for the young cohort, whereas none of the populations for the old cohort showed significant deviation from mutation-drift equilibrium (Table 1).

The results of the AMOVA showed that most of the genetic variation was found within populations for both cohorts (Table 3).

Discussion

Our results showed that *E. pleiospermum* populations in disturbed forest on the Shennongjia Mountain still

Table 2 Pairwise F_{ST} (below diagonal) and D_{EST} (above diagonal) values between populations for young and old cohorts and between cohorts at the five sampling sites for *Euptelea pleiospermum*

	YA	YB	YC	YD	YE	OA	OB	OC	OD	OE
YA	–	0.079	0.101	0.142	0.102	0.032	–	–	–	–
YB	0.046*	–	0.005	0.044	0.013	–	0.020	–	–	–
YC	0.064*	0.001	–	0.020	0.011	–	–	0.008	–	–
YD	0.092*	0.033*	0.017*	–	0.034	–	–	–	0.013	–
YE	0.058*	0.011	0.011	0.042*	–	–	–	–	–	0.007
OA	0.041	–	–	–	–	–	0.066	0.074	0.056	0.076
OB	–	0.008	–	–	–	0.074*	–	0.001	0.019	0.010
OC	–	–	0.003	–	–	0.065*	–0.001	–	0.052	0.000
OD	–	–	–	0.010	–	0.074*	0.022*	0.035*	–	0.036
OE	–	–	–	–	0.005	0.072*	0.005	–0.004	0.041*	–

YA–YE represent young cohort at population fragment A–E, respectively; OA–OE represent old cohort at population fragment A–E, respectively

* Significance for F_{ST} ($P < 0.05$)

Table 3 Analysis of molecular variance (AMOVA) for young and old cohorts in the five populations of *Euptelea pleiospermum*

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Young cohort				
Among populations	4	33.457	0.120	4.34***
Within populations	235	622.818	2.650	95.66
Total	239	656.275	2.770	
Old cohort				
Among populations	4	30.054	0.112	4.11***
Within populations	263	685.065	2.605	95.89
Total	267	719.119	2.717	

*** $P < 0.001$

harbor moderately high levels of genetic diversity and that habitat fragmentation has limited genetic effects on this old rare tree species. No significant differences in either genetic diversity (A_R , H_E , and H_O) or genetic differentiation (F_{ST} and D_{EST}) were detected between pre- and post-fragmentation cohorts. Although contrasting results have been reported in many common or recently rare tree species (Aldrich et al. 1998; Dayanandan et al. 1999; Jump and Peñuelas 2006; Fernández-M and Sork 2007; André et al. 2008; Farwig et al. 2008; Moreira et al. 2009), the consistency of genetic diversity (Mathiasen et al. 2007; Yao et al. 2011) or genetic divergence (Kettle et al. 2007) in different cohorts has been reported in recently fragmented populations of other naturally rare tree species.

The threshold effect of habitat fragmentation is an important factor determining the impact of habitat

fragmentation on genetic diversity and structure (Parker and Mac Nally 2002; Bacles and Jump 2011). The existence of fragmentation threshold suggests a trade-off between increased isolation and dispersal distance of pollen and seed (Kramer et al. 2008). Unchanged or increased gene flow in the more open forests with disturbance may counteract the impacts of fragmentation (White et al. 2002; O'Connell et al. 2006; Wang et al. 2010). However, when distances between isolated populations exceed the maximum dispersal distance (past the fragmentation threshold), negative genetic effects are expected (Bacles and Jump 2011). Therefore, two contrasting hypotheses were proposed to explain the limited genetic impacts of habitat fragmentation on *E. pleiospermum*.

First, increased isolation of fragmented populations did not reach the fragmentation threshold. In the case

of *E. pleiospermum*, two factors may prevent the occurrence of a fragmentation threshold. (i) As an “old rare” tree species that occurs naturally in small and isolated fragments within undisturbed habitats, *E. pleiospermum* may have developed some strategies to cope with isolation and small population size. Therefore, similar to other naturally rare tree species (Kamm et al. 2011), its threshold local density before any negative impacts is low. (ii) As a wind-pollinated tree species with airborne seed, gene flow of *E. pleiospermum* can be increased in fragmented forest because fragmentation removes vegetation which potentially impedes its pollen or seed dispersal.

However, the elimination of rare alleles in the young cohort is an early warning of negative genetic impacts. In both cohorts, most of the genetic variation was explained within populations (Table 3). This indicates that *E. pleiospermum* is an obligate or mainly outcrossing tree species. An immediate consequence of habitat fragmentation is the decrease of population size, thereby leading to a genetic bottleneck in outcrossing plants, which will have crucial effects on the maintenance of rare alleles (Lande 1988; Aguilar et al. 2008). The genetic bottleneck detected in the young cohort of *E. pleiospermum* (Table 1) offers one explanation for the loss of rare alleles. The marginally significant increase of inbreeding coefficient in the young cohort is another early warning of negative genetic impacts. This is consistent with many other outcrossing plant species (Honnay and Jacquemyn 2007; Bacles and Jump 2011). Therefore, we argue against the first hypothesis.

Second, when the fragmentation threshold has been reached, it needs several generations to make the negative genetic impacts become detectable. One common explanation for the weak genetic effects of fragmentation on tree species is the short time (e.g., mostly not more than one generation) elapsed since fragmentation (Aguilar et al. 2008; Andrianoelina et al. 2009; Bacles and Jump 2011; Yao et al. 2011). The argument is that post-fragmentation individuals did not have sufficient time to experience genetic drift, inbreeding, and inbreeding depression, thereby making the loss of genetic diversity and increase of genetic differentiation undetectable (Lowe et al. 2005; Kettle et al. 2007; Kramer et al. 2008). In the case of *E. pleiospermum*, the limited genetic effects of fragmentation may result from too few fragmented generations, because the time between the start of

fragmentation (year 1970) and our study (year 2008) is less than one generation of this tree species. It should be mentioned that clonal reproduction by sprouting is a mechanism of *E. pleiospermum* to buffer the genetic impacts of fragmentation by delaying the time between generations and thereby moderating the loss of alleles through genetic drift. Vegetative reproductive is a special mechanism of many “old rare” plant species to survive in unstable habitats (Tang and Ohsawa 2002; Rasmussen and Kollmann 2004) and this phenomenon is very common in *E. pleiospermum* (Wei et al. 2010b).

Conclusions

It is evident that the remnant fragmented populations of *E. pleiospermum* can provide a buffer to the negative genetic impacts of deforestation and fragmentation. Therefore, we conclude that this “old rare” tree species is unsusceptible to recent habitat fragmentation. However, the elimination of rare alleles and increase of inbreeding coefficient in the young cohort are early warnings of deleterious genetic consequences of deforestation and fragmentation. In a recent review, Lowe et al. (2005) concluded that inbreeding is observed immediately following disturbance and genetic diversity is lost slowly over subsequent generations. Kettle et al. (2007) inferred that time needed for the observation of direct impacts on population differentiation is longer than that for the change of inbreeding coefficient. Therefore, a possible scenario is that the genetic diversity will decrease and the genetic differentiation will increase in the future if effective restoration practices are not adopted. Thus, effective conservation strategies for this “old rare” tree species are: (1) impeding further deforestation or fragmentation to prevent the potential genetic erosion; (2) restoring the fragmented populations to rescue the lost rare alleles.

In this study, *E. pleiospermum* was chosen to represent a group of “old rare” Tertiary-relict tree species distributed in south-central China. One implication of our study is that many other naturally rare tree species in this area are likely to be weakly impacted by recent habitat fragmentation in the short term, which was confirmed by other studies in this area (Wang et al. 2010; Yao et al. 2011). To fully understand the relationship between fragmentation

and rare or common species' population genetics, studies on more rare species and comparisons with common species in the same area are required. Here, it should be mentioned that the use of a moderate number of putatively neutral markers in this study precluded assessment of adaptive diversity, so genomic approach (e.g., next-generation sequencing) (Ouborg et al. 2010) should be adopted in the future study.

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