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Genetic Evaluation of the Efficacy of In Situ and Ex Situ Conservation of *Parashorea chinensis* (Dipterocarpaceae) in Southwestern China

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The majority of research in genetic diversity yields recommendations rather than actual conservation achievements. We assessed the efficacy of actual in situ and ex situ efforts to conserve Parashorea chinensis *(Dipterocarpaceae) against the background of the geographic pattern of genetic variation of this species. Samples from seven natural populations, including three in a nature reserve, and one ex situ conservation population were studied. Across the natural populations, 47.8% of RAPD loci were polymorphic; only 20.8% on average varied at the population level. Mean population genetic diversity was 0.787 within natural populations and 1.410 for the whole species. Significant genetic differentiation among regions and isolation by distance were present on larger scales (among regions). AMOVA revealed that the majority of the among-population variation occurred among regions rather than among populations within regions. Regression analysis, Mantel test, principal coordinates analysis, and cluster analysis consistently demonstrated increasing genetic isolation with increasing geographic distance. Genetic differentiation within the region was quite low compared to that among regions. Multilocus spatial autocorrelation analysis of these three populations revealed random distribution of genetic variation in two populations, but genetic clustering was detected in the third population. The ex situ conserved population contained a medium level of genetic variation compared with the seven natural populations; it contained 77.1% of the total genetic variation of this species and*

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*91% of the moderate to high frequency RAPD fragments (*f > 0.05*). Exclusive bands were detected in natural populations, but none were found in the ex situ conserved population. The populations protected in the nature reserve contained most of the genetic variation of the whole species, with 81.4% of the total genetic variation and 95.7% of the fragments with moderate to high frequency (*f > 0.05*) of this species conserved. The results show that the ex situ conserved population does not contain enough genetic variation to meet the need of release in the future, and that more extensive ex situ sampling in natural populations TY, NP, HK, and MG is needed. The in situ conserved population contains representative genetic variation to maintain long-term survival and evolutionary processes of* P. chinensis.

KEY WORDS: genetic evaluation; biodiversity conservation; isolation by distance; multilocus spatial autocorrelation analysis; *Parashorea chinensis*.

INTRODUCTION

Geographic differentiation of genetic variation results from limited gene dispersal in many plant species and is summarized in isolation by distance, increased geographic distance leading to increasing genetic isolation of population (Wright, 1943). An extensive investigation of genetic structure over different geographic distances would demonstrate the consequences of isolation by distance and gene flow. Examination of patterns of genetic variation, particularly in combination with ecological and geographic data, can provide insights into the species' recent evolutionary and biogeographic history. In the case of rare or endangered plant species, the analysis of population genetic structure and diversity over different geographic distances can provide important information for the development of a conservation program (e.g., Kang and Chung, 1997; Chung *et al*., 1998; Gemmill *et al*., 1998; Godt and Hamrick, 1998; Li *et al*., 2002; Jin *et al*., 2003). The number of investigations of genetic diversity is large, but the majority of that research yields recommendations rather than actual conservation achievements. Few attempts have been made to use such genetic information to evaluate and guide actual conservation efforts (e.g., Manuder *et al*., 1999; Storme *et al*., 2004).

Parashorea chinensis (Dipterocarpaceae) is a rare dipterocarp restricted to southwest China and adjacent areas of Laos and Vietnam (Fig. 1). On favorable sites, *P. chinensis* can reach diameters of 1.5 m and heights of more than 80 m (Xu and Yu, 1982; Zhu, 1992). Seeds of this species are dispersed mainly by gravity and germinate quickly after falling to the ground, and the seedlings require light for establishment (Ying and Shuai, 1990). *P. chinensis* produces high-quality timber but has a highly fragmented distribution and is currently subject to specific

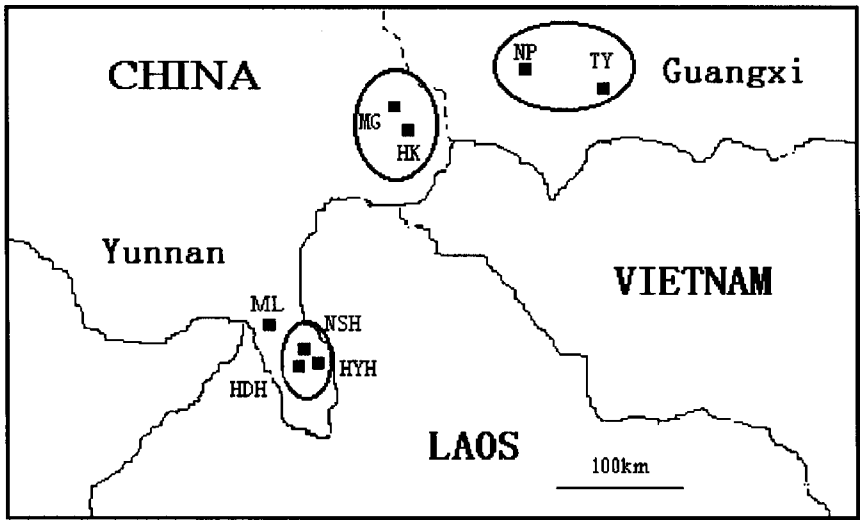

Fig. 1. Relative locations of populations of *Parashorea chinensis* sampled for this study. Ovals encircle the three main distribution ranges of this species.

protection and management plans (Fu, 1992). A comprehensive conservation program has been in place to protect this species since the 1950s, when a national nature reserve was established in Mengla County, protecting 60% of the individuals of *P. chinensis* and many other endangered and rare wildlife species. In the 1980s an ex situ conserved population was established with the transplantation of more than 200 seeds and 50 seedlings in Xishuangbanna Tropical Botanical Garden (China). Little is known, however, about the genetic profile of this ex situ population or about the pattern of genetic variation in the entire species; therefore, the long-term survival of the ex situ population and the efficacy of the in situ conservation efforts are uncertain, leaving further conservation of this species in the dark. More broadly, the spatial and temporal scales at which genetic processes occur in tropical forest ecosystems are largely unknown. The complexity of species composition and ecological diversity can be used for sampling strategies for monitoring genetic diversity of an in situ conservation area and the ex situ conservation of rare and threatened species. There is increasing interest in the management and preservation of genetic diversity in such tropical forest ecosystems. A better understanding of gene flow and genetic isolation within this species will assist in defining the relationships between populations as well as estimating their potential contribution to future evolution. As a result, information on the genetic structure and ecology of *P. chinensis* will be fundamental to the development of long-term efficient conservation strategies.

The aim of this investigation was to assess the geographic pattern of genetic variation within and among populations of *P. chinensis* and to reveal the genetic consequences of geographic isolation at different spatial scales. The ultimate aim is to evaluate the efficiency of current conservation strategies by identifying the amount of genetic variation contained in ex situ and in situ conserved populations and then inferring strategies for further conservation efforts, e.g., determination of in situ conservation site variation and the need for further material collection to reinforce ex situ populations. For this purpose, randomly amplified polymorphic DNA (RAPD) markers were used (Williams *et al*., 1990). The use of RAPDs as markers in population genetic studies for tree species has been well established (Chalmers *et al*., 1992; Yeh *et al*., 1995; Gillies *et al*., 1997; Allnutt *et al*., 1999). The ease and efficiency of this method makes it a desirable option when appropriate statistical analyses are used (Lynch and Milligan, 1994; Stewart and Excoffier, 1996; Parker *et al*., 1998; Smouse and Peakall, 1999), and it is recommended for use in small low-tech laboratories (Kjolner *et al*., 2004). It is particularly attractive because DNA sequence information is not required prior to investigating a previously unstudied species, which facilitates its application to a wide range of taxa that are currently threatened with extinction worldwide (Newton *et al*., 1999).

MATERIALS AND METHODS

Field Sampling

Seven natural populations and one ex situ conserved population were sampled from three main distribution ranges of *P. chinensis* in southwestern China (Fig. 1 and Table I). Leaves were harvested from plants with a diameter at breast height (dbh) greater than 5 cm for each population (Table I). Leaves were dried in the field in silica gel and transported to the laboratory for DNA extraction. Population

Table I. Location of *Parashorea chinensis* Populations Analyzed in This Study

Locality	Code	*N*	*n*	Latitude, Longitude	Altitude (m)
Tianyang, Guangxi AR	TY	120	24	23°42′N, 106°54′E	460
Napo, Guangxi AR	NP	45	24	23°18′N, 105°52′E	650
Hekou, YP	HK	35	24	22°47′N, 103°58′E	600
Maguan, YP	MG	50	24	23°01′N, 104°24′E	600
Nanshahe, Mengla, YP	NSH	32	24	21°31′N, 101°35′E	850
Huiduhe, Mengla, YP	HDH	45	34	21°32′N, 101°34′E	850
Huiyinghe, Mengla,YP	HYH	80	40	21°30′N, 101°36′E	850
Menglun, Mengla, YP	ML	205	24	21°55′N, 101°06′E	850

Note. *N*: estimated population size. *n*: sample size. AR: Autonomous Region. YP: Yunnan Province.

size was estimated roughly by counting individuals with dbh greater than 5 cm. Three populations from the south Yunnan region (NSH, HDH, and HYH) were chosen to investigate the spatial genetic variation in fine geographic scale (i.e., within-population genetic structure). In these three populations, *P. chinensis* forms small, dominant populations, providing a unique opportunity to undertake spatial analysis. The relative physical position of each sampled tree was recorded for those three populations.

RAPD Amplification

Genomic DNA was isolated using a modified CTAB extraction method (Doyle and Doyle, 1990). Twenty arbitrary primers that yielded reproducible and clear amplification products were selected from 132 primers (Shengong Inc.) and were employed in PCR amplification. DNA amplification was performed in a Rapid-cycler 1818 (Idaho Tech.), programmed for an initial 1 min at 94°C, 10 s at 35°C, 20 s at 72°C for two cycles, followed by 40 cycles of 0 s at 94°C, 0 s at 35°C, and 1 min at 72°C, and a final step for 7 min at 72°C (0 s at a temperature means that the target temperature is reached but not maintained for any length of time). Reactions were carried out in a volume of 10 μL containing 50 mM Tris-HCl, pH 8.3, 500 μg/mL BSA, 10% Ficoll, 1 mM Tartrazine, 2 mM $MgCl_2$, 200 μM dNTP, 1 μM primer, 5 ng of DNA template, and 0.5 U *Taq* polymerase. Amplification products were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide and imaged by Bio-Rad imaging devices (Gel Doc 2000 Gel Documentation System) supported by Quantity One (version 4.2). With software and manual verification, fragment size was estimated using the 100–3000 bp DNA ladder as a marker. All PCR reactions were prepared in sterile conditions, and a negative control (in which DNA was omitted) was included with each PCR run.

Data Analysis

Only RAPD bands that could be unequivocally scored were counted in the analysis. Amplified products were scored as a discrete banding state (1 as present and 0 as absent) for each individual tree. Shannon's index of phenotypic diversity (H_0), estimated as $-\sum P_i \log 2P_i$, where P_i is the frequency of the band's presence or absence, was used to quantify the degree of population diversity. Shannon's H is frequently used for RAPD studies because the index is insensitive to bias that may be introduced into data by undetectable heterozygosity (Black-Samuelsson and Andersson, 1997; Parani and Parida, 1997; Gustafson *et al.*, 1999; Maki and Horie, 1999).

We used the AMOVA procedure (Excoffier *et al.*, 1992) to estimate the variance of components of RAPD phenotypes associated with the geographic

nested structure of natural populations, with the partitioning of variation in the genetic diversity among individuals within populations, among populations within regions, and among regions. A further principal coordinates analysis based on genetic distance (Euclidean distance) was used to illustrate the pattern of genetic variation within and among populations (GenAlEx V5.1, Peakall and Smouse, 2001).

Pairwise unbiased genetic distances (Nei, 1978) calculated by Popgen V3.22 (Yeh *et al*., 1999) were used to construct a neighbor-joining tree using the program MEGA 2 (Kumar *et al*., 2001). To test the hypothesis of isolation by distance, geographic distances were obtained with a global positioning system (GPS). Pairwise F_{ST} values were linearly transformed $[F_{ST}/(1 - F_{ST})]$ and regressed on pairwise natural logarithm transformation of geographic distances. This transformation was made because populations are not distributed along a linear transect (Rousset, 1997). Since population pairs are not independent, a Mantel test was used to evaluate if the significance was consistent. A Mantel test on the transformed matrices of pairwise F_{ST} and geographic distance was conducted by GenAlEx V5.1 (Peakall and Smouse, 2001).

For within-population spatial genetic structure analysis, we employed a multivariate approach to the microspatial autocorrelation analysis developed by Smouse and Peakall (1999) for multiallelic dominant loci such as RAPD. Unlike classical spatial autocorrelation analysis, which is usually executed one allele at a time, the procedure is intrinsically multivariate, avoiding the need for an allele-by-allele, locus-by-locus analysis. By combining alleles and loci, this approach strengthens the spatial signal by reducing stochastic noise (Smouse and Peakall, 1999; Peakall *et al*., 2003). In brief, pairwise individual-by-individual genetic distances for the dominant RAPD loci were calculated via the method of Smouse and Peakall (1999). Genetic distance matrices for each locus were summed across loci, under the assumption of statistical independence. A linear pairwise geographic matrix was calculated as the Euclidean distance between x and y coordinates at a site. The spatial autocorrelation coefficient, r, was calculated according to Smouse and Peakall (1999). Then 1000 random permutations were performed to test for statistical significance and to define the upper and lower bounds of the 95% confidence interval. Multilocus spatial autocorrelation analyses were conducted for all three populations separately. All spatial genetic autocorrelations were performed using the software package GenAlEx V5.1 (Peakall and Smouse, 2001).

To evaluate the efficiency of genetic conservation, the amount of genetic diversity in the ex situ conserved population (ML) was compared with that in seven natural populations (NSH, DH, HYH, HK, MG, TY, NP), and the genetic diversities of in situ conserved populations (NSH, HDH, HYH) were compared with that of the entire species.

RESULTS

RAPD Profile and Genetic Diversity Estimates in Natural Populations

The 20 primers revealed polymorphic bands ranging between 0 and 13. The size of amplified bands ranged between 160 and 2080 bp. Of the 253 reliable bands generated by the 20 chosen primers, 121 bands (47.8%) were polymorphic across the seven populations, and the percentage of polymorphic bands varied from 15.4 to 30.0% (with an average of 20.8%) at the population level (Table II).

The highest genetic diversity values were obtained with primer S307, in the population NP; the lowest values were obtained with primer S314, which amplified only monomorphic bands across the seven populations. Interestingly, primer S366 amplified only monomorphic bands in seven populations, while the size of amplified bands varied among populations. The highest and lowest genetic diversity values were obtained in the populations NP ($H_0 = 1.464$) and HDH ($H_0 = 0.473$), respectively. The mean diversity within the seven populations of *P. chinensis* was $H_{pop} = 0.787$, and the total diversity was $H_{sp} = 1.410$ (Table II).

AMOVA Partition

Populations of *P. chinensis* were grouped into three regions, south Yunnan (NSH, HYH, HDH), southeast Yunnan (HK, MG), and southwest Guangxi (TY, NP). Of the total molecular variance, 37.7% was partitioned to regional diversity, 11.4% to the population differences within regional diversity, and 50.9% to individual differences within populations. When the total variance was partitioned without considering the regional distribution of the populations, 43.4% was attributed to population divergence and 56.7% to individual differences within populations. On

Table II. Summary of Genetic Diversity in Eight Populations of *Parashorea chinensis*

Population	PPB (%)	H_0
TY	19.8	0.7656
NP	30.0	1.4640
HK	18.6	0.6870
MG	20.2	0.8133
NSH	17.4	0.5721
HDH	15.4	0.4730
HYH	24.5	0.7215
ML	20.6	0.7029
Population average	20.7	0.7870
Species	47.8	1.4100

Note. PPB: percentage of polymorphic bands, H_0: Shannon's index of phenotypic diversity.

Table III. Analysis of Molecular Variance of Seven Populations of *Parashorea chinensis*

Source of variation	Variance component	% Total variance
Nested analysis		
Variance among groups	0.0265	37.7
Variance among populations within groups	0.0080	11.4
Variance within populations	0.0359	50.9
Analysis among populations		
Variance among populations	0.0274	43.4
Variance within populations	0.0359	56.7
Analysis among groups		
Variance among groups	0.0300	42.5
Variance within groups	0.0406	57.5

Note. Total of 194 individuals sampled from seven populations, and 253 RAPD markers employed. Nested analysis was carried out on all populations.

the other hand, if the total variance was partitioned considering only the regional distribution of individuals, 42.5% would be attributable to regional divergence and 57.5% to individual differences within regions (Table III).

Genetic Consequences of Isolation at Large Scale of Spatial Distance

Regression analyses indicated a significant positive correlation between transformed F_{ST} and geographic distances (Fig. 2; $r^2 = 0.7109$, $p < 0.001$). In

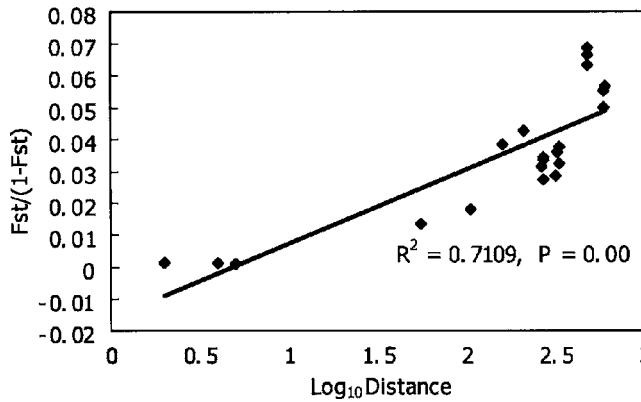

Fig. 2. Plot of transformed F_{ST} and geographic distance matrices showing the pattern of isolation by distance.

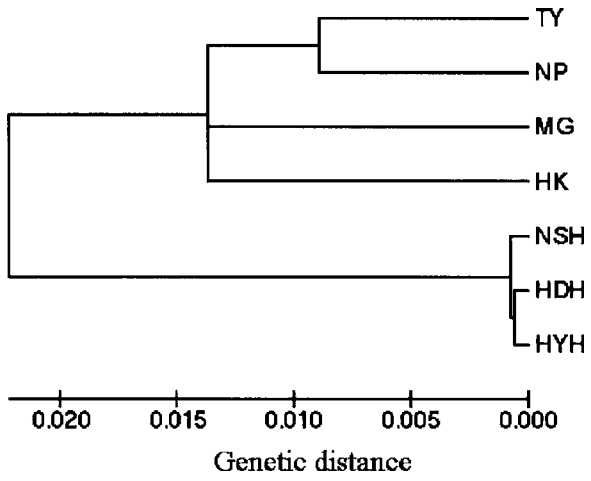

Fig. 3. Neighbor-joining tree of genetic distance, showing the distinctive branches corresponding to the three distribution areas.

addition, the results from the Mantel test indicated that genetic and geographic distance were significantly related ($r = 0.853$, $p = 0.008$). Cluster analysis with neighbor-joining approach of pairwise genetic distance was performed; three distinctive branches, corresponding to three regions, were identified (Fig. 3). A further principal coordinate analysis illustrated the pattern of within- and between-regions RAPD diversity; two principal coordinates distinguished the genetic diversity from three regions, and populations NSH, HYH, and HDH (South Yunnan region) could not be distinguished (Fig. 4).

Size of Individual Tree in Three Populations and Within-Population Genetic Structure

Frequency distributions of tree size in populations NSH and HDH were similar (Fig. 5). Population HYH has more individuals in the second size category but very few in the bigger category. Only two trees (5%) in population HYH were assigned to the biggest group; eight trees (26%) in population NSH and nine trees (27%) in HDH were assigned to the biggest group.

Multilocus spatial autocorrelation analysis revealed different patterns of genetic structure within population in three populations (Fig. 6). The multilocus autocorrelation coefficient r-values were not significantly positive for the first distance class in either population NSH or population HDH. Although not statistically significant, positive r values extend to 25 m in population NSH and 38 m in population HDH, indicating weak spatial clustering of genetic variation

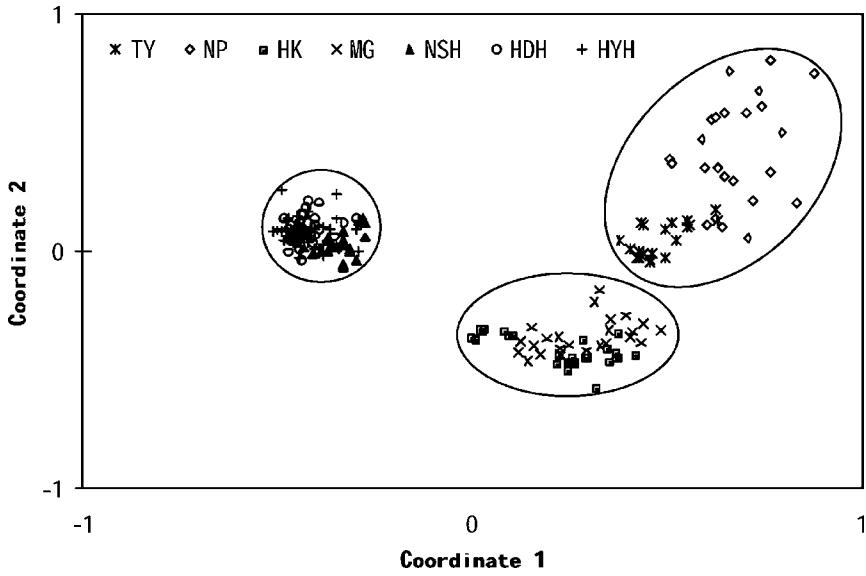

Fig. 4. Principal coordinates analysis showing the genetic pattern of RAPD variation within and among populations. The ellipses indicate the relativity of the three groups and do not represent levels of confidence.

in both populations. Though both correlograms show oscillation of high and low autocorrelation, the clustering patterns are not statistically significant.

In population HYH, r values are positive and significant for the first two distance classes (17 and 27 m), with an x intercept at 36 m, which suggests significant genetic clustering in this population. The correlogram shows clearly the correlation coefficient r as a function of distance, and there is no apparent

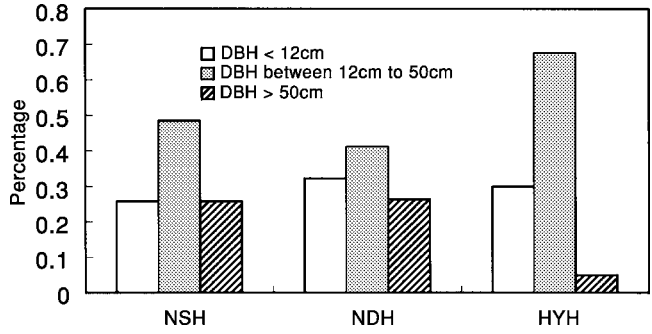

Fig. 5. Frequency of size of sampled trees in three populations. The standard for age classification is arbitrary.

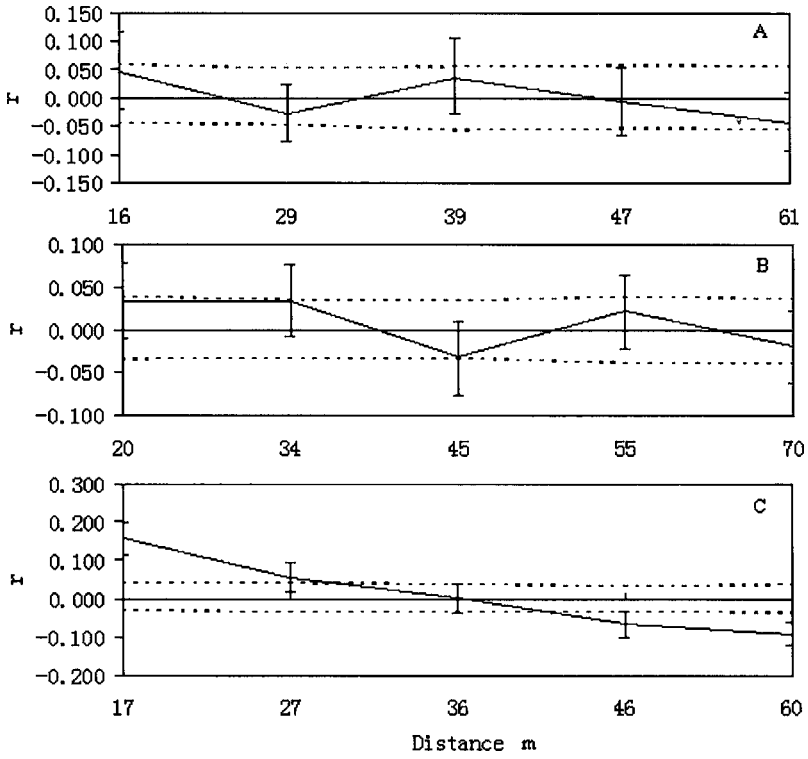

Fig. 6. Genetic correlation *r* as a function of distance in three populations, 95% CI, about the null hypothesis of a random distribution of genotypes shown by dashed line, and 95% confidence error bars about *r* as determined by bootstrapping. (A) Autocorrelation for population NSH. (B) Autocorrelation for population HDH. (C) Autocorrelation for population HYH.

oscillation of high and low autocorrelation. Instead, there is a general decline in *r* with distance. Consistently negative *r* values are found after 36 m.

Genetic Evaluation of ex situ and in situ Conservation

The ex situ population ML generated a total of 195 bands, ranging from 160 to 2080 bp. Of those, 26.7% (52 out of 195) were polymorphic. If the genetic data from the seven natural populations are considered, however, the ex situ population ML represents intermediate genetic variation compared with seven natural populations, with 20.6% of bands (52 out of 253) being polymorphic. The percentage of polymorphic bands in population ML was lower than that in populations NP and HYH, but higher than in populations TY, HK, MG, NSH, and HDH.

Of the 253 bands amplified in the entire species, 195 were detected in the ex situ population ML, suggesting that 77.1% of the total genetic variation is conserved in that population. Also, 187 bands had frequencies greater than 0.05, and 171 were detected in ML, indicating that 91.44% of the alleles with moderate to high frequency ($f > 0.05$) were conserved in that ex situ population. In population TY, 192 bands were generated, of which 170 occurred in population ML; 167 TY bands had frequencies greater than 0.05, and 156 ($f > 0.05$) occurred in population ML, implying that 88.5% of the total population genetic variation and 93.4% of alleles with moderate to high frequency were conserved in ex situ population ML (Table IV). In the other six natural populations, the percentage of the total population genetic variation conserved in ex situ population ML ranged from 82.03% to 92.5%, and the percentage of alleles with moderate to high frequency conserved in this population ranged from 87.6% to 98.8% (Table IV). Of the low-frequency RAPD bands ($f < 0.05$), the natural population TY had 25 bands, and 14 bands occurred in population ML, indicating that about 56.0% of the rare loci were also conserved in population ML (Table IV). The frequencies of the low-frequency alleles of the other six natural populations conserved in population ML ranged from 37.5 to 55.6% (Table IV).

RAPD analysis also revealed exclusive bands in natural populations. Seven RAPD primers produced 16 exclusive bands in populations TY, NP, MG, NSH, and HYH, and the frequencies of exclusive bands ranged from 0.0211 to 0.5000. None, however, was detected in population ML.

Genetic diversities of the populations conserved in the nature reserve (NSH, HYH, and HDH) were lower than those of the populations (TY, NP, HK, and MG) outside the reserve (Table V). In NSH, HYH, and HDH, 30.8% RAPD bands were polymorphic; 44.7% of bands were polymorphic in the populations outside the nature reserve (Table V). Considering the whole species, 221 of 253 RAPD bands were detected in populations NSH, HYH, and HDH, implying that 87.4% of the total genetic variation was conserved in in situ populations. Among the

Table IV. The Amount of Genetic Variation of Natural Populations of *Parashorea chinensis* Conserved in Artificial Population ML

Population	Total bands	Bands detected in ML (%)	Bands with $f > 0.05$	Bands > 0.05 in ML (%)	Bands with $f < 0.05$	Bands < 0.05 in ML (%)
TY	192	170 (88.54)	167	156 (93.41)	25	14 (56.00)
NP	217	178 (82.03)	193	169 (87.56)	24	9 (37.50)
HK	196	171 (87.24)	173	159 (91.91)	23	12 (52.17)
MG	199	176 (88.44)	179	164 (91.62)	20	11 (55.00)
NSH	190	174 (91.58)	167	162 (97.01)	23	12 (52.17)
HDH	187	173 (92.51)	164	161 (98.17)	23	12 (52.17)
HYH	206	186 (90.29)	170	168 (98.82)	36	20 (55.56)
Mean	198	175 (88.38)	173	163 (94.07)	25	13 (51.51)
Species	253	195 (77.08)	187	171 (91.44)	66	25 (37.88)

Table V. Genetic Variation Conserved in In Situ Populations of *Parashorea chinensis*

Population (sample size)	*N* (PPB)	% Bands detected	N_a	N_b (% of species)	N_c	N_d (% of species)
Out of reserve (96)	246 (44.7%)	97.23	189	186 (99.47)	57	60 (90.91)
In reserve (98)	221 (30.8%)	87.35	168	179 (95.72)	53	42 (63.64)
Species (194)	253 (48.2%)	—	187	—	66	—

Note. *N* (PPB): total number of bands (percentage of polymorphic bands). % Bands detected: Percentage of bands detected compared to the whole species. N_a: Number of bands with frequency > 0.05. N_b: Number of bands > 0.05 detected. N_c: Number of bands with frequency <0.05. N_d: Number of bands < 0.05 detected.

total of 253 bands, 187 had frequencies greater than 0.05, and 179 occurred in in situ conserved populations, indicating that 95.7% of alleles with moderate to high frequency ($f > 0.05$) were conserved. Of the low-frequency alleles ($f < 0.05$), 66 RAPD bands were detected, with 42 bands occurring in in situ conserved populations, indicating that about 63.6% of the rare loci were also conserved (Table V).

DISCUSSION

Geographic Pattern of Genetic Variation in *P. chinensis*

Increasing geographic distance will lead to increasing genetic isolation of populations; this is clearly illustrated in our investigation of the genetic pattern along different spatial scales. Isolation by distance is obviously responsible for the genetic distance patterns over large geographic distance found in *P. chinensis*. Significant genetic differentiation is the result of limited capability of long-distance seed dispersal and perhaps subsequent genetic drift in geographically isolated regions. Seeds of *P. chinensis* are gravity-dispersed, with most of them falling within a short distance of the tree crown and with little secondary dispersal. Seeds of *P. chinensis* are recalcitrant and remain viable for only several days (Ying and Shuai, 1990), which further limits effective seed dispersal. Understandably, successful long-distance seed dispersal events will probably be extremely limited. Recent timber harvesting by humans would affect the population size negatively, and small populations are more likely to experience genetic drift than large populations (Ellstrand and Elam, 1993). In addition, adaptation to a different habitat is probably another cause of significant regional genetic differentiation. A conclusion can be drawn from the amplified pattern of primer S366, which amplified different monomorphic bands in different populations. Moreover, ecological and morphological differences between different regions were demonstrated in this species (Zhu, 1992). Supportably, relatively high levels of regional genetic differentiation are not uncommon in tropical tree species with a wide geographical range. For

instance, Spanish cedar (*Cedrela odorata*) is a highly valued timber species native to the American tropics. Analysis indicated that 55% of the total variation recorded was maintained among rather than within populations using RAPDs (Gillies *et al*., 1997). Studies of the leguminous tree *Gliricidia sepium* have also recorded a relatively high degree of population differentiation, where 60% of the variation recorded was among, rather than within, populations (Chalmers *et al*., 1992).

The results of the AMOVA suggest considerable genetic differentiation among regions, but little genetic divergence among populations within regions. Furthermore, random distribution of genetic variation in fine scale was detected in two (out of three) populations of *P. chinensis*. Though no detailed work has been done on the pollination biology of *P. chinensis,* this species is a canopy tree and its flowers are 20–30 m above the secondary canopy, which would be a distinctive attraction for the pollinators over long distances. The present results indicate that despite the high level of fragmentation and small size of subpopulations in *P. chinensis,* there was an extensive network of genetic exchange over the spatial scale of the study (within 3–5 km).

Genetic clustering within populations was expected in populations of *P. chinensis*. Seed dispersal in this species is limited, with seeds being primarily gravity dispersed (Ying and Shuai, 1990). Limited patterns of seed dispersal have been shown to have a significant effect on patterns of spatial genetic structure within plant populations (Chung *et al*., 2000). The existing randomness of genetic variation in *P. chinensis* may result from overlapping seed shadows. Because adult trees are 50–80 m tall, seed may fall some distance away from the maternal trees, and variation in the density of the adults could explain the different patterns of spatial genetic structure in three of the populations. Population HYH has a much lower density of large trees than the other two sites, thus there should be less overlap of seed shadow and greater relatedness at short distance. In addition, these results may be influenced by the exclusion of seedlings with a dbh smaller than 5 cm from the analysis. The significant spatial clustering of genetic variation detected in population HYH may reflect the high ratio of young to old trees recorded in this population. Many tree species exhibit strong genetic structure in young stages, and juvenile cohorts were more structured than adult cohorts (Hamrick *et al*., 1993; Hamrick and Nason, 1996; Doligez and Joly, 1997; Epperson and Alvarez-Buylla, 1997; Aldrich *et al*., 1998; Ueno *et al*., 2000). Such results may be anticipated due to extensive mortality and selection between seedling and adult stages. Theoretical simulations by Doligez *et al*. (1998) showed that the occurrence of symmetric overdominance selection at a few loci might reduce spatial genetic structure at all loci, as would be expected if selection against inbred individuals were involved. No information is available regarding the distance of pollen flow in *P. chinensis,* although pollen dispersal is less likely to influence the spatial pattern of genetic structure compared to seed dispersal (Chung *et al*., 2000).

The total diversity level of *P. chinensis* (percentage of polymorphic bands PPB = 47.8%) and the mean population diversity (PPB = 20.8%) are substantially lower than those of other dipterocarps in tropical areas. Recent studies based on analysis of variation at isozyme loci have revealed considerable genetic variation in natural populations of many dipterocarps. In *Stemonoporus oblongifolius,* the percent of polymorphic loci ranges from 88.9 to 100% (Murawski and Bawa, 1994). Similarly, a high level of genetic variation has been observed in *Shorea megistophylla* (Murawski *et al*., 1994) and *Hopea odorata* (Wickneswari *et al*., 1994). Moreover, considerable variation was found within populations of many Malaysian species of *Hopea* and *Shorea* using RAPD (Wickneswari *et al*., 1996; Harada *et al*., 1994). Recently, a high level of genetic variation has been observed in *Shorea leprosula* (Lee *et al*., 2000b) and *Dryobalanops aromatica* (Lee *et al*., 2000a; Lim *et al*., 2002). Although it is difficult to compare different studies, especially those using different methods of data generation, in our study relatively low levels of genetic variation were revealed in populations of *P. chinensis*. Population genetic structure has a close link to evolutionary history, geographic distribution, and life history. Many researchers (e.g., Ashton, 1982; Xu and Yu, 1982; Zhu, 1996) have suggested that dipterocarps originated from the old Gondwana continent in the early Tertiary and that the Guinea continent was the center of diversity for this family. Currently *P. chinensis* is mainly distributed throughout the south and southeast of Yunnan, southwest of Guangxi Autonomous Region, and scattered in adjacent Laos and Vietnam, where the northern margin of the range of the dipterocarps occurs. The current pattern of distribution could therefore be the result of postglacial recolonization, resulting in reduced genetic variability as a consequence of repeated bottleneck events (Seitz, 1995; Boulton *et al*., 1998).

Efficiency of Genetic Conservation of *P. chinensis*

The goal of ex situ conservation programs is to maintain the species in captivity until habitat restoration allows its release back to nature. Such restoration could take decades or even centuries; therefore, captive populations must be managed as a long-term, multigenerational breeding program. The restored environments will undoubtedly differ from the original habitats and communities. It is therefore critical that the released populations have sufficient genetic variability to provide adaptive flexibility in an uncertain future (Templeton, 1982, 1991).

Assuming that a population's short-term viability has been assured, its long-term viability will probably depend, in part, on the amount of genetic variability it retains. The importance of the absolute level of genetic variability for conservation is debatable. Some suggest that all genetic variation within a species should be captured (Hawkes, 1976), but as Brown and Briggs (1991) have pointed out, this is unrealistic, and it is also unnecessary. First, many low-frequency alleles are unconditionally deleterious and are maintained only as a result of recurrent mutation,

many low-frequency alleles might actually contribute to genotypes that lower the average viability of individuals, and low-frequency alleles are likely to be lost in just a few generations. Second, most adaptively significant variation is contained in alleles found at moderate to high frequency. In short, maintenance of long-term population viability requires an attempt to preserve a representative sample of moderate- to high-frequency alleles, whether the population is managed in its natural habitat or samples are collected for off-site preservation (Templeton, 1991). The current local rarity of such alleles implies their insignificant contribution to present adaptation.

Marshall and Brown (1975) suggested that the objective of genetic conservation is to maintain the population that will contain 95% of all the alleles with a frequency greater than 0.05 at a random locus occurring in the target population. In our study, the ex situ population ML conserves only part of the genetic variation of the whole species (77.1% of the total genetic variance of the species, and 91.4% for the alleles with frequency greater than 0.05), which is lower than the Marshall and Brown standard. Our results implied that the amount of genetic variation conserved in population ML is not enough to sustain long-term and multigenerational survival of *P. chinensis*. To meet the needs of future releases, more genetic variation must be included in this population. On the other hand, the in situ conserved populations maintain most of the genetic variation of the entire species (87.4% of the total genetic variation of the species, and 95.7% for the alleles with frequency greater than 0.05), indicating that the in situ conserved populations in the nature reserve contain sufficient representative genetic variation to maintain the long-term survival and evolutionary process of *P. chinensis*.

Exclusive bands occurring in natural populations were not detected in the ex situ population ML. Five out of 16 exclusive bands occurred in the natural populations with moderate to high frequency. The populations that are distinctive in their DNA traits should have high conservation status (Avise, 1989; Dizon *et al*., 1992). Consequently, to add those exclusive traits to the ex situ population and to assure adaptation to the future restoration environment, more extensive sampling is needed from populations TY, NP, HK, and MG. Considering the high genetic diversity of the populations outside the nature reserve and the extensive population differentiation among the regions, more attention should also be paid to the populations outside the reserve.

Ex situ conservation of rare tropical trees is thought to be difficult, because only a limited number of large woody plants can be cultivated in botanical gardens (Bawa and Ashton, 1991). In our study, even with as many as 200 individuals surviving in botanical gardens, the genetic diversity of *P. chinensis* was not fully represented. On the other hand, limited facilities are available, and inevitable genetic changes from random genetic drift and selection in artificial environments may make it difficult for captive populations to be reestablished in the wild (Soulé, 1987). Although many species are being rescued by ex situ methods, and then being

reintroduced, the primary method for their conservation must be in situ protection and management. It conserves not only the plant species and the habitat in which it lives but also the associated animals on which it may depend for pollination and dispersal of its diasporas, and also the animals, particularly insects, that might depend on the plant species. As suggested in the present work, in situ conservation maintains genetic diversity more efficiently than the ex situ strategy.

Implications for Further Efforts in Conservation of *P. chinensis*

The geographic pattern of genetic variation in *P. chinensis* has important practical implications for conservation and management efforts. First, strategies for the conservation of genetic diversity need to consider not only current threats to a particular region but also the level of diversity in an area. Our data indicate that the area of population NP contains a significant amount of the genetic diversity within *P. chinensis* and that this area should be a priority for conservation, either for traditional in situ approaches or ex situ collecting to reinforce the genetic variation in the ex situ conserved population. In the case of ex situ collection, the distribution pattern of genetic variation within the population could provide guidance. Meanwhile, materials going into an ex situ population could be screened to select individuals that would increase the overall diversity. Second, significant genetic differentiation was revealed between regions in *P. chinensis*. Outcrossing depression may be an important risk in ex situ conservation of this species, which suggests that ex situ collection of enough individuals from different regional populations to assure possible matings between individuals from the same ecological type is needed to avoid outbreeding depression. Third, extensive gene flow within populations and among adjacent populations on a small geographic scale (<4 km) implies that policymakers need to be aware of the importance and complementary role that remnant forest patches and trees play in providing connectivity and enhancing population variability when designing the nature reserve.

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