

A population genetic evaluation of ecological restoration with the case study on *Cyclobalanopsis myrsinaefolia* (Fagaceae)

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Abstract The ultimate goal of ecological restoration is to create a self-sustaining ecosystem that is resilient to perturbation without further assistance. Genetic variation is a prerequisite for evolutionary response to environmental changes. However, few studies have evaluated the genetic structure of restored populations of dominant plants. In this study, we compared genetic variation of the restored populations with the natural ones in *Cyclobalanopsis myrsinaefolia*, a dominant species of evergreen broadleaved forest. Using eight polymorphic microsatellite loci, we analyzed samples collected from restored populations and the donor population as well as two other natural populations. We compared the genetic diversity of restored and natural populations. Differences in genetic composition were evaluated using measurements of genetic differentiation and assignment tests. The mean number of alleles per locus was 4.65. Three parameters (A , A_R , and expected heterozygosity) of genetic variation were found to be lower, but not significantly, in the restored

populations than they were in the natural populations, indicating a founder effect during the restoration. Significant but low F_{ST} (0.061) was observed over all loci, indicating high gene flow among populations, as expected from its wind-pollination. Differentiation between the two restored populations was smallest. However, differences between the donor population and the restored populations were higher than those between other natural populations and the restored populations. Only 13.5% and 25.7% individuals in the two restored populations were assigned to the donor population, but 54.1 and 40% were assigned to another natural population. The genetic variation of the donor population was lowest, and geographic distances from the restoration sites to the donor site were much higher than the other natural populations, indicating that the present donor likely was not the best donor for these ecological restoration efforts. However, no deleterious consequences might be observed in restored populations due to high observed heterozygosity and high gene flow. This study demonstrates that during the restoration process, genetic structures of the restored populations may be biased from the donor population. The results also highlight population genetic knowledge, especially of gene flow-limited species, in ecological restoration.

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Introduction

Conversion of natural habitats into agricultural and industrial landscapes, and ultimately into degraded land, is one major impact of human activities on the natural environment (Dobson et al. 1997). To reverse this trend, there has been an increase in efforts to restore the degraded land throughout the world (Anderson 1995; Miyawaki 1993). Early reclamation of degraded land was made with a few species that were often exotic to the disturbed area. Recently there is a growing awareness that restoring ecosystem function and biodiversity often requires the deployment of native species (Lesica and Allendorf 1999).

The ultimate goal of ecological restoration is to create a self-sustaining ecosystem that is resilient to perturbation without further assistance (Urbanska et al. 1997). Dominant species play vital roles in community persistence, and their restoration is of vital significance to ecosystem restoration. Many efforts are focused on restoring dominant species, and a major goal is to restore their census population sizes to levels that will allow them to persist over the long term within a dynamic landscape and to retain the capacity to undergo adaptive evolutionary change (Montalvo et al. 1997). Genetic variation is of fundamental significance to the latter. It has been recognized that the long-term consequences of sustainable forest management should not lead to the decline in the genetic diversity of forest species (Chen 1999; Namkoong et al. 1996). There are some studies comparing the genetic structure of managed or planted populations with native populations. However, most of them have focused on commercially important species (Gomory 1992; Knowles 1985; Stoehr and El-Kassaby 1997), and few on the dominant species that play critical roles in ecological restoration (Travis et al. 2002; Williams and Davis 1996).

The potential natural vegetation is that which would have been present in the absence of human disturbance, and can be predicted based on the climate and soil conditions (Song 2001). Based on the Potential Vegetation Theory (PVT) (Tuxen 1956), Miyawaki proposed a method to restore forests (Miyawaki 1993; Miyawaki 1998). Like the “Framework Species Method” (Tucker and Murphy 1997), the PVT method involves planting mixtures of native dominant species, which provide suitable habitats for

the establishment of other species. This method emphasizes collection of seeds or seedlings of dominant species from natural forests near restoration sites and high-density planting of seedlings so as to promote competition and height growth. This approach to reforestation aims to restore forests as close as possible to their expected natural status.

Although there is growing consensus about the importance of ecological restoration, there is a lack of agreement on what is a successful restoration project (Anand and Desrochers 2004; Palmer et al. 2005). There are dozens of papers evaluating vegetation restoration success from species composition and diversity, and ecosystem processes (Martin et al. 2005). Restoring genetic structure, especially that of dominant species, is rarely considered in restoration practice, even though its role in the persistence of restored systems has been recognized (Lesica and Allendorf 1999; Montalvo et al. 1997). Genetic variation in dominant species is an important factor that determines the endurance of a planted forest: high genotypic diversity in dominant species can enhance the recovery of an ecosystem (Reusch et al. 2005). There are several studies comparing the genetic composition of restored and natural populations of dominant species of poor-species seagrass or salt marsh (Travis et al. 2002; Williams and Davis 1996). However, no study examines the genetic structure of dominant species for ecological restoration of terrestrial communities.

Cyclobalanopsis myrsinaefolia (Blume.) Oersted. (Fagaceae), an evergreen oak, is an important canopy species in evergreen broad-leaved forests in eastern China and Japan. It is capable of attaining heights of 25 m and a DBH of 0.8 m. The species exhibits wide ecological amplitude and is a dominant species in evergreen broad-leaved forests or mixed evergreen and deciduous broad-leaved forests in mainland China (Wu 1980). Species of *Cyclobalanopsis* are monoecious and are pollinated by wind. Moderate genetic variation, low genetic differentiation and a mixed mating system were found in the congener *C. glauca* (Chen and Song 1997; Chen et al. 1997). Recently, Isagi and Suhandono (1997) surveyed nine microsatellite markers in *C. myrsinaefolia*. Based on only 20 screened trees, three to 17 alleles were found and the expected heterozygosity ranged from 0.16 to 0.92 among these polymorphic loci (Isagi and Suhandono 1997).

In 2000, a restoration project started and two artificial forests were set up in Pudong New Area of Shanghai Municipality using indigenous species, such as *Cyclobalanopsis myrsinaefolia*, *C. glauca*, *Castanopsis sclerophylla*, and *Machilus thunbergii*. In 2004, these forests reached an average height of about 4 m (unpublished data). In this article we compare the genetic composition of restored *C. myrsinaefolia* populations with natural ones, with the aims to (1) examine the effects of a particular restoration effort on genetic diversity and (2) explore the genetic implications for ecological restoration.

Methods

Study sites and sample collections

Following Miyawaki's method (Miyawaki 1993) of ecological restoration, a forest restoration project was started in 2000 in Pudong New District, Shanghai Municipality. *Cyclobalanopsis myrsinaefolia* is one of the dominant species of the native evergreen broad-leaved forests in the studied region. Therefore, *C. myrsinaefolia* seeds were collected by local villagers in October of 1999 from an area of about 10 km² in Western Huangshan of Anhui Province, and were germinated in greenhouses. When seedlings reached about 10 cm in height, they were transferred into small pots with a diameter of about 10 cm. Each pot contained one seedling. In the spring of 2001, seedlings of *C. myrsinaefolia* and other eight species were transplanted to the two afforestation sites, Sanling and Sunqiao (Fig. 1), at a density of about four seedlings per m². In April 2003, there were about 2,300 *C. myrsinaefolia* individuals remaining and the survival rate was about 93% in Sanling (Da et al. 2004). About 2,000 seedlings were planted at another restored site, Sunqiao, and no record about the survival rate was available. Leaves were collected from random samples of 37 and 35 saplings from the two sites respectively. To reduce contamination with fungi and bacteria, the fresh leaves were cleaned using wet gauze before drying with silica gel.

To represent the genetic variation of natural populations, the donor population of the seeds, Western Huangshan of Anhui Province, and two other wild populations (Tianmu Mountain and Tiantong) (Fig. 1) were also sampled. In Western Huangshan and

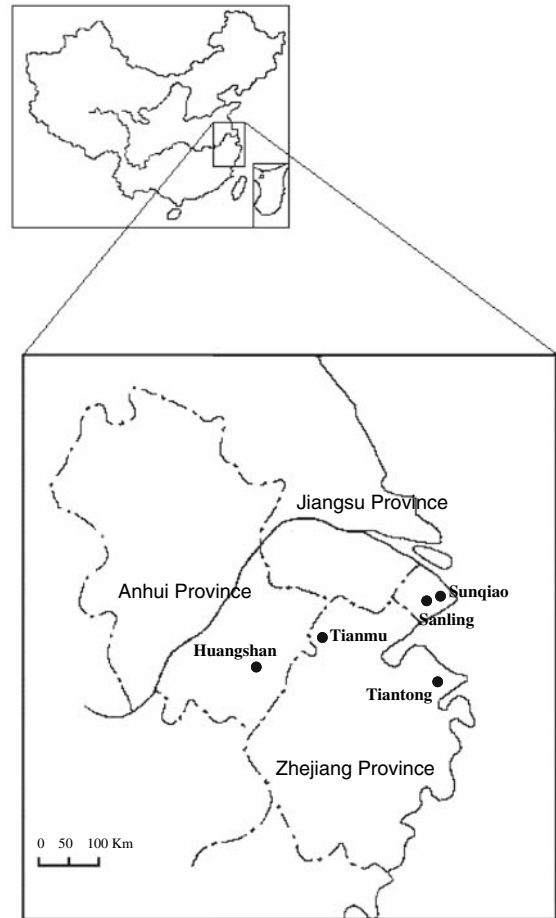


Fig. 1 Sampling sites of the *Cyclobalanopsis myrsinaefolia* populations investigated in the present study. The two populations in Shanghai are restored, and the natural population Huangshan was the donor. Populations Tianmu and Tiantong are natural populations

Tianmu, *C. myrsinaefolia* is the dominant species, whereas in Tiantong *C. myrsinaefolia* is scattered within communities dominated by *Castanopsis fargesii*, *Ca. carlesii*, and *Lithocarpus glaber*. At each of the three wild populations, leaf samples were collected randomly from 28 to 32 *C. myrsinaefolia* trees separated by a minimum distance of 20 m.

Microsatellite DNA analysis

Silica gel-dried *C. myrsinaefolia* leaves were grounded in liquid nitrogen and DNA was extracted according to a modified Doyle and Doyle (1987)

protocol (Fan et al. 2004). Nine SSR primer-pairs developed for this species (Isagi and Suhandono 1997) were tested. However, one primer-pair (QM33GA1) did not amplify well, so only the remaining eight pairs were used further.

SSR PCRs were carried out in a total volume of 20 μ l consisting of 50 ng of template DNA, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.2 μ M of each primer, 0.6 units of Taq polymerase and double distilled water on a MJ Research thermal cycler PTC-220. Cycling conditions were as follows: one cycle of 94°C for 1 min, followed by 35 cycles of 15 s at 94°C, 45 s at 54.8–68.5°C (depending on which primer pair; Table 1), and 45 s at 72°C, with a final extension of 5 min at 72°C. PCR products were separated on 6% denaturing polyacrylamide gels and detected by staining with silver nitrate.

Data analysis

Data were checked for misprint, scoring errors, and deviations from Hardy–Weinberg equilibrium (HWE) due to the presence of null alleles using MICRO-CHECKER (Van Oosterhout et al. 2004). For each population of *C. myrsinaefolia*, genetic diversity was estimated based on standard genetic parameters: mean numbers of alleles (A), allelic richness (A_R , a measure independent of sample size), and expected heterozygosity (H_E) under the hypothesis of Hardy–Weinberg (H–W) genotypic proportions (Nei 1978). Deviations from H–W genotypic proportions were calculated by Wright's F_{IS} (Weir and Cockerham 1984) and heterozygote deficiency was tested via randomization of alleles within

samples using FSTAT (Goudet 1995). Observed heterozygosity (H_O) was calculated using TFPGA (Miller 1997).

Population differentiation was assessed by estimation of F_{ST} (Weir and Cockerham 1984) and R_{ST} (Slatkin 1995) using FSTAT (Goudet 1995). Genetic distances and identities (Nei 1978) were calculated with the program TFPGA (Miller 1997). In order to visualize the genetic relationship among populations, a matrix of genetic distance (Nei 1978) was calculated and a clustering dendrogram was constructed using the UPGMA method with the program TFPGA (Miller 1997). Confidence levels for the phenogram were constructed by bootstrapping the original data 10,000 times with replacement over all loci.

Since the restored populations are offspring populations of the donor population, we used assignment tests to further investigate if the offspring are representative of the donor population. Assignment tests were performed with GENECLASS version 2.0 (Piry et al. 2004).

Results

All eight primer pairs from *Cyclobalanopsis myrsinaefolia* amplified polymorphic loci. A total of 51 alleles were detected among the total sample of 162 trees from the five populations. The number of alleles per primer pair ranged from 4 for QM57 and QM69 to 10 for QM55, with a mean of 6.4 ± 2.1 (Table 1).

The mean number of alleles per locus per population (A) was 4.65. Population Tianmu and Tiantong, with two private alleles, had the highest observed mean number of alleles per locus (5.25). Allelic richness (A_R) was 5.6 per population and followed a

Table 1 Primer pair characteristics and heterozygosity of eight simple sequence repeat (SSR) loci used in the present study

	QM69-2M1	QM57-3M	QM50-3M	QM67-3M1	QM63-2M3	QM51GA1	QM58TGT	QM55GA	Mean
No. of alleles	4	6	4	5	6	8	8	10	6.4
H_O	0.385	0.570	0.205	0.289	0.188	0.698	0.393	0.494	0.403
H_T	0.457	0.710	0.370	0.338	0.346	0.807	0.748	0.850	0.578
G_{ST}	0.003	0.082	0.026	0.018	0.066	0.055	0.037	0.071	0.050
θ	0.004	0.103	0.032	0.021	0.082	0.065	0.047	0.084	0.061
R_{ST}	-0.004	0.133	0.018	0.027	0.063	0.052	0.059	0.081	0.067

H_O : observed heterozygosity; H_T : expected heterozygosity; G_{ST} : gene differentiation coefficient; R_{ST} : distance measure that incorporate the stepwise mutation process in microsatellites (Slatkin 1995)

similar trend to *A* across populations. Each population was significantly biased from HWE at one to three loci. It was most likely due to the presence of null allele(s), indicated by MICRO-CHECKER test. Genetic variation (H_E) across all populations varied from 0.494 in population Huangshan to 0.611 in population Tiantong (Table 2), and the global expected heterozygosity (H_T) was 0.578. The overall observed heterozygosity (H_O) was 0.403, and the average H_O among the natural populations was 0.383.

Mean allele number (4.188), allele richness (4.064) per locus, and expected heterozygosity (0.544) of the restored populations were lower than those of the natural populations (4.958, 4.892, and 0.552, respectively) (Table 2). The mean observed heterozygosity was higher than that of the natural populations. However, no significance was found for the above differences with *t*-test.

Four alleles in the donor population were absent in samples from the restored populations. Eight alleles present in one or both of the restored populations were not detected in the donor population, but most of them were rare alleles with frequencies lower than 0.05. Given the presumption that alleles in the descendent, restored populations were actually present in the donor population but just absent in the sampled individuals, allele number and allelic richness in the restored populations were much lower than the donor population as well as the other natural populations (Table 2). However, the observed and expected heterozygosities of the restored populations were higher than

those of the donor population, and one of the restored populations had the highest H_O and second highest H_E (Table 2).

Although the value of F_{ST} (0.061) was not high, genetic differentiation among all populations was highly significant ($P < 0.001$). There was a similar value of R_{ST} (0.067). Significant genetic differentiation was also observed between the restored populations and between natural populations (Table 3). The F_{ST} values indicated a difference between the two management types was larger than those within each management type. Restored populations were the least differentiated ($F_{ST} = 0.037$, $P < 0.001$). Bootstrap analysis of the phenogram provides strong support ($P < 0.001$) for differences among populations according to their management types (Fig. 2). The populations were clustered into two groups. The first group comprises three natural populations, and the two restored populations were clustered in the second group. In the first group, the populations Tiantong and Tianmu were more similar.

Pair-wise, genetic identities between the restored populations and the donor population (0.896 and 0.860 for Sanling and Sunqiao, respectively) were smaller than those between the restored and other natural populations (0.930–0.942 for Sanling and 0.884–0.893 for Sunqiao, respectively) (Table 4), which indicates that the restored populations were more genetically similar to other natural populations than to the donor. Differentiation values based on assignment tests produced similar results. The

Table 2 Genetic diversity at SSR markers in the five *Cyclobalanopsis myrsinaefloia* populations

	Restored			Natural			
	Sanling	Sunqiao	Mean	Huangshan	Tianmu	Tiantong	Mean
No. of individuals	37	35	36	28	32	30	30
No. of alleles	31	36	33.5	35	42	42	39.7
No. of Common alleles ($P \geq 0.05$)	26	27	26.5	28	35	35	32.7
Private alleles	0	1	0.5	1	2	2	1.7
<i>A</i>	3.875	4.500	4.188	4.375	5.250	5.250	4.958
A_R	3.752	4.375	4.064	4.351	5.131	5.195	4.892
H_O	0.409	0.455	0.432	0.334	0.419	0.397	0.383
H_E	0.505	0.583	0.544	0.494	0.554	0.611	0.553
F_{IS}	0.191**	0.220**		0.324**	0.242**	0.351**	

A: number of alleles per locus; A_R : allelic richness; H_O : observed heterozygosity; H_E : expected heterozygosity; F_{IS} : inbreeding coefficient. * $P < 0.05$, ** $P < 0.01$

Table 3 Multilocus estimates of hierarchical F -statistics and their statistical significance after 10,000 permutation tests between populations under the same management type, between different management types, and for all populations of *Cyclobalanopsis myrsinaefolia*

Management type	F_{IS}	F_{IT}	F_{ST}
Restored	0.206***	0.235***	0.037***
Wild	0.306***	0.336***	0.044***
Between different management types	0.279***	0.315***	0.049***
Total	0.261***	0.306***	0.061***

*** $P < 0.001$

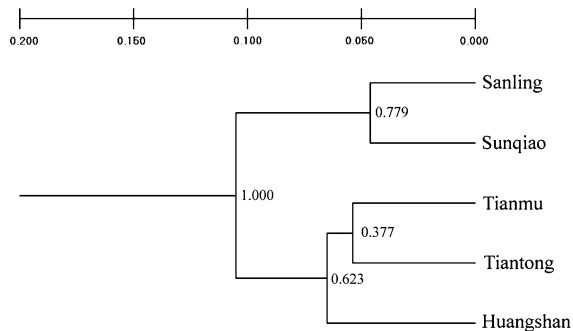


Fig. 2 UPGMA phenogram based on Nei's (1978) genetic distances estimated for five populations of *Cyclobalanopsis myrsinaefolia*. Proportions of similar replicates after 10,000 permutations are shown for each grouping

percentage of correctly assigned individuals in the restored populations, Sanling and Sunqiao, to the donor, Huangshan population, were only 13.5% and 25.7%, respectively, while 54.1 and 40% of individuals in Sanling and Sunqiao were assigned to population Tiantong (Fig. 3). This indicates that the genetic composition of the restored populations was not representative of the donor population.

Table 4 Estimates of Nei's unbiased (1978) identity (lower diagonal) and distance (upper diagonal) for *Cyclobalanopsis myrsinaefolia* populations based on eight polymorphic SSR markers

Population	Sanling	Sunqiao	Huangshan	Tianmu	Tiantong
Sanling	–	0.0461	0.1102	0.0722	0.0603
Sunqiao	0.9549	–	0.1511	0.1235	0.1133
Huangshan	0.8956	0.8598	–	0.0545	0.0757
Tianmu	0.9304	0.8838	0.9469	–	0.0538
Tiantong	0.9415	0.8929	0.9271	0.9476	–

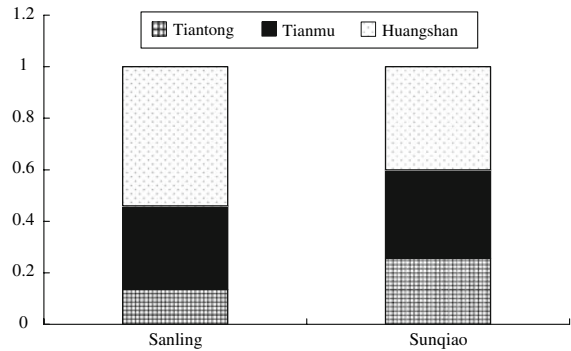


Fig. 3 Percentages of assignment to each population of *Cyclobalanopsis myrsinaefolia* according to Baudoin and Lebrun (2000)

Discussion

Genetic diversity and structure of *Cyclobalanopsis myrsinaefolia*

Six of eight loci in the present study exhibited significant deviations from Hardy–Weinberg expectations, most likely due to the presence of null alleles. The high within natural population F_{IS} of 0.31 (range 0.24–0.35) and most F_{IS} s in each population were positive, though some were not significant, indicated that, besides the presence of null alleles, pronounced inbreeding might be another cause. *Cyclobalanopsis myrsinaefolia* is wind-pollinated species. In its close relative, *C. glauca*, the outcrossing rate was estimated to be 0.332 based on allozyme data (Chen and Song 1997). These data indicated that a mixed mating system in the studied species.

A total of 51 alleles were detected at the eight microsatellite loci employed in this study, with a mean of 6.4 alleles ($n = 162$), which is somewhat higher than that of a group of 20 individuals from Kyoto City, central Japan (6.13 alleles over the same

eight loci, Isagi and Suhandono 1997). The difference is likely due to the larger sample size in the present study. Allelic diversity of *C. myrsinaefolia* is moderate to low and falls at the lower end of the range observed in other tree species (Blakesley et al. 2004; Goto et al. 2004; Kelly et al. 2004; Litrico et al. 2005; Ueno et al. 2000).

The expected heterozygosity was much lower in our study populations (0.49–0.61) than that found in a study of a Japanese population (0.63) (Isagi and Suhandono 1997). It varies considerably among loci, ranging from 0.32 for locus QM63-2M3 to 0.79 for locus QM55GA (Table 1), suggesting that overall diversity values depend on loci which are selected. Hence, a sample of only eight loci may not accurately reflect genome-wide SSR diversity. For wind-pollinated plants with mixed mating system (such as *C. myrsinaefolia*), Hamrick and Godt (1989) reported a mean G_{ST} of 0.2 based on allozymes, similar to that of mixed-mating (0.20) and long-lived perennial (0.19) plants. Due to higher mutation rates, microsatellites usually give decreased values of F_{ST} s compared with allozyme estimates (Balloux and Lugon-Moulin 2002). The estimated F_{ST} (0.044) among natural *C. myrsinaefolia* populations is slightly smaller than an allozyme-based estimate of 0.056 in *C. glauca* (Chen et al. 1997), a dominant tree species with similar distribution. However, the size of the sampling area affects estimates of F_{ST} . Nevertheless, our estimate of population differentiation is similar to those obtained of other long-lived species (Galeuchet et al. 2005; Gustafsson 2000; Otero-Arnaiz et al. 2005).

Effects of ecological restoration on genetic composition

Three of the four parameters of genetic variation were lower in the restored than in the natural populations, indicating a founder effect during the restoration. Compared with the donor populations of *C. myrsinaefolia*, one restored population had lowered number of alleles and allelic richness while another restored population had slightly increased number of alleles per locus and allelic richness. It is interesting that, compared to the donor population, four alleles were absent and eight new alleles were observed in the restored populations. There might be

two sources contributing to the difference. First, sampled individuals of the donor population in our study are not exactly the same as those for collecting seeds. Second, even if all of the individuals that seeds were collected from were sampled for genetic analysis, the seeds still may have been more diverse than the maternal trees because pollen would have also contributed to the gene pool of the seed (Morris et al. 2002).

Unlike the situation of number of alleles and allelic richness, increased heterozygosity was found in the restored populations in comparison to the donor population. This difference was due to the fact that heterozygosity depended more on the evenness of the allele frequencies than on the number of alleles per locus. Differential responses between allele richness and heterozygosity has been observed in many situations, such as in *Rutidosia leptorrhynchoides* (Young et al. 1999), *Primula veris* and *P. vulgaris* (Van Rossum et al. 2004). In each case, the species suffered a population size decrease.

Although no reduction of genetic diversity in restored or regenerated populations was found in some cases (El-Kassaby 2000; Knowles 1985; Thomas et al. 1999; Travis et al. 2002), a reduction of genetic diversity in artificial populations relative to natural populations has been observed in some other species, including *Picea glauca* (Gomory 1992; Rajora 1999), *Zostera marina* (Williams and Davis 1996), *Scaphium macropodum* (Ratnam et al. 2000), *Eucalyptus considianiana* (Glaubitz et al. 2003), *Metasequoia glyptostroboides* (Li et al. 2005), and *Inga edulis* (Hollingsworth et al. 2005). Diverse results of artificial regeneration on genetic diversity of plant populations can generally be explained by differing propensities of the regeneration methods to cause founder effects (Montalvo et al. 1997). For example, vegetative regeneration, such as via cuttings, may lead to low diversity in the artificial populations (Chen 1999).

The fact that the lowest degree of differentiation was found between the two restored populations indicated that these populations are genetically more similar to each other than to the natural populations. This situation has also been observed in restored populations of other plants, for example, in the co-dominant grass species *Andropogon gerardii* and *Sorghastrum nutans* (Gustafson et al. 2004), and in the tree species *Metasequoia glyptostroboides* (Li

et al. 2005). Low differentiation between the restored populations in the present study was easy to understand because they were restored using seedlings germinated from the same seed-lot. However, we did not expect that the differences between the restored populations and the donor population would be larger than those between the restored populations and the other two natural populations (Table 4 and Fig. 3). In accordance with Miyawaki's method, the seeds used for restoration were collected as widely as possible from the donor population and the samples we obtained for genetic analysis were sampled at random from the donor and the restored populations. Furthermore, the artificial populations are thought to be offspring populations of the donor. Previous studies indicated that temporal differentiation is usually much lower than spatial differentiation (Chen et al. 2003; Gregorius et al. 1986; Linhart et al. 1981; Morris et al. 2002; Ueno et al. 2002). Therefore, we expected a high similarity between the restored populations and the donor before conducting the present study. The observed differences are likely the result of unrepresentative seed collection. Although the seeds were collected as widely as possible from the donor population, unequal seed production among the maternal individuals, viability differences among half-sib families and strong competition in the restored sites might lead to changes in gene frequencies in the descendent populations. Biased genetic composition in newlyfounded populations had been observed in many systems (Frankham et al. 2002; Gugerli et al. 2001; Ledig 2000).

Implications for ecological restoration

Ecological restoration has been emphasized in the last two decades (Dobson et al. 1997), and methods have been proposed with the aim of establishing communities that mimic natural ones (for example, Miyawaki 1993; Tucker and Murphy 1997). However, restoration efforts might lead to a decreased and unrepresentative genetic composition in the restored populations as a result of founder effects, as observed here in *C. myrsinaefolia* and in other plants (Li et al. 2005; Williams and Davis 1996).

Genetic characteristics of the population should be well considered in ecological restoration because they might play a critical role in the survival and

persistence of the restored system (Krauss and Koch 2004). In eelgrass, low genetic diversity might have caused restoration failure (Williams and Orth 1998). An extreme example was found in English elm, which was a very common tree in Britain before 1970s. The outbreak of Dutch elm disease ravaged elm populations, and killed more than 25 million trees in Britain. Recent analysis indicated that the tree's genetic uniformity may have helped to fell the entire English population (Gil et al. 2004). For successful restoration, appropriate population genetic characteristics depend on the degree and scale of disturbance (Lesica and Allendorf 1999). Lesica and Allendorf (1999) suggested that local plants or shade-tolerant plants from environments that match the habitat to be restored are best suited to restore sites where degree of disturbance has been low. Mixtures of genotypes from different sources populations at an earlier successional stage may provide the best strategy for restoring highly disturbed sites to which local climax-stage vegetation have not adapted.

Among the studied natural populations, the Huangshan population has the lowest genetic variation according to all parameters. Furthermore, the spatial distance between Shanghai, the restored site, and Huangshan is larger than those between Shanghai and either of other two natural populations. The populations near the restored site are usually in similar climate and soil conditions. Therefore these populations are preferable donors rather than the other populations due to potential local adaptation (Hufford and Mazer 2003; Lesica and Allendorf 1999). Based on the above reasons, whether considering within-population genetic variation or local adaptation (which is usually related to the spatial distance), the Huangshan population is not the best donor for ecological restoration of forests dominated by *C. myrsinaefolia* in Shanghai. Rather, the Tianmu and Tiantong populations are preferable donors. This suggested that, in practice, project managers failed to collect appropriate seeds in ecological restoration, although Huangshan is not far away from Tianmu and Tiantong.

Although the biased genetic composition from the donor was found in the populations of restored vegetations, no deleterious consequences might be expected in this species. First, only small founder effects were found in the restored populations, and the observed heterozygosity is higher in the restored

population, indicating at least in the short-term the genetic diversity is maintained. Second, *Cyclobalanopsis myrsinaefolia* is a wind-pollinated species, and a high gene flow is expected among populations. Gene flow over successive generations will restore the genetic diversity. However, for species with limited gene flow, wrongly selected donors will lead to some deleterious problems, and may lead to restoration failure (Lesica and Allendorf 1999; Montalvo et al. 1997). Therefore, we highlight the population genetic knowledge of dominant species before starting ecological restoration.

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References

- Anand M, Desrochers RE (2004) Quantification of restoration success using complex systems concepts and models. *Restor Ecol* 12:117–123
- Anderson P (1995) Ecological restoration and creation: a review. *Biol J Linn Soc* 56(suppl.):187–211
- Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. *Mol Ecol* 11:155–165
- Blakesley D, Pakkad G, James C, Torre F, Elliott S (2004) Genetic diversity of *Castanopsis acuminatissima* (Bl.) A. DC. in northern Thailand and the selection of seed trees for forest restoration. *New Forest* 27:89–100
- Chen XY (1999) Population genetics considerations for ecological restoration. *Resour Environ Yantze Basin* 9:313–319
- Chen XY, Li YY, Wu TY, Zhang X, Lu HP (2003) Size-class differences in genetic structure of *Metasequoia glyptostroboides* Hu et Cheng (Taxodiaceae) plantations in Shanghai. *Silvae Genet* 52:107–109
- Chen XY, Song YC (1997) Mating system and inferred inbreeding depression of a *Cyclobalanopsis glauca* population in Diaoqiao, Huangshan. *Acta Ecol Sin* 17:462–468
- Chen XY, Wang XH, Song YC (1997) Genetic diversity and differentiation of *Cyclobalanopsis glauca* populations in East China. *Acta Bot Sin* 39:149–155
- Da LJ, Yang YC, Chen M (2004) The method of ecological greening and its application in the construction of the approaching nature plant community in Shanghai. *J Chin Landscape Archit* 4:38–40
- Dobson AP, Bradshaw AD, Baker AJM (1997) Restoration ecology and conservation biology. *Science* 277:515–522
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19:11–15
- El-Kassaby YA (2000). Impacts of industrial forestry on genetic diversity of temperate forest trees. In: Matyas C (ed) *Forest genetics and sustainability*, V63. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 155–169
- Fan XX, Shen L, Zhang X, Chen XY, Fu CX (2004) Assessing genetic diversity of *Ginkgo biloba* L (Ginkgoaceae) populations from China by RAPD markers. *Biochem Genet* 42:269–278
- Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to conservation genetics*. Cambridge University Press, Cambridge
- Galeuchet DJ, Perret C, Fischer M (2005) Microsatellite variation and structure of 28 populations of the common wetland plant, *Lychnis flos-cuculi* L., in a fragmented landscape. *Mol Ecol* 14:991–1000
- Gil L, Fuentes-Utrilla P, Soto A, Cervera MT, Collada C (2004) English elm is a 2,000-year-old Roman clone. *Nature* 431:1053
- Glaubitz JC, Wu HX, Moran GF (2003) Impacts of silviculture on genetic diversity in the native forest species *Eucalyptus sieberi*. *Conserv Genet* 4:275–287
- Gomory D (1992) Effects of stand origin on the genetic diversity of Norway spruce (*Picea abies* Karst.) populations. *Forest Ecol Manage* 54:215–223
- Goto S, Tsuda Y, Nagafuji K, Uchiyama K, Takahashi Y, Tange T, Ide Y (2004) Genetic make-up and diversity of regenerated *Betula maximowicziana* Regel. sapling populations in scarified patches as revealed by microsatellite analysis. *Forest Ecol Manage* 203:273–282
- Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. *J Hered* 86:485–486
- Gregorius H-R, Krauhansen J, Mueller-Starck G (1986) Spatial and temporal genetic differentiation among the seeds in a stand of *Fagus sylvatica* L. *Heredity* 57:255–262
- Gugerli F, Sperisen C, Buchler U, Magni F, Geburek T, Jeandroz S, Senn J (2001) Haplotype variation in a mitochondrial tandem repeat of Norway spruce (*Picea abies*) populations suggests a serious founder effect during postglacial re-colonization of the western Alps. *Mol Ecol* 10:1255–1263
- Gustafson DJ, Gibson DJ, Nickrent DL (2004) Conservation genetics of two co-dominant grass species in an endangered grassland ecosystem. *J Appl Ecol* 41:389–397
- Gustafsson S (2000) Patterns of genetic variation in *Gymnadenia conopsea*, the fragrant orchid. *Mol Ecol* 9:1863–1872
- Hamrick JL, Godt MJW (1989). Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) *Plant population genetics, breeding, and genetic resources*. Sinauer, Sunderland, Massachusetts, USA, pp 43–63
- Hollingsworth PM, Dawson IK, Goodall-Copestake WP, Richardson JE, Weber JC, Montes CS, Pennington RT

- (2005) Do farmers reduce genetic diversity when they domesticate tropical trees? A case study from Amazonia. *Mol Ecol* 14:497–501
- Hufford KM, Mazer SJ (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. *TREE* 18:147–155
- Isagi Y, Suhandono S (1997) PCR primers amplifying microsatellite loci of *Quercus myrsinifolia* Blume and their conservation between oak species. *Mol Ecol* 6:897–899
- Kelly BA, Hardy OJ, Bouvet J-M (2004) Temporal and spatial genetic structure in *Vitellaria paradoxa* (shea tree) in an agroforestry system in southern Mali. *Mol Ecol* 13:1231–1240
- Knowles P (1985) Comparison of isozyme variation among natural stands and plantations: jack pine and black spruce. *Can J Forest Res* 15:902–908
- Krauss SL, Koch JM (2004) Rapid genetic delineation of provenance for plant community restoration. *J Appl Ecol* 41:1162–1173
- Ledig FT (2000) Founder effects and the genetic structure of Coulter pine. *J Hered* 91:307–315
- Lesica P, Allendorf FW (1999) Ecological genetics and the restoration of plant communities: mix or match? *Restor Ecol* 7:42–50
- Li YY, Chen XY, Zhang X, Wu TY, Lu HP, Cai YW (2005) Genetic differences between wild and artificial populations of *Metasequoia glyptostroboides* Hu et Cheng (Taxodiaceae): Implications for species recovery. *Conserv Biol* 19:224–231
- Linhart YB, Mitton JB, Sturgeon KB, Davis ML (1981) Genetic variation in space and time in a ponderosa pine. *Heredity* 46:407–426
- Litrico I, Ronfort J, Verlaque R, Thompson JD (2005) Spatial structure of genetic variation and primary succession in the pioneer tree species *Antirhea borbonica* on La Reunion. *Mol Ecol* 14:1575–1584
- Martin LM, Moloney KA, Wilsey BJ (2005) An assessment of grassland restoration success using species diversity components. *J Appl Ecol* 42:327–336
- 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
- Miyawaki A (1993) Restoration of native forests from Japan to Malaysia. In: Lieth H, Lohmann M (eds) Restoration of tropical forest ecosystems. Kluwer Academic Publishers, Dordrecht, pp 5–24
- Miyawaki A (1998) Restoration of urban green environments based on the theories of vegetation ecology. *Ecol Eng* 11:157–165
- Montalvo AM, Williams SL, Rice KJ, Buchmann SL, Cory C, Handel SN, Nabhan GP, Primack R, Robichaux RH (1997) Restoration biology: a population biology perspective. *Restor Ecol* 5:277–290
- Morris AB, Baucom RS, Cruzan MB (2002) Stratified analysis of the soil seed bank in the cedar glade endemic *Astragalus bibullatus*: Evidence for historical changes in genetic structure. *Am J Bot* 89:29–36
- Namkoong G, Boyle T, Gregorius HR, Joly H, Savolainen O, Ratman W, Young A (1996) Testing criteria and indicators for assessing the sustainability of forest management: genetic criteria and indicators. CIFOR Working Paper No. 10
- Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–590
- Otero-Arnaiz A, Casas A, Hamrick JL, Cruse-Sanders J (2005) Genetic variation and evolution of *Polaskia chichipe* (Cactaceae) under domestication in the Tehuacan Valley, central Mexico. *Mol Ecol* 14:1603–1611
- Palmer MA, Bernhardt ES, Allan JD, Lake PS, Alexander G, Brooks S, Carr J, Clayton S, Dahm CN, Follstad Shah J, Galat DL, Loss SG, Goodwin P, Hart DD, Hassett B, Jenkinson R, Kondolf GM, Lave R, Meyer JL, O'donnell T.K, Pagano L, Sudduth E (2005) Standards for ecologically successful river restoration. *J Appl Ecol* 42:208–217
- Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: A software for genetic assignment and first-generation migrant detection. *J Hered* 95:536–539
- Rajora OP (1999) Genetic biodiversity impacts of silvicultural practices and phenotypic selection in white spruce. *Theor Appl Genet* 99:954–961
- Ratnam W, Lee CT, Muhammad N, Boyle TJB (2000) Impact of logging on genetic diversity in humid tropical forests. In: Matyas C (eds) Forest genetics and sustainability, vol 63. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 171–181
- Reusch TBH, Ehlers A, Hammerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *PNAS* 102:2826–2831
- Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139:457–462
- Song YC (2001) Vegetation ecology. East China Normal University Press, Shanghai
- Stoehr MU, El-Kassaby YA (1997) Levels of genetic diversity at different stages of the domestication cycle of interior spruce in British Columbia. *Theor Appl Genet* 94:83–90
- Thomas BR, Macdonald SE, Hicks M, Adams DL, Hodgetts RB (1999) Effects of reforestation methods on genetic diversity of lodgepole pine: an assessment using microsatellite and randomly amplified polymorphic DNA markers. *Theor Appl Genet* 98:793–801
- Travis SE, Proffitt CE, Lowenfeld RC, Mitchell TW (2002) A comparative assessment of genetic diversity among differently-aged populations of *Spartina alterniflora* on restored versus natural wetlands. *Restor Ecol* 10:37–42
- Tucker NIJ, Murphy TM (1997) The effects of ecological rehabilitation on vegetation recruitment: some observations from the Wet Tropics of North Queensland. *Forest Ecol Manage* 99:133–152
- Tuxen R (1956) Die huetige potentielle natuerliche Vegetation als Gegestand der Vegetationskarierung. *Angew Pflanz* 13:5–42
- Ueno S, Tomaru N, Yoshimaru H, Manabe T, Yamamoto S (2002) Size-class differences in genetic structure and individual distribution of *Camellia japonica* L. in a Japanese old-growth evergreen forest. *Heredity* 89:120–126
- Ueno S, Yoshimaru H, Kawahara T, Yamamoto S (2000) Isolation of microsatellite markers in *Castanopsis*

- cuspidata* var. *sieboldii* Nakai from an enriched library. Mol Ecol 9:1188–1190
- Urbanska K, Webb N, Edwards P (1997) Restoration ecology and sustainable development. Cambridge University Press, Cambridge
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538
- Van Rossum F, De Sousa SC, 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*. Conserv Genet 5: 231–245
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370
- Williams SL, Davis CA (1996) Population genetic analyses of transplanted eelgrass (*Zostera marina*) reveal reduced genetic diversity in southern California. Restor Ecol 4:163–180
- Williams SL, Orth RJ (1998) Genetic diversity and structure of natural and transplanted eelgrass populations in the Chesapeake and Chincoteague Bays. Estuaries 21:118–128
- Wu ZY (1980) Vegetation of China. Science Press, Beijing
- Young AG, Brown AHD, Zich FA (1999) Genetic structure of fragmented populations of the endangered daisy *Rutidosia leptorrhynchoides*. Conserv Biol 13:256–265