Biodiversity and Conservation (2006) 15:1339–1351
DOI 10.1007/s10531-005-4875-1

Effects of fragmentation of evergreen broad-leaved forests on genetic diversity of *Ardisia crenata* var. *bicolor* (Myrsinaceae)

AI-LIAN ZHAO[1], XIAO-YONG CHEN[1,2,*], XIN ZHANG[1] and DONG ZHANG[1]

[1]*Department of Environmental Sciences, East China Normal University, Shanghai 200062, P. R. China;* [2]*Shanghai Key Laboratory for Ecological Processes and Restoration in Urban Areas, Zhongshan R. (N.) 3663, Shanghai 200062, P. R. China; *Author for correspondence (e-mails: xychen@des.ecnu.edu.cn, xychens@sh163.net; phone: +86-21-62232697; fax: +86-21-62233669)*

Received 11 May 2004; accepted in revised form 20 March 2005

Key words: *Ardisia crenata* var. *bicolor*, Differentiation, Forest fragmentation, Genetic diversity, Population size, RAPD markers

Abstract. Due to the long generation times and high densities, dominant tree species usually did not respond consistently with theoretical predictions to the recent fragmentation. Genetic structures of shrubs and herbs, especially those with low densities, may be more sensitive to forest fragmentation. We studied the genetic structure of a self-compatible subshrub, *Ardisia crenata* var. *bicolor* (Myrsinaceae) in a recently fragmented landscape. Ten RAPD primers used for analysis generated a total of 76 bands. We found that *A. c.* var. *bicolor* had relatively low species-level (P_{95} = 63.2%; H = 0.106; Shannon diversity index (SI) = 0.246) and within-population diversity (P_{95} = 5.3–46.1%; H = 0.026–0.175; SI = 0.032–0.253), and significant population differentiation (G_{ST} = 0.445). Significantly positive relationships were found between measures of diversity (P_{95}, H and SI) and the log of estimated population size. No significant relationship was observed between Nei's genetic distance and spatial distance of pairwise populations, indicating no isolation-by-distance. Given most species of forests are shrubs and herbs with short generation times, our observation indicated that distinct genetic consequences of recent fragmentation may be expected for quite a number of plant species.

Introduction

Due to increased urbanization, intensive agricultural practices and habitat destruction, many plant species occur in highly fragmented habitats (Van Rossum et al. 2004). Usually, consequences of habitat fragmentation consist of reduced population size and increased isolation (Saunders et al. 1991; Van Rossum et al. 2004), leading to genetic erosion and increased genetic differentiation among populations, through random drift, increased levels of inbreeding and reduced gene flow (Young et al. 1996; Chen 2000; Van Rossum et al. 2004). Ultimately these genetic processes may result in fitness declines and extinction (Keller and Waller 2002; Bacles et al. 2004).

There have been increasing studies concerning genetic effects in plants of habitat fragmentation, and loss of genetic diversity and increased differen-

tiation have been found in some systems (e.g., Raijmann et al. 1994; Hall et al. 1996; Morden and Loeffler 1999; Frankham et al. 2002). However, these responses to increased fragmentation are unlikely to be common, and other factors may influence the genetic consequences of fragmentation. First, most studies were conducted on long-lived species in a recently fragmented landscape and there were not sufficient time for bottleneck and inbreeding to take action (Young et al. 1993; Cardoso et al. 1998). Long-lasting, dormant seed banks also can buffer against genetic effects for decades or centuries (Morris et al. 2002). Second, many plant species studied were dominant species with high density. Thus, populations in fragmented habitats were large enough to maintain relatively high genetic diversity. Thirdly, some species are naturally rare species, and have evolved mechanisms to overcome the disadvantages of small population size. Thus, genetic consequences of recent fragmentation may not be detectable for a long time (England et al. 2002).

Shrubs and herbs constitute the main part of species composition of forests. Therefore, genetic effects observed in dominant species of forests might not be general for most forest species. Shrubs and herbs – especially those with low densities – may be more genetically sensitive to forest fragmentation because of their much shorter life span. A shorter life span means they pass many generations even in recently fragmented habitats and, given their low densities, they therefore experience large declines in population size even if the fragmentation is not serious. However, much fewer studies have been conducted on herbs and shrubs than on tree species. For example, populations of herbaceous *Swertia perennis* in small, isolated habitats had reduced genetic variability and the highest within-population inbreeding coefficients (Lienert et al. 2002). In the herb, *Scutellaria montana*, populations that were less than 100 individuals tended to have lower proportions of polymorphic loci than that of populations more than 100 individuals (Cruzan 2001).

Ardisia (Myrsinaceae) is a tropical and subtropical genus and includes about 200 species. Coral ardisia, *A. crenata*, native to Japan to north India, is an insect-pollinated and self-compatible evergreen subshrub (Cheon et al. 2000). In China, a variant, *A. crenata* var. *bicolor*, was identified according to the purple color of the lower side of its leaves, whereas some researchers thought it as a distinct species, i.e. *A. bicolor*. *A. crenata* var. *bicolor* is a small upright-growth shrub. Although outcrossing rate of *A. c.* var. *bicolor* was estimated to be about 1 based on allozyme using Ritland's (1990) MLT program (Chen et al. 2001), bag-pollination treatments indicated that it is self-compatible (unpublished data). This species can reproduce vegetatively via rhizome, but spreading to a short distance, usually less than 1 m (personal observations).

In the present study, populations of *A. c.* var. *bicolor* in a fragmented landscape were selected to determine whether there is a relationship between population size and the level of variation and to evaluate the degree of

population subdivision and differentiation, using RAPD markers. Although RAPDs have some limitations – such as dominant allelic expression and occasionally low reproducibility – they have advantages in investigating genetic variation, such as random sampling in the whole genome, high levels of polymorphism, and fast and easy to perform, and have been widely used in estimating genetic variation of plant populations (Nybom and Bartish 2000; Nybom 2004).

Methods

Population sampling

The study sites were located in Tiantong Forest Park (TFP) and adjacent areas (Figure 1). TFP was distributed by evergreen broad-leaved forests (EBLFs) dominated by Fagaceae species, such as *Castanopsis fargesii*, *Ca. carlesii*, *Ca. sclerophylla*, *Lithocarpus glaber*, *L. henryi*, *Cyclobalanopsis nubium*, and species of Theaceae (*Schima superba*) and Lauraceae (*Machilus thunbergii*) (Song and Wang 1995). Around TFP, there were EBLFs fragments of previous continuous forests or recovered from abandoned or unmanaged plantations. These fragmented EBLFs were usually dominated by *Cyclobalanopsis glauca*, *Cy. gilva*, *Ca. sclerophylla*, *L. glaber*, *Ca. carlesii*, *M. thunbergi*. Surrounding these EBLFs, there were *Cunninghamia lanceolata* plantations, *Phyllostachys pubescens* forests, and shrubs dominated by *Quercus fabra* and bamboos.

In TFP and adjacent areas, *A. c.* var. *bicolor* usually appears in forests of lower than 300 m above sea-level. Based on detailed surveys, 10 populations of *A. c.* var. *bicolor* were sampled (Figure 1). The estimated sizes of each population ranged from ~5 to about 1000 individuals (Table 1). Leaves were collected randomly from individuals with a distance of at least 2 m between each other in medium and large populations, avoiding collecting the same clones. In small populations, as many as possible individuals were collected with a distance of at least 2 m between sampled individuals.

DNA extraction and PCR condition

We isolated DNA with modified Doyle and Doyle's (1987) procedure (Fan et al. 2004). A set of random 10-mer primers was purchased from Sagon Inc., Shanghai. After screening more than 100 arbitrary primers, 10 primers that consistently amplified clear banding patterns were chosen for further studies (Table 1). RAPD assays were performed using the conditions described by Fan et al. (2004). Samples were amplified at least two replicates and same pattern was obtained by the primers used in this study. Five μl amplification product was separated on 1.6% agarose gel in 0.5× TBE buffer and visualized by

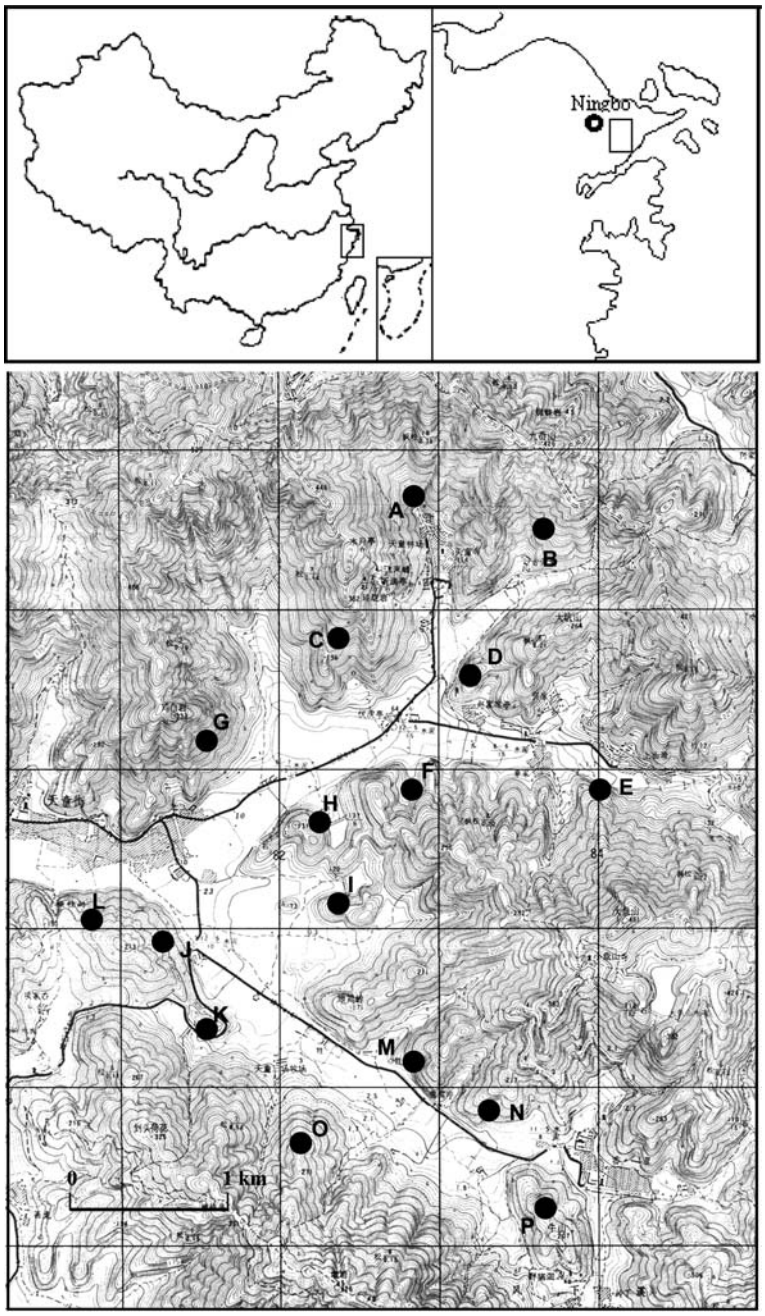

Figure 1. Locations of sampling sites of *Ardisia crenata* var. *bicolor* in Tiantong Forest Park and adjacent areas.

Table 1. RAPD primers used in the survey of *Ardisia bicolor* and number of scored bands.

Primer	Sequence 5′-3′	Number of scored bands	Primer	Sequence 5′-3′	Number of scored bands
S59	CTGGGGACTT	8	S1361	TCGGATCCGT	10
S1200	GTGAACGCTC	9	S2068	CATACGGGCT	8
S1221	CACACCGTGT	3	S2084	CCCAAGCGAA	4
S1238	GTTGCGCAGT	11	S2100	CAAAGGCGTG	9
S1341	GTCCACCTCT	4	S2160	CACCGACATC	10

staining with ethidium bromide and photographed under UV light with Bio-RAD Gel Doc2000^{TM}.

Data analysis

Each PCR product was assumed to represent a single locus and was scored for presence and absence. The resulting data matrix was analyzed using Popgene 1.31 (Yeh et al. 1999). Gene diversities (H) at population and at the species level were calculated based on Lynch and Milligan's (1994) Taylor expansion estimate using TFPGA (Tools For Population Genetic Analyses) v1.3 (Miller 1997).

Nei's unbiased genetic identity (I) and genetic distance (D) between populations were also analysed using Popgene 1.31. Because the data were larger than the up-limit of AMOVA, coefficient of gene differentiation (G_{ST}) was calculated to estimate population differentiation. Shannon diversity index (Lewontin 1972), $SI = -\sum p_i \log_2 p_i$, was calculated to provide a relative estimate of the degree of variation at population and species levels using Popgene 1.31 (Yeh et al. 1999). The proportion of diversity among populations was estimated as $(SI_{sp}-SI_{pop})/SI_{sp}$, whereas SI_{sp} and SI_{pop} were SI at species and population level, respectively. A Mantel type matrix randomization test (Mantel 1967) was performed to evaluate the relationship between the matrix of genetic distances and the matrix of geographic distances using TFPGA (Miller 1997).

Relationships between measures of within-population genetic variation and population size were analyzed using Regression methods in Microsoft® Excel program.

Results

The RAPD profile

The 10 primers used for analysis generated a total of 76 bands, among which polymorphic bands were 48 (or 63.2%) and 51 (or 67.1%) based on 95 and

Table 2. Patterns of genetic diversity for Ardisia bicolor populations.

Population	Estimated population size	Sample size	P_{95}	P	H	SI
A	1000	34	35.5%	40.8%	0.144	0.208
B	1000	33	32.9%	35.5%	0.122	0.178
C	100	15	29.0%	30.3%	0.120	0.168
D	100	14	30.3%	32.9%	0.128	0.179
E	1000	37	46.1%	47.4%	0.175	0.253
F	500	34	32.9%	38.2%	0.127	0.186
G	5	2	7.89%	7.9%	0.039	0.048
H	200	21	30.3%	31.6%	0.122	0.174
I	5	2	5.3%	5.3%	0.026	0.032
J	100	32	22.4%	27.6%	0.073	0.112
K	50	22	27.6%	32.9%	0.113	0.163
L	10	5	15.8%	15.8%	0.065	0.089
M	100	25	21.1%	23.7%	0.077	0.112
N	200	23	34.2%	36.8%	0.129	0.186
O	300	13	39.5%	40.8%	0.171	0.236
P	30	8	19.7%	19.7%	0.072	0.103
Mean	294	20	26.9%	29.2%	0.106	0.152
Total	4700	320	63.2%	67.1%	0.192	0.246

P_{95} and P are percentages of polymorphic bands based on 95 and 100% criteria, respectively; H denotes mean Nei's gene diversity based on Nei's (1972) unbiased estimates; SI, Shannon's diversity index.

100% criteria, respectively (Table 2). The number of scored bands ranged from 3 for primer S1221 to 11 for primer S1238. 308 of the 320 individuals from the 16 populations were found to have a unique multilocus genotype, and six genotypes have two individuals. The individuals having the same multilocus genotypes belonged to same populations. No population-specific band was observed in the data set.

Genetic diversity

Percent polymorphic RAPD loci varied from 5.3 (population I) to 46.1 % (E), based on 95% criterion, with a mean of 26.9% (Table 2). The Nei's gene diversity ranged from 0.026 to 0.175 with a mean of 0.106 and the pooled species-level value was 0.192. The relative degree of diversity in each population as measured by Shannon's index varied from 0.032 to 0.253 (Table 2). The mean Shannon diversity for all populations was 0.152 and the pooled species-level value was 0.246. Among the 16 populations, population E exhibited the highest level of genetic variability (P, H and SI) and population O was the next, whereas population I was the lowest (Table 2). Regression analysis indicated significantly positive relationships between measures of within-population genetic variation (P_{95}, H and SI) and the log of population sizes (Figure 2).

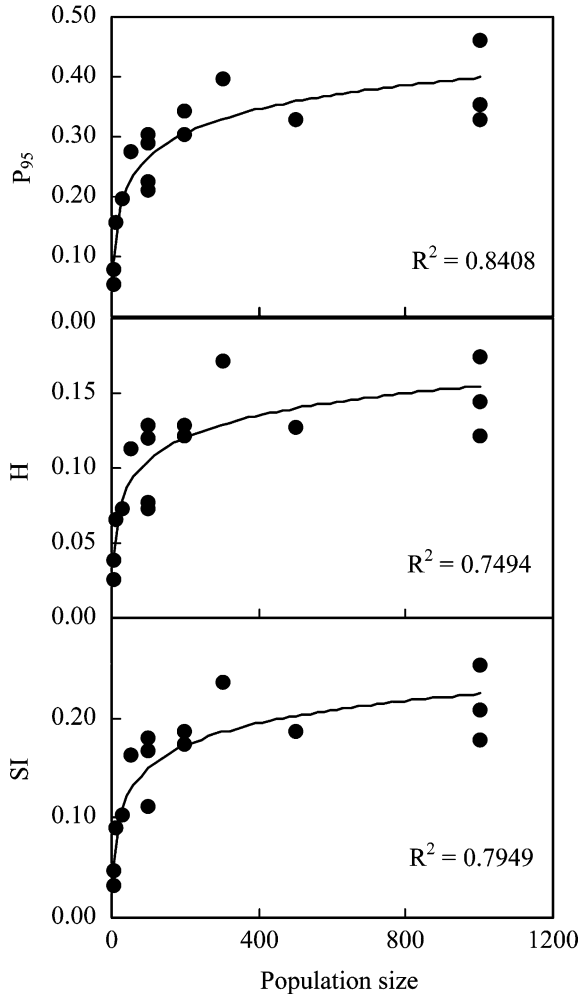

Figure 2. Relationships between measures of within-population genetic variation and population size in *Ardisia crenata* var. *bicolor*. P_{95}, H and SI were the percentage of polymorphic loci at 95% criterion, expected heterozygosity and Shannon diversity index, respectively.

Genetic differentiation

The coefficient of genetic differentiation between populations (G_{ST}) was 0.445, indicating a high differentiation among populations. The Shannon's index analysis partitioned 38.4% of the total variation among populations. Genetic distances (D) between populations varied from 0.018 to 0.149 (Table 3) with a mean of 0.055 ± 0.023. The level of gene flow (Nm) was estimated to be 0.312. Mantel test indicated no significant relationship between genetic distance and spatial distance ($r = 0.021$, $P = 0.422$).

Table 3. Nei's unbiased genetic identity (below diagonal) and genetic distance (above diagonal) between populations of *Ardisia crenata* var. *bicolor*.

Population	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
A	–	0.0372	0.0361	0.0212	0.0441	0.0254	0.1092	0.0429	0.0558	0.0603	0.0345	0.0383	0.0354	0.0382	0.0581	0.0445
B	0.9635	–	0.0397	0.0380	0.0442	0.0481	0.0893	0.0300	0.0552	0.0538	0.0300	0.0490	0.0296	0.0247	0.0676	0.0606
C	0.9646	0.9611	–	0.0324	0.0505	0.0452	0.0959	0.0372	0.0479	0.0655	0.0418	0.0626	0.0464	0.0296	0.0716	0.0631
D	0.9790	0.9627	0.9682	–	0.0426	0.0370	0.0922	0.0395	0.0532	0.0589	0.0398	0.0393	0.0356	0.0331	0.0462	0.0530
E	0.9569	0.9567	0.9507	0.9583	–	0.0389	0.0895	0.0338	0.0707	0.0574	0.0471	0.0725	0.0519	0.0281	0.0287	0.0457
F	0.9749	0.9530	0.9558	0.9636	0.9618	–	0.0953	0.0294	0.0718	0.0548	0.0499	0.0624	0.0414	0.0456	0.0660	0.0584
G	0.8965	0.9145	0.9085	0.9119	0.9144	0.9091	–	0.0892	0.1155	0.0858	0.0894	0.1216	0.0827	0.0608	0.0722	0.1487
H	0.9580	0.9705	0.9635	0.9613	0.9668	0.9711	0.9147	–	0.0411	0.0555	0.0397	0.0676	0.0385	0.0339	0.0526	0.0341
I	0.9458	0.9463	0.9532	0.9482	0.9317	0.9307	0.8909	0.9597	–	0.0732	0.0570	0.0771	0.0562	0.0526	0.0754	0.0447
J	0.9415	0.9477	0.9366	0.9428	0.9442	0.9467	0.9178	0.9460	0.9294	–	0.0184	0.0397	0.0387	0.0398	0.0713	0.1064
K	0.9661	0.9704	0.9590	0.9610	0.9540	0.9513	0.9145	0.9611	0.9446	0.9818	–	0.0304	0.0290	0.0296	0.0609	0.0789
L	0.9625	0.9522	0.9393	0.9614	0.9301	0.9395	0.8855	0.9346	0.9258	0.9610	0.9701	–	0.0257	0.0608	0.0926	0.1034
M	0.9652	0.9709	0.9547	0.9650	0.9494	0.9595	0.9206	0.9623	0.9454	0.9620	0.9714	0.9746	–	0.0413	0.0755	0.0876
N	0.9625	0.9756	0.9709	0.9675	0.9723	0.9555	0.9411	0.9667	0.9488	0.9609	0.9708	0.9410	0.9595	–	0.0518	0.0565
O	0.9435	0.9347	0.9309	0.9548	0.9717	0.9361	0.9303	0.9488	0.9274	0.9312	0.9409	0.9115	0.9273	0.9495	–	0.0536
P	0.9565	0.9412	0.9389	0.9484	0.9553	0.9433	0.8618	0.9665	0.9563	0.8991	0.9242	0.9018	0.9161	0.9451	0.9478	–

Discussion

The present study reveals relative low genetic diversity in *A. c.* var. *bicolor* compared to other species based on RAPD markers. Percentage of polymorphic bands, Nei's gene diversity and Shannon index of the pooled data were 67.1%, 0.192 and 0.246, respectively (Table 2). These values were even lower than many endangered species, *Metasequoia glyptostroboides* (*P*: 87.9%, $H = 0.318$, SI = 0.476) (Li et al. 2005), *Caesalpinia echinata* ($P = 95.7\%$) (Cardoso et al. 1998), *Leucadendron elimense* ($P = 98.8\%$) (Tansley and Brown 2000), *Boloria aquilonaris* ($H = 0.402$) (Vandewoestijne and Baguette 2002), but higher than *Haplostachys haplostachya* ($H = 0.166$) (Morden and Loeffler 1999), *Dryopteris cristata* ($P = 2.5\%$) (Landergott et al. 2001).

Our results were not in accordance with the predictions based on the association of life history traits and genetic variation. According to the data of RAPDs, there were strong associations between genetic diversity and breeding system or successional status (Nybom and Bartish 2000; Nybom 2004). Outcrossing species had significantly high genetic diversity than selfers. Higher genetic diversity was found in late- than early - successional species. Species of ingested seed dispersal also possessed relatively high genetic diversity (Nybom 2004). Given such considerations, high genetic diversity was expected based on its high outcrossing rate (Chen et al. 2001), mid to late-successional status and bird dispersal manner.

Relatively low genetic diversity in the studied *A. c.* var. *bicolor* populations might be explained by their geographical positions. The studied populations are located on the eastern margin of its distribution in mainland China (Figure 1). Due to effects of founder events, genetic drift and inbreeding, marginal populations usually possess relative low genetic variation, which had been confirmed in diverse species (Chen et al. 1997; Tyler 2002; Cassel and Tammaru 2003). Restricted geographical range in the present study might be another explanation of the low genetic diversity. Though 10 populations were sampled, their spatial distances were small. The largest distance of pairwise populations is 4.6 km. If populations were sampled in a large range, more genetic variation might be expected.

Though some studies failed to observe the distinct genetic consequences of forest fragmentation on plant populations (Ellstrand and Elam 1993; Young et al. 1996), our results indicated that habitat fragmentation had played a vital role in genetic structure of *A. c.* var. *bicolor* populations. Fragmentation led to the loss of genetic diversity. In small populations, significantly lower diversity was observed than large and medium ones. Significant relationship was found between estimated population size and within-population genetic variation as measured by *P*, *H* and Shannon index.

Decreased genetic diversity in small populations was due to various reasons. Firstly, the instantaneous effects of fragmentation (i.e., sampling effects) lead to stochastic loss of rare alleles because only a small portion of the original

gene pool remains after the decrease. Buchert et al. (1997) had compared the genetic diversity in pre-harvest and post-harvest gene pools of two virgin stands of eastern white pine (*Pinus strobus*). They found total and mean number of alleles was reduced by 25% after tree density reductions of 75%. About 40% of the low frequency alleles and 80% of the rare alleles were lost because of harvesting (Buchert et al. 1997). Secondly, inbreeding and genetic drift further decreased genetic variation in small populations (Young et al. 1996). In the present study, most small populations experienced bottleneck for more than 10 generations, given a generation of 3 years and deforestation of at least 50 years. It is enough for inbreeding and drift to virtually change the genetic composition of small populations of less than 50 individuals. Thirdly, founder effect might also contribute to low genetic diversity in some small populations (Frankham et al. 2002). Population G, for instance, located in dense high shrubs, had only two small individuals, indicating a recent founding event.

High genetic differentiation was observed among populations of *A. c.* var. *bicolor*. G_{ST} indicated that about 44.5% of the genetic variation occurred among populations with short spatial distances. This value is higher than the RAPD-based estimates of other widespread, or animal-dispersal species (Nybom and Bartish 2000). High genetic differentiation among populations was also in accordance with theoretical prediction of fragmentation, indicating the effects of bottleneck and inbreeding.

No significant relationship between genetic distance and spatial distance was found in the present study. This is usually interpreted that selection or drift plays a more significant role than gene flow. In this study, no distinct difference in habitats was found among populations, though the dominant species were different. At local scale, populations from different communities usually showed similar genetic composition in studied species, such as, *Cyclobalanopsis glauca* (Chen and Song 1998). Therefore, selection plays a minor role in the differentiation of *A. c.* var. *bicolor* populations, and drift led by fragmentation contributed to the high differentiation.

Our findings in *A. c.* var. *bicolor* give a gloomy implication for forest species because most species of forests are shrubs and herbs and among them most are moderate- or low-density species. For example, there were about a dozen of species in tree layer of EBLFs; among them, usually less than 3 species dominated the community. However, the number of species in shrub and herb layers was about three to four folds of that in tree layer (Song and Wang 1995). Among them, most are moderate or low density, like *A. c.* var. *bicolor*, and are vulnerable to fragmentation. This situation is also common in tropical, temperate or boreal forests. Thus, although some studies showed no distinct effects on long-lived tree species which have survived hundreds of years of fragmentation, our study indicated that distinct genetic consequences of recent fragmentation may be expected for quite a number of plant species. More attention should be paid to these species and conservation efforts are needed.

Acknowledgements

We thank Mr Ling-jian Li and Ms Xiao-xia Fan for the assistance in sample collection and preparation. We thank J. P. Sniadecki of Grand Valley State University for English improvement and helpful comments and reviewers for their critical comments and suggestions. This study was supported by Natural Science Foundation of China (39870128, 30170060), The State's Tenth Five-year "211 Project" and Shanghai Priority Academic Discipline.

References

Bacles C.F.E., Lowe A.J. and Ennos R.A. 2004. Genetic effects of chronic habitat fragmentation on tree species: the case of *Sorbus aucuparia* in a deforested Scottish landscape. Molecular Ecology 13: 573–584.

Buchert G.P., Rajora O.P., Hood J.V. and Dancik B.P. 1997. Effects of harvesting on genetic diversity in old-growth eastern white pine in Ontario, Canada. Conservation Biology 11: 747–758.

Cardoso M.A., Provan J., Powell W., Ferreira P.C.G. and de Oliveira D.E. 1998. High genetic differentiation among remnant populations of the endangered *Caesalpinia echinata* Lam. (Leguminosae-Caesalpinioideae). Molecular Ecology 7: 601–608.

Cassel A. and Tammaru T. 2003. Allozyme variability in central, peripheral and isolated populations of the scarce heath (*Coenonympha hero*: Lepidoptera, Nymphalidae); implications for conservation. Conservation Genetics 4: 83–93.

Chen X.Y. 2000. 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., Li N. and Shen L. 2001. The mating system of *Ardisia crenata* var. *bicolor* (Myrsinaceae), a subtropical understory shrub, in Tiantong National Forest Park, Zhejiang Province. Acta Phytoecologica Sinica 25: 161–165.

Chen X.Y. and Song Y.C. 1998. Microgeographic differentiation in a *Cyclobalanopsis glauca* poplation in western Huangshan, Anhui Province. Journal of Plant Resources Environment 7: 10–14.

Chen X.Y., Wang X.H. and Song Y.C. 1997. Genetic diversity and differentiation of *Cyclobalanopsis glauca* populations in East China. Acta Botanica Sinica 39: 149–155.

Cheon C.P., Chung M.Y. and Chung M.G. 2000. Allozyme and clonal diversity in Korean populations of *Ardisia japonica* and *Ardisia crenata* (Myrsinaceae). Israel Journal of Plant Science 48: 239–245.

Cruzan M. 2001. Population size and fragmentation thresholds for the maintenance of genetic diversity in the herbaceous endemic *Scutellaria montana* (Lamiaceae). Evolution 55: 1569–1580.

Doyle J.J. and Doyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Ellstrand N.C. and Elam D.R. 1993. Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics 24: 217–242.

England P.R., Usher A.V., Whelan R.J. and Ayre D.J. 2002. Microsatellite diversity and genetic structure of fragmented populations of the rare, fire-dependent shrub *Grevillea macleayana*. Molecular Ecology 11: 967–977.

Fan X.X., Shen L., Zhang X., Chen X.Y. and Fu C.X. 2004. Assessing genetic diversity of *Ginkgo biloba* L. (Ginkgoaceae) populations from China by RAPD markers. Biochemical Genetics 42: 269–278.

Frankham R., Ballou J.D. and Briscoe D.A. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge.

Hall P., Walker S. and Bawa K. 1996. Effect of forest fragmentation on genetic diversity and mating system in a tropical tree, *Pithecellobium elegans*. Conservation Biology 10: 757–768.

Keller L.F. and Waller D.M. 2002. Inbreeding effects in wild populations. Trends in Ecology and Evolution 17: 230–241.

Landergott U., Holderegger R., Kozlowski G. and Schneller J. 2001. Historical bottlenecks decrease genetic diversity in natural populations of *Dryopteris cristata*. Heredity 87: 344–355.

Lewontin R.C. 1972. The apportionment of human diversity. Evolutionary Biology 6: 381–398.

Li Y.-Y., Chen X.-Y., Zhang X., Wu T.-Y., Lu H.-P. and Cai Y.-W. 2005. Genetic differences between wild and artificial populations of *Metasequoia glyptostroboides* Hu et Cheng (Taxodiaceae): Implications for species recovery. Conservation Biology 19: 224–231.

Lienert J., Fischer M., Schneller J. and Diemer M. 2002. Isozyme variability of the wetland specialist *Swertia perennis* (Gentianaceae) in relation to habitat size, isolation, and plant fitness. American Journal of Botany 89: 801–811.

Lynch M. and Milligan B.G. 1994. Analysis of population genetic structure with RAPD markers. Molecular Ecology 3: 91–99.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27: 209–220.

Miller M.P. 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.

Morden C.W. and Loeffler W. 1999. Fragmentation and genetic differentiation among subpopulations of the endangered Hawaiian mint *Haplostachys haplostachya* (Lamiaceae). Molecular Ecology 8: 617–625.

Morris A.B., Baucom R.S. and Cruzan M.B. 2002. Stratified analysis of the soil seed bank in the cedar glade endemic *Astragalus bibullatus*: Evidence for historical changes in genetic structure. American Journal of Botany 89: 29–36.

Nybom H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Molecular Ecology 13: 1143–1155.

Nybom H. and Bartish I.V. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspectives in Plant Ecology, Evolution and Systematics 3/2: 93–114.

Raijmann L.E.L., Van Leeuwen N.C., Kersten R., Oostermeijer J.R.B., Den Nijs H.C.M. and Menken S.B.J. 1994. Alleleic variation and outcrossing rate in relation to population size in *Gentiana pneumonanthe* L. Conservation Biology 8: 1014–1026.

Ritland K. 1990. A series of FORTRAN computer programs for estimating plant mating systems. Journal of Heredity 81: 235–237.

Saunders D.A., Hobbs R.J. and Margules C.R. 1991. Biological consequences of ecosystem fragmentation: A review. Conservation Biology 5: 18–32.

Song Y.C. and Wang X.R. 1995. Vegetation and Flora of Tiantong National Forest Park, Zhejiang Province. Shanghai Scientific Documentary Press, Shanghai.

Tansley S.A. and Brown C.R. 2000. RAPD variation in the rare and endangered *Leucadendron elimense* (Proteaceae): implications for their conservation. Biological Conservation 95: 39–48.

Tyler T. 2002. Large-scale geographic patterns of genetic variation in *Melica nutans*, a widespread Eurasian woodland grass. Plant Syst. Evol. 236: 73–87.

Van Rossum F., De Sousa S.C. and Triest L. 2004. Genetic consequences of habitat fragmentation in an agricultural landscape on the common *Primula veris*, and comparison with its rare congener, *P. vulgaris*. Conservation Genetics 5: 231–245.

Vandewoestijne S. and Baguette M. 2002. The genetic structure of endangered populations in the Cranberry Fritillary, *Boloria aquilonaris* (Lepidoptera, Nymphalidae): RAPDs vs allozymes. Heredity 89: 439–445.

Yeh F.C., Yang R.C. and Boyle T. 1999. Popgene Version 1.31: Microsoft Window-Based Freeware for Population Genetic Analysis. Department of Renewable Resources, University of Alberta, Edmonton, AB Canada.

Young A., Boyle T. and Brown T. 1996. The population genetic consequences of habitat fragmentation for plants. Trends in Ecology and Evolution 11: 413–418.

Young A., Merriam H.G. and Warwick S.I. 1993. The effects of forest fragmentation on genetic variation in *Acer saccharum* Marsh. (sugar maple) populations. Heredity 71: 277–289.