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A preliminary study of genetic variation in the endangered, Chinese endemic species *Dysosma versipellis* (Berberidaceae)

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Abstract. This study represents a preliminary analysis of allozyme variation in *Dysosma versipellis* (Berberidaceae), an endangered plant species endemic to China. Five populations of *D. versipellis* and one population of *D. pleiantha* were sampled and analyzed using starch gel electrophoresis of nine enzymes that corresponded to nine interpretable loci. Levels of genetic polymorphism within populations (means: P = 15.54%, A = 1.16, He = 0.045) were much smaller than values for seed plants in general (P = 34.2%, A = 1.53, He = 0.113), as well as values for other endemic species (P = 26.3%, A = 1.39, He = 0.063). Mean values for the F_{ST} across all *D. versipellis* populations tended to be high (F_{ST} = 0.468). An indirect estimate of the number of migrants per generation (Nm = 0.284) indicated that gene flow is low among populations of *D. versipellis*. Additionally, analysis of genetic variation revealed a substantial heterozygosity deficiency in all analyzed populations except HB. Genotype frequencies within *D. versipellis* populations indicate that they may be severely inbred, making inbreeding depression a possible explanation for the low seed set observed in this species. Likewise, the low level of genetic diversity observed within *D. versipellis* populations suggests that clonal reproduction might be more important than sexual reproduction for *D. versipellis*. In comparison, genetic variation observed in one population of the closely related species *D. pleiantha* was much higher than the variation within *D. versipellis* populations. On the basis of these observations, we suggest that in situ conservation will be an important and practical measure for maintaining this species. If ex situ conservation is pursued, sampling should cover all populations across the species' distribution so as to retain as much genetic diversity as possible.

Keywords: Allozyme; Asexual reproduction; *Dsma versipellis*; Endangered species; Genetic variation.

Introduction

Plants in the genus *Dysosma* (Berberidaceae), with seven species, occur solely in China. Populations of the endangered species *D. versipellis*, an herbaceous perennial species that grows in the understory of mixed evergreen and deciduous forests (Ying et al., 1993), have a further restricted distribution in the East and South China. *Dysosma pleiantha* is another rare species in the genus, but little is known about its biology. However, it is clear that these two species have non-overlapping distributions and differ substantially in both leaf and flower morphology (Ying et al., 1993). These observations suggest that the breeding systems of some *Dysosma* species may have undergone divergent evolution resulting from adaptation to different ecological conditions. *Dysosma versipellis* reproduces sexually through cross-pollination and asexually by spreading rhizomes. Field observations indicates that although the plants flower almost every year, seed production is limited, and seedling establishment is poor. Propagation, therefore, appears to occur mostly by vegetative means (Ma, 2000; Qiu and Qiu, 2002). In recent years, natural populations of this species have declined considerably due to anthropogenic activities like habitat destruction and overcollecting for medicinal applications. The rhizome of *Dysosma* has been found to be a source of podophyllotoxin, the active ingredient used as a starting compound for the chemical synthesis of etoposide (VP-16-213) and teniposide (VM-26), effective agents in the treatment of lung cancer, a variety of leukemias, and other solid tumor diseases (Jackson and Dewick, 1984, 1985). Nearly all remaining populations of *D. versipellis* are now located within protected nature reserves, and the species is classified as endangered in the Chinese Plant Red Book (Fu, 1992). Because the species is endangered, and harvesting of rhizomes continues to exceed the rate of natural regeneration, immediate attention should be given to conserving the species through in situ and/or ex situ approaches.

Resource managers responsible for the conservation and recovery of threatened plant species frequently need to make choices regarding which areas are in need of greatest protection, which populations should have priority for

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preservation, what the minimum number of individuals required to avoid inbreeding and to sustain genetic variation is, as well as how germplasm should be collected from a population to capture representative genetic diversity for ex situ conservation (Torres et al., 2003). Thus, understanding how genetic variation is distributed among individuals and populations must be considered together with evolutionary history, population dynamics, breeding system, and species/population structure in evaluating the critical attributes of rare species, and such knowledge is of paramount importance for developing recovery plans that can meaningfully sample and preserve genetic diversity (Falk and Holsinger, 1991). To our knowledge, this study represents the first attempt to characterize the genetic diversity within and among populations of *D. versipellis*.

Among the many molecular techniques available for evaluating plant genetic variation, allozyme electrophoresis is more than adequate to address many conservation genetics issues. This technique is amenable for conservation genetic surveys because data can be obtained quickly for many individuals. The approach involves analysis of single-gene codominant molecular markers (loci) that are biparentally inherited and easily assessed by visualizing band patterns and intensities on starch electrophoretic gels. This approach has made it possible to better understand the spatial distribution of clones and genetic diversity maintained within and among plant populations (e.g., Parker and Hamrick, 1992; Berg and Hamrick, 1994).

In the present study, we used enzyme electrophoresis to assess whether patterns of genetic diversity are consistent with clonal structure and low levels of sexual reproduction observed across *D. versipellis* populations in the field. Specifically, we addressed the following questions: (1) Are *D. versipellis* populations typically composed of one or multiple genets? (2) What are the levels and distribution of genetic variability within and among populations of *D. versipellis*? (3) What are the possible factors that might explain the patterns and levels of genetic variation observed? Additionally, we present measures of allozyme variation within and among populations of *D. versipellis*, and compare these to data published for other plant taxa with similar characteristics (Ellstrand and Roose, 1987; Hamrick and Godt, 1990). It is our hope that such information will be used by conservation biologists to formulate effective management strategies based on genetic data for the conservation of this endangered species.

Materials and Methods

Plant Material and Population Sampling

Dysosma versipellis is a perennial rhizomatous herb that undergoes both sexual and asexual reproduction. Individual plants grow from rhizome and typically reach 40 to 150 cm in height, with an unforked stem bearing one or two alternately arranged leaves, which are rounded, centrally peltate, and 4-9-lobed with finely dentate margin. Plants remain in a juvenile phase for 4-5 years. When mature, plant sends out a shoot in early March, and a terminal cyme emerges on the shoot in April with 5 to 8 drooping red-purple flowers. Fruits mature in late June (Figure 1) (Ying et al., 1993; Qiu and Qiu, 2002).

Dysosma versipellis occurs in the undergrowth of subtropical forests between 200 and 3,500 m elevation, and is distributed across Sichuan, Anhui, Hunan, Guanxi, Jianxi and Hubei Provinces. From 1998 to 2002 several extensive collecting trips for *D. versipellis* were conducted across China. The sampling included five fragmented populations located across the species entire range. Samples from one population of *D. pleiantha* were also collected (Figure 2). Due to the different sizes of the populations, the number of samples collected ranged from 12 to 22. The distance between adjacent samples was at least 10 m to increase the likelihood of sampling non-clonal, inter-individual variation within each population. Samples were taken from the edges, as well as the interior of populations. A total of 100 young leaf samples were taken from randomly chosen putative individuals. The leaf material was transported in a hand cooler to the laboratory, where it was stored at -80°C.

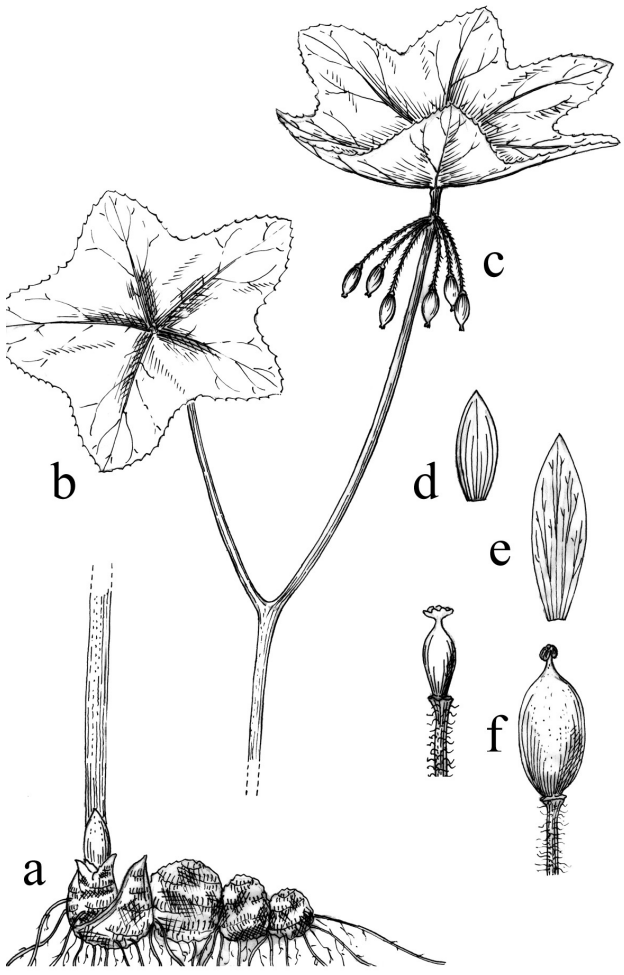

Figure 1. *Dysosma versipellis.* a, rhizome and roots; b, leaf; c, cyme; d, sepals; e, petal; f, fruit.

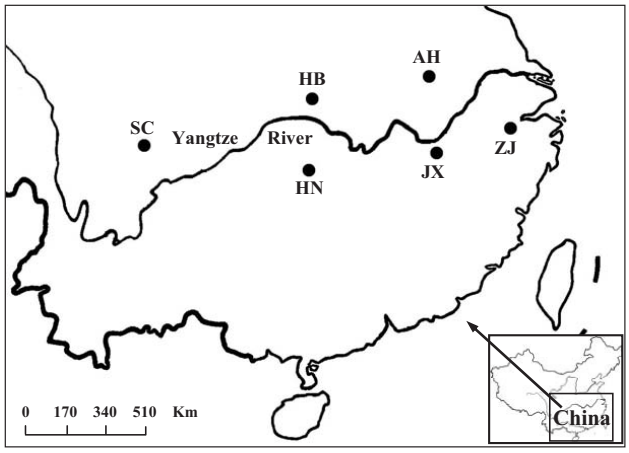

Figure 2. Location of the five natural populations of *Dysosma versipellis* and one population of *Dysosma pleiantha* (ZJ) included in this study. SC = Sichun population; HB = Hubei population; HN = Hunan population; AH = Aihui population; JX = Jiangxi population; and ZJ = Zhejiang population.

Enzyme Extraction and Electrophoresis

Leaves were ground in cold extraction buffer consisting of 0.2 M Tris-HCl (pH = 7.5), 2 mM EDTA, 0.12 M $Na_2S_2O_5$, 1 M $MgCl_2$, 80 mg/mL, PVP, and 40 µL/mL mercaptoethanol. Extracts were absorbed onto Whatman No. 1 filter papers subjected to electrophoresis on horizontal 12.5% starch gels. Enzymes were resolved and scored by starch-gel electrophoresis. The electrophoresis methods followed those of Soltis et al. (1983). After initially screening 15 enzymes, nine were chosen for further analysis. Alcoholdehydrogenase (*Adh*), isocitrate dehydrogenase (*Idh*) and malic enzyme (*Me*) were resolved on buffer system 6 (S11) (Soltis et al., 1983) while phosphoglucoisomerase (*Pgi*), nadh-diaphorase (*Dia*), shikimate dehydrogenase (*Skd*), 6-phosphogluconate dehydrogenase (*Pgd*), triose-phosphate isomerase (*Tpi*) and phosphoglucomutase (*Pgm*) were resolved on buffer system 12 (S12) according to Soltis et al. (1983). After electrophoresis, gels were sliced into layers for staining following the protocols reported by Weeden and Wendel (1989) with slight modifications. Putative loci were designated sequentially. (The most anodally migrating isozyme was designated "1", the next "2", and so on.) Similarly, allelic variation at a locus was coded alphabetically, with the most anodally migrating allozyme designated as allele "a".

Data Analysis

Allozyme diversity was estimated for each subpopulation using BIOSYS-1, Version 1.7 (Swofford and Selander, 1989). Data were entered as genotype numbers from which allele frequencies were calculated. Unbiased estimates of Nei's genetic identity (*I*) and genetic distance (*GD*) (Nei, 1972) were calculated. The levels of genetic variability within subpopulations were estimated using four variables: the mean number of alleles (*A*) per locus, proportion of polymorphic loci (*P*) (95% criterion) per subpopulation, the observed heterozygosity (*Ho*), and the mean expected heterozygosity (*He*). Differences in both population and sample size confound direct comparison of the genetic diversity in this sample site with that of other sites. To more directly compare levels of genetic diversity, we controlled for differences in sample size by randomly choosing twelve individuals from each site and calculating all genetic diversity summary statistics on this reduced data set. This comparison allowed us to determine whether the observed levels of diversity in the small population were comparable to those found in equivalent samples from larger populations.

Wright's *F* [*F* = (1- *Ho* / *He*)], the inbreeding coefficient, measures the deviation of population genotypic composition from Hardy-Weinberg (H-W) expectations. The inbreeding coefficient was calculated at each polymorphic locus and tested for significant deviation using chi-square tests (Li and Horvitz, 1953). The average fixation indices were also calculated for each population and tested for significant difference from zero.

The partition of total genetic diversity into within- and among-population components was examined using Nei's (1973, 1978) genetic diversity statistics. For each polymorphic locus, total genetic diversity (H_T) was partitioned into diversity within populations (H_S) and diversity among populations (and D_{ST}) as $H_T = H_S + D_{ST}$. A measure of genetic differentiation among populations relative to the total genetic diversity (G_{ST}) was calculated at each polymorphic locus ($G_{ST} = D_{ST} / H_T$). The genetic structure within and among populations was also evaluated using Wright's (1965) *F*-statistics: F_{IT}, F_{IS} and F_{ST}. The F_{IT} and F_{IS} coefficients measure excesses of homozygotes or heterozygotes relative to the panmictic expectations within the entire samples and within populations, respectively. The F_{ST} coefficient estimates relative population differentiation. Deviation of F_{IT}, F_{IS} and F_{ST} from zero were tested using chi-square tests (Li and Horvitz, 1953; Workman and Niswander, 1970). A rough estimation of the quantity *N*m (*N* = population size, m = migration rate) was also estimated using Wright's (1951) formula $F_{ST} = 1 / (1+4Nm)$.

To examine the genetic relationship among populations, a dendrogram was constructed using a UPGMA analysis as implemented by NTSYS-pc, Version 2.02c (Rohlf, 1997). In order to test for a correlation between genetic distances (*GD*) and geographical distances (measured in km) among populations, a Mantel test was performed using the program TFPGA (Miller, 1997). The null hypothesis refers to the absence of association between the elements of the pairs of matrices. A normalized *Z* test was performed in which the observed value after 1000 permutations should be significantly larger than that expected by chance in order for an association to be accepted as valid.

Clonal diversity (Fager, 1972; Ellstrand and Roose, 1987) was evaluated by the following indices: (1) number of genotypes, *G*; (2) the mean clone size, *N*c = *N* / *G*, where *N* represents the sample size; (3) proportion of distinguishable genotypes, *PD* = *G* / *N* ; (4) a modified version of the

Simpson diversity index, $D = 1- [Ni(Ni - 1) / N(N - 1)]$, where *Ni* is the number of samples of the *i*th genotype; and (5) a Fager index, $E = (D - D_{min}) / (D_{max} - D_{min})$, where $D_{min} = (G - 1)(2N - G) / N(N - 1)$ and $D_{max} = (G - 1) N / G (N - 1)$. The Simpson diversity index (*D*) was originally developed as a measure of species diversity and has been employed to measure clonal diversity within populations (Parker, 1979; Ellstrand and Roose, 1987). The *D* value ranges from 0 to 1, where 0 reflects all individuals containing the same multilocus genotype, and 1 is for each individual having a unique multilocus genotype. In addition, Fager's *E* value describes the evenness of distribution of genotypes within a population and varies between 0 (when all individuals in a population possess the same genotype) and 1 (when a population has completely uniform genotype frequencies).

Results

Within-Population Variation and Population-level Homozygosity

Of the nine loci resolved, seven were polymorphic across the range of *D. versipellis*. Percentage of polymorphic loci at the population level for *D. versipellis* ranged from 0.0% at JX, to 33.3% at AH, with a mean of 15.54% across populations. The mean number of alleles (*A*) per locus ranged from 1.0 at JX, to 1.3 at AH for *D. versipellis*. The mean within population genetic diversity (*He*) was 0.045. Population AH had the highest expected diversity (0.061) while Population JX had the lowest (0.000) (Table 1). Genetic variation within the one sampled population of *D. pleiantha* (means: *P* = 55.60%, *A* = 1.80, *He* = 0.208) was much higher than that of examined populations of *D. versipellis*. Population level values for *D. versipellis* (means: *P* = 15.54%, *A* = 1.16, *He* = 0.045) were much smaller than average values measured for other seed plants (*P* = 34.2%, *A* = 1.53, *He* = 0.113), as well as for other endemic species (*P* = 26.3%, *A* = 1.39, *He* =0.063) (see Hamrick and Godt, 1990). Across all populations, the mean number of alleles per locus for *D. versipellis* was 1.80 (*N* = 84), and the percentage of polymorphic loci (95% criterion) was 77.78%. Since the genetic diversity results may correlate with sample size of a population, an equal number of individual (twelve) randomly selected from each population was analyzed. When *N* = 12 in all populations, the average value of *P* was 15.54%, *A* was 1.14, *He* was 0.047, and *Ho* was 0.022. Results showed that the values of *A* and *Ho* obtained from the twelve random individuals of each population were a little lower and *He* a little higher than the original ones, but the trend was the same (Table 1). Thus, the apparently high levels of diversity at AH population (Table 1) appear not to be the result of a large sample size.

Table 1. Genetic variability in five populations of *Dysosma versipellis* and one population of *Dysosma pleiantha*.

Population	Genetic diversity indices								H-W deviations			
	N	A	*P* [1]	*Ho*	*Ho**	*He*[2]	*He**	*F*	Tests	HE	HD	NS
Dysosma versipellis												
HN	22	1.20	11.10	0.030	0.037	0.055	0.056	0.455	2	0	2	0
		(0.1)		(0.030)	(0.034)	(0.045)	(0.056)					
JX	15	1.00	0.00	0.000	0.000	0.000	0.000	–	0	0	0	0
		(0.1)		(0.000)	(0.000)	(0.000)	(0.000)					
AH	15	1.30	33.30	0.022	0.019	0.061	0.068	0.639	3	0	3	0
		(0.2)		(0.022)	(0.019)	(0.034)	(0.036)					
SC	12	1.10	11.10	0.000	0.000	0.052	0.052	1.000	1	0	1	0
		(0.1)		(0.000)	(0.000)	(0.052)	(0.052)					
HB	20	1.20	22.20	0.067	0.056	0.059	0.061	-0.136	2	0	1	1
		(0.1)		(0.067)	(0.056)	(0.048)	(0.045)					
Mean		1.16	15.54	0.024	0.022	0.045	0.047	0.467				
		(0.1)		(0.027)	(0.024)	(0.025)	(0.027)					
Species	84	1.80	77.78	0.028		0.085						
		(0.1)		(0.017)		(0.036)						
Dysosma pleiantha												
ZJ	16	1.80	55.60	0.000	0.000	0.208	0.230	1.000	5	0	5	0
		(0.3)		(0.000)		(0.077)	(0.079)					

N, sample sizes; *A*, observed number of alleles per locus; *Ho*, observed heterozygosity; *Ho**, *Ho* standardized by sample size; *He*, expected heterozygosity; *He**, *He* standardized by sample size; *P*, percentage of polymorphic loci; *F*, fixation index. Standard errors in parentheses. Tests indicate the number of loci with a significant deficiency of heterozygotes; HE, the number of loci with a significant excess of heterozygotes; HD, the number of loci with a significant deficiency of heterozygotes; and NS, the number of loci with nonsignificant inbreeding coefficients. The abbreviations HN, AH, HB, SC, JX and ZJ see Figure 2.

[1]A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

[2]Unbiased estimate (see Nei, 1978).

In general, observed genotype frequencies were significantly different from H-W expectations. Indeed, of eight inbreeding coefficients calculated, only one was not significantly different from zero. Such results were obtained from locus *Skd* in population HB, which showed a negative but insignificant *F* index (F = -0.429; P = 0.119). Accordingly, the average fixation index (F = 0.467) is significantly higher than zero for the analyzed populations, except for HB (Table 1).

Clonal Diversity

The clonal diversity and evenness of genotype distributions of the five populations of *D. versipellis* are summarized in Table 2. Ten genotypes were identified in 84 individuals of *D. versipellis*. Four populations were comprised of more than one genet while the JX population appeared to consist of only one. The largest number of genets, five, was found in the AH population. Among the five populations of *D. versipellis*, average clone sizes ranged from 3.00 (AH) to 15.00 (JX), and the mean values of *D* and *E* were 0.458 and 0.528, respectively (Table 2).

Distribution of Genetic Variation and Population Divergence

The estimates of population genetic structure using Nei's genetic diversity statistics are shown in Table 3. The average of total heterozygosity (H_T) and intrapopulation genetic diversity (H_S) were 0.083 and 0.046, respectively. The coefficient of genetic differentiation among population (G_{ST}) varied from 0.004 (*Idh*) to 0.668 (*Dia*), with a mean of 0.445. The results indicated that 44.5% of total genetic diversity is among populations, 55.5% representing intrapopulation genetic diversity. In the five populations of *D. versipellis* studied here, the mean overall F_{IS} of 0.455 was statistically different from zero, suggesting that most of the populations deviate from Hardy-Weinberg expectation within populations (Table 3). The mean overall F_{IT} of 0.710 was also statistically different from zero, indicating nonequilibrium conditions across populations of the species and a deficiency of heterozygotes. The mean F_{ST} over all *D. versipellis* populations was 0.468. The *D. pleiantha* population (from ZJ) differs considerably from all *D. versipellis* populations, as depicted in the UPGMA clus-

Table 2. Clonal diversity and distribution uniformity in five populations of *Dysosma versipellis* and one population of *Dysosma pleiantha.*

	N	*G*	*Nc* (=*N* / *G*)	*PD* (=1/ *Nc*)	*D*	*E*
Dysosma versipellis						
HN	22	4	5.50	0.182	0.636	0.716
JX	15	1	15.00	0.067	0.000	0.000
AH	15	5	3.00	0.033	0.629	0.401
SC	12	2	6.00	0.167	0.485	0.840
HB	20	3	6.67	0.150	0.542	0.685
Mean		3	7.23	0.120 (0.066)	0.458 (0.264)	0.528 (0.336)
Species	84	10	8.40	0.119	0.655	0.6375
Dysosma pleiantha						
ZJ	16	10	1.60	0.625	0.942	0.867

N, Sample size; *G*, number of genotypes; *N/G*, average size of genotypes; *PD*, proportion of distinguishable genotypes; *D*, Simpson index; *E*, Fager index; Standard errors in parentheses. The abbreviations HN, AH, HB, SC, JX and ZJ see Figure 2.

Table 3. Nei's (1973) genetic diversity indices, *F* statistics for 7 polymorphic loci of 5 populations in *Dysosma versipellis.*

Locus	Nei's genetic diversity indices				*F* statistics		
	H_T	H_S	D_{ST}	G_{ST}	F_{IS}	F_{IT}	F_{ST}
Adh	0.020	0.020	0.000	0.011	1.000 ***	1.000 ***	0.040 **
Dia	0.249	0.083	0.166	0.668	0.313 ***	0.781 ***	0.681 ***
Gdh	0.252	0.121	0.130	0.518	1.000 ***	1.000 ***	0.545 ***
Idh	0.027	0.027	0.000	0.004	1.000 ***	1.000 ***	0.054 ***
Pgi	0.065	0.058	0.007	0.108	0.280 ***	0.379 ***	0.138 ***
Pgm	0.019	0.018	0.001	0.014	1.000 ***	1.000 ***	0.037 ***
Skd	0.113	0.086	0.028	0.243	-0.429 ns	-0.064 ns	0.255 ***
Mean	0.083	0.046	0.037	0.445	0.455 ***	0.710 ***	0.468 ***

F_{IS}, deviations from Hardy-Weinberg expectations within individual populations; F_{IT}, deviations from Hardy-Weinberg expectations across all populations; H_T, total genetic diversity; H_S, mean within-population genetic diversity; D_{ST}, genetic diversity among populations; G_{ST}, proportion of total genetic diversity partitioned among populations; Significance level: ***= p<0.001, **= p<0.01, * p = < 0.05, ns = nonsignificant.

ter analysis (Table 4; Figure 3). The mean genetic identity between the two species was 0.885 (Nei's genetic distance = 0.115). No significant correlation was found between genetic distance and geographic distance (r=0.155; P = 0.652) based on the Mantel test. The one-tail probability, P coefficient [random $Z \leq$ observed Z], indicated that the null hypothesis can not be rejected, suggesting no clear geographical pattern of isolation-by-distance in the distribution of the species' genetic variability.

Discussion

As it has been well documented that breeding system and geographic range are good predictors of genetic parameters at the population level (Hamrick and Godt, 1990), it is useful to compare the level of genetic variation in *D. versipellis* to the levels documented for other seed plants with a similar life history, geographic range, and breeding system. The percentage of polymorphic loci (15.54%) found in *D. versipellis* is lower than what was reported for other endemic species (26%) and predominantly clonal species (29%). Similarly, the genetic diversity value (*H*e = 0.045) is much lower than the mean value calculated by Hamrick and Godt (1990) for 338 species of dicotyledonous plants (*H*e = 0.09) and 56 species of sexual and asexual taxa (*H*e = 0.10). The mean "proportion of distinguishable genotypes" per population for *D. versipellis* was 0.119, which is also lower than the value of 0.17 for the 21 species summarized by Ellstrand and Roose (1987), and well below the mean of 0.27 for 45 species reported by Widen et al. (1994). However, it is quite similar to the mean value of 0.13 for asexual species in which sexual reproduction is uncommon (Widen et al., 1994).

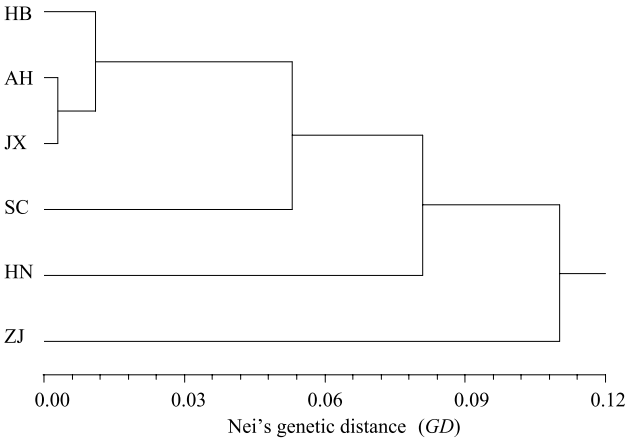

Figure 3. UPGMA dendrogram showing relationships among five populations of *Dysosma versipellis* (HB-HN) and one of *Dysosma pleiantha* (ZJ) based on Nei's unbiased genetic distances (*GD*).

In addition, the fixation indices for the sampled *D. versipellis* populations indicate that most deviate from Hardy-Weinberg equilibrium, and that there is a substantial deficiency of heterozygotes. The high F_{IS} levels in the species probably indicate some selfing and/or intra-clone outcrossing events caused by clonal reproduction and limited seedling recruitment. The species is self-incompatible, and insect pollinators tend to visit adjacent flowers (Qiu and Qiu, 2002). Fruits and developing seeds resulting from these pollinations may abort at various stages of development as a result of self-incompatibility in this taxon. Limited seedling recruitment undoubtedly also contributes to the maintenance of low within-population heterozygosity in *D. versipellis*. Further loss of genetic variability may also result from succession because of reduced seedling establishment combined with the elimination of genotypes via competition, poor adaptation, or stochastic events (McNeilly and Roose, 1984).

Clonal diversity and genetic structure in populations of clonal species varies greatly. Some endangered plant species, especially those with little or no sexual reproduction, such as *Taraxacum obliquum* (Van Oostrum et al., 1985; Ellstrand and Roose, 1987) and *Haloragodendron lucasii* (Sydes and Peakall, 1998), have only one or a few genets across all populations. For other species, the number of genets varies greatly among populations, with some populations consisting of only one genet and others supporting many (Aspinwall and Christian, 1992; Eckert and Barrett, 1993; Ayres and Ryan, 1997). In asexual populations, genotypic diversity is expected to reach a climax over time until a single genotype reaches fixation (Parker, 1979). *Dysosma versipellis* is described as self-incompatible, and Ma (2000) believed that colonies in the wild may derive from a single seedling, which corresponds to a single genotype growing in clonal patches. However, contrasting this view, only the JX popu-

Table 4. Coefficients of Nei's (1978) unbiased genetic distance (below diagonal) and unbiased genetic identity (above diagonal) for five populations (HB-HN) of *Dysosma versipellis* and one population (ZJ) of *Dysosma pleiantha*.

Population	HB	SC	AH	JX	HN	ZJ
HB	****	0.937	0.985	0.990	0.927	0.927
SC	0.065	****	0.954	0.950	0.886	0.855
AH	0.015	0.047	****	0.996	0.933	0.914
JX	0.010	0.052	0.004	****	0.940	0.917
HN	0.076	0. 120	0.069	0.062	****	0.845
ZJ	0.073	0.156	0.090	0.087	0.168	****

The abbreviations HN, AH, HB, SC, JX and ZJ see Figure 2.

lation of *D. versipellis* comprised one genet while others appeared to consist of several genets in this study. Plausible explanations for such results could be (a) mutation; (b) occasional occurrence of sexual reproduction resulting in unique genotypes; or (c) differential selection in a spatially heterogenous environment (Ellstrand and Roose, 1987). Mutation is unlikely to play an important role in maintaining genetic variation in *D. versipellis* owing to low frequency of mutations. Occasional occurrence of sexual reproduction leading to seed set may contribute towards maintaining genetic diversity in some populations of *D. versipellis*. Overall, the low level of genetic diversity in *D. versipellis* provides evidence of a bias towards asexual reproduction although some sexual reproduction apparently does take place.

For species that reproduce sexually, populations usually contain many genets (Ellstrand and Roose, 1987; Eriksson and Bremer, 1993). The ZJ population of *D. pleiantha* that we examined appears to consist of multiple genets. The relatively high clone diversity and small clone sizes of *D. pleiantha* indicate that sexual reproduction is likely more important than clonal reproduction in this particular species; indeed, the high fruit production observed in natural populations of *D. pleiantha* confirms this hypothesis.

Further comparison of the observed genetic variation among *D. versipellis* populations with values reported for other plant taxa (reviewed by Hamrick and Godt, 1990) indicates that *D. versipellis* populations are highly differentiated. As stated earlier, the population genetic differentiation in the present study (G_{ST} = 0.455) is much higher than the average for perennial-herbaceous plants (G_{ST} = 0.233) and endemic plants (G_{ST} = 0.25). A high level of population differentiation may be explained by several factors, including the species' breeding system, genetic drift, or geographic isolation of populations (Hogbin and Peakall, 1999). Low levels or absence of gene flow among populations is also characteristic of many rare species (Slatkin, 1985), and a number of studies have documented high levels of genetic differentiation among populations of rare species (Fischer and Matthies, 1998; Sun and Wong, 2001). *N*m, the number of migrants per generation (*N*m = 0.284), for *D. versipellis* was less than one successful migrant per generation, indicating limited gene flow among populations. This value corresponds well with the frequent inbreeding, considerable clonal reproduction, and isolated nature of *D. versipellis* populations. However, it is noteworthy that genetic differentiation among populations of *D. versipellis* does not appear to be correlated with geographic distance among populations.

The relatively low genetic diversity and high genetic differentiation documented for *D. versipellis* are most likely a result of habitat fragmentation (Qiu and Qiu, 2002). The subtropical forests necessary for the survival of this species have been destroyed and disturbed by anthropogenic activity at an alarming rate during the past century. As a result, the numbers and sizes of extant *D. versipellis* populations have decreased greatly, which in turn has led to the loss of genetic diversity and alteration of population genetic structure (Ellstand and Elam, 1993). Overall, the low allozyme diversity documented for this species is likely a result of habitat fragmentation followed by genetic drift and limited gene flow among small populations.

Continued exploitation of wild individuals for the traditional Chinese medicine trade remains an important factor affecting the rarity and endangered status of the species. Since low clone diversity and large clone sizes indicate that vegetative reproduction is more important than sexual reproduction for *D. versipellis*, the species may be able to survive in the wild despite being collected by humans, as long as harvesting is managed in a sustainable manner and habitats are preserved. In this regard, in situ conservation and regular monitoring of natural populations will be an important strategy for conserving this species. If ex situ strategies are developed and pursued, we recommend that sampling include all known localities across the species' distribution, since the species now occurs in fragmented and genetically distinct populations.

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濒危特有植物八角蓮（小檗科）遺傳變異的初步研究

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利用等位酶分析方法，對中國特有濒危植物八角蓮（*Dysosma versipellis*）5 個居群和六角蓮（*Dysosma pleiantha*）1 個居群的遺傳變異進行了分析。採用澱粉凝膠電泳方法，分析了 9 個酶系統，獲得了 9 個能解釋的基因位元點的資料。研究的結果表明，八角蓮居群水準的遺傳變異（P = 15.54%, A = 1.16, He = 0.045）比種子植物的平均值（P = 34.2%, A = 1.53, He = 0.113）要低得多，甚至比特有植物的平均值（P = 26.3%, A = 1.39, He = 0.063）還要低。在總的基因位點變異中，有 46.8% 的變異來自居群間，53.2% 的變異存在於居群內。間接估算的居群間基因流較低（Nm = 0.284）。在所分析的八角蓮居群中，除 HB 居群以外的所有居群遺傳雜合度均嚴重不足。從基因型頻率看，八角蓮居群內植株間存在近交現象，因此，近交衰退是八角蓮結實率低的可能原因。八角蓮居群內具有較低的克隆多樣性，也表明了八角蓮的繁殖策略是以營養繁殖為主。浙江居群的六角蓮比八角蓮所有居群具有更高的遺傳變異和分化。通過以上研究，我們認為原地保護策略對八角蓮非常重要，遷地保護時應該對所有居群進行取樣以盡可能保護該種更多的遺傳多樣性。

關鍵詞：等位酶；營養繁殖；八角蓮；濒危物種；遺傳變異。