Population genetics and conservation of the critically endangered *Clematis acerifolia* (Ranunculaceae)

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Abstract: Allozyme electrophoresis was used to evaluate the levels of genetic diversity and population genetic structure of the critically endangered *Clematis acerifolia* Maximowicz (Ranunculaceae), a narrow endemic species in China. On the basis of variation at 19 putative loci in nine populations covering the entire distribution of this species, low values of genetic diversity were detected (P = 20.5%, A = 1.27, and H_e = 0.072). A significant deficiency of heterozygotes was found in all populations. Most loci showed deviations from the Hardy–Weinberg equilibrium, probably as a result of population genetic structuring. The high genetic divergence among populations (F_{ST} = 0.273) can be interpreted as an effect of the extinction of local populations and genetic drift within extant populations, and has probably been enhanced by habitat fragmentation in recent decades. Threats to this species are mainly anthropogenic (road works, construction of holiday resorts, and extraction activities), although stochastic risks cannot be ignored. Therefore, to preserve extant genetic variation of *C. acerifolia*, in situ strategies, such as the preservation of its habitat or at least the most diverse populations, and ex situ measures, such as the collection and long-term storage of seeds, should be adopted.

Key words: Clematis acerifolia, conservation, allozyme electrophoresis, genetic diverstity, habitat fragmentation.

Résumé : Les auteurs ont utilisé l'électrophorèse des allozymes, pour évaluer les degrés de diversité génétique et la structure génétique des populations du *Clematis acerifolia* Maximowicz (Renonculacées) éminemment en danger, une espèce étroitement endémique de la Chine. Sur la base de 19 présumés loci, de neuf populations couvrant la totalité de la distribution de cette espèce, les auteurs ont observé une faible diversité génétique (P = 20,5 %, A = 1,27 et H_e = 0,072). On note une déficience significative en homozygotes. La plupart des loci montrent des déviations par rapport à l'équilibre de Hardy-Weinberg, résultant probablement d'une structuration de la population génétique. La forte divergence génétique entre les populations (F_{ST} = 0,273) peut être interprétée comme la suite de l'extinction de populations locales et d'une dérive génétique dans les populations existantes, et a probablement été renforcée par la fragmentation de l'habitat au cours des récentes décennies. Les menaces qui pèsent sur cette espèce sont surtout anthropogènes (travaux routiers, construction de stations de vacance, et activités d'extraction), bien que les risques stochastiques ne doivent pas être ignorés. Conséquemment, pour préserver la variation génétique actuelle du *C. acerifolia*, on devrait adopter des stratégies in situ, comme la conservation des habitats visant au moins les populations les plus diversifiées, et des mesures ex situ, comme la récolte et la conservation à long terme des graines.

Mots clés : Clematis acerifolia, conservation, électrophorèse d'allozymes, diversité génétique, fragmentation de l'habitat.

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Introduction

The main causes of plant and animal extinction are the loss, degradation, and subsequent fragmentation of once continuous habitats (Holsinger and Gottlieb 1991; Falk and Olwell 1992; Hughes et al. 1997; Tilman and Lehman 2001). Habitat fragmentation is becoming progressively more common for many species, especially those in and around urban centers (Allphin et al. 1998). Habitat destruction and fragmentation have delimited an increasing number of plant species to small and isolated populations. Even in intact habitat remnants, these populations face an increased risk of extinction because of demographic and genetic stochasticity, which leads to decreased effective population sizes and increased rates of inbreeding and genetic drift (Barrett and Kohn 1991; Young et al. 1996; Fisher and Matthies 1998).

The preservation of genetic diversity in endangered species is a priority in conservation planning since long-term survival of species depends on the maintenance of sufficient genetic variability within and among populations to accommodate new selection pressures brought about by environmental change (Barrett and Kohn 1991). Studies that address genetic diversity and the way in which it is structured within rare and endangered plant species may contrib-

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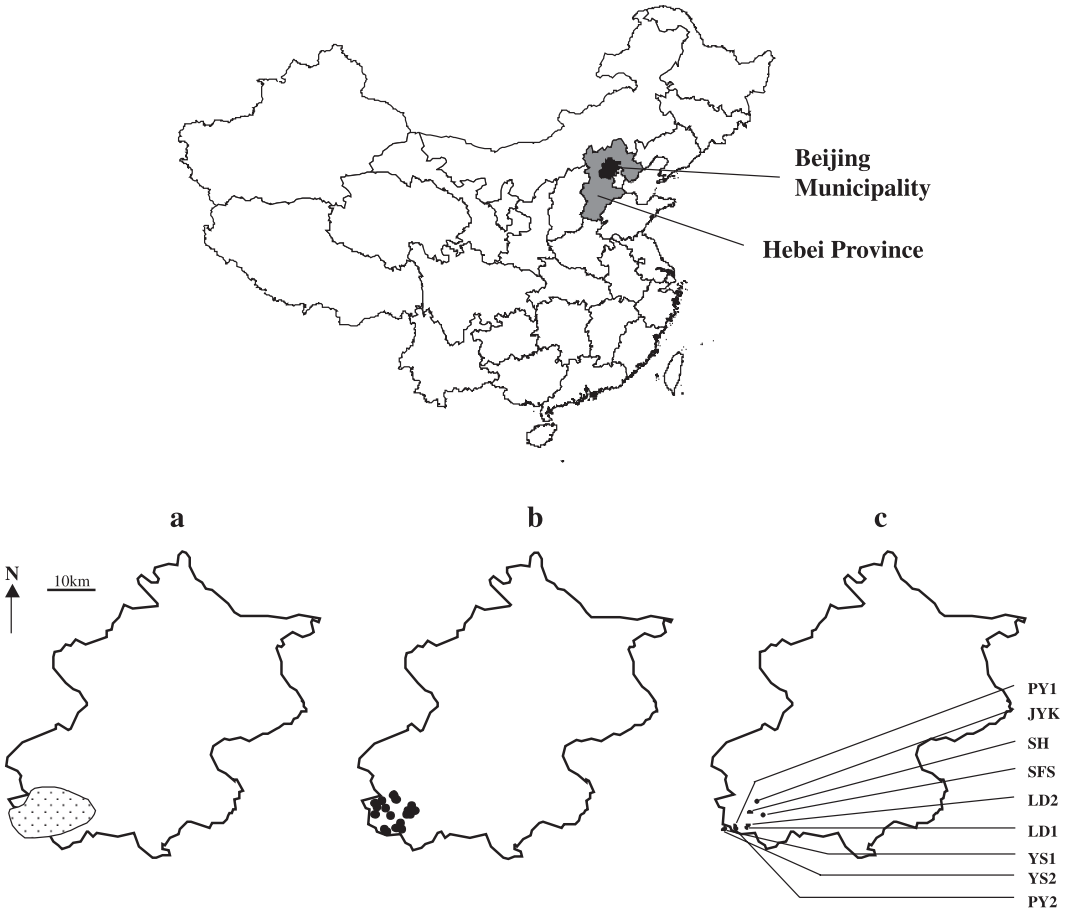
Fig. 1. (*a*) Historical distribution of *Clematis acerifolia* based on literature and our field observations. (*b*) Historical localities based on the herbaria records. (*c*) Sampled populations of *C. acerifolia*. In Beijing Municipality: JYK, Jayukou; LD, Liudu; PY, Pingyu; SH, Sihe; SFS, Shangfangshan; in Hebei Province: YS, Yesanpo.



ute to knowledge of their evolutionary history and potential. Moreover, these studies are critical for the conservation and management of these plants (Schaal et al. 1991; Hamrick and Godt 1996; Torres et al. 2003). The structure of genetic diversity within and among populations also has important implications for developing sampling strategies for species recovery, and when appropriate, for reintroduction (Chang et al. 2004).

China is one of the world's richest countries in plant diversity. The unique, abundant, and diversified flora of the country results from the great diversity of geographical, climatological, and topographical features, in addition to a complex geological history (Fu 1992). China is estimated to hold more than 30 000 species of higher plants (Gu 1998; Li 2003) and more than 10 000 of these are considered endemic (Fu 1992; Dinerstein and Wikramanayake 1993). Nevertheless, recent overexploitation of plant resources as a result of agricultural practices, traditional chinese medicine, and other human activities have led to severe habitat destruction and fragmentation, which has endangered more than 3000 species (Fu 1992; Wan and Liu 1994; Gu 1998).

The genus *Clematis* L. (Ranunculaceae) consists of 147 species in China, and over 60% of these are endemic. Of these species, *Clematis acerifolia* Maximowicz has an extremely narrow distribution range, confined to the southwestern part of the Beijing Municipality and to an adjacent small area in Hebei Province, along the Baihuashan and Shangfangshan mountains (Fig. 1). This species is a small, erect perennial shrub, 20–60 cm tall, with palmately 5-lobed leaves and flowers with 5–8 white to pinkish glabrous sepals (Wang 2002). Like all the species of the genus *Clematis*, *C. acerifolia* is a diploid of $2n = 16$ (Gong et al. 1985; Yang 2002). Unfortunately, information concerning pollination mechanisms, breeding system, and seed dispersal for this species is scarce. Suitable habitats for *C. acerifolia* are located on vertical cliffs in gorges and narrow passes that contain fissures in the rock and have a certain degree of humidity. Owing to its striking morphological differences from other species in the genus and its unique habitat, the taxonomic rank of *C. acerifolia* has not been questioned since its original description by Maximowicz in 1879 (Wang 2002). In a recent revision of the genus *Clematis* by Wang (2002), *C. acerifolia* was included in the sect. §Cheiropsis as a single species of the subsect. Acerifoliae.

Although many continuously distributed large populations of *C. acerifolia* were recorded in this area several decades ago, severe habitat destruction and fragmentation caused by human activities, especially tourism and mining, have led to a rapid decline in the total number and size. For example, on the basis of the specimens of *C. acerifolia* collected from the 1950s to the 1970s and stored at the PE (Herbarium Pekinensis), the number and size of its populations

Table 1. Studied populations of *Clematis acerifolia*.

Population code	Locality	Altitude (m)	Latitude, longitude	Population size	Sample size
SFS	Shangfangshan, Beijing	605	39°40′33″N, 115°49′29″E	70–80	20
LD1	Liudu, Beijing	258	39°39′50″N, 115°37′32″E	50–60	20
LD2	Liudu, Beijing	269	39°39′55″N, 115°37′36″E	50	22
PY1	Pingyu, Beijing	202	39°39′44″N, 115°31′25″E	30	20
PY2	Pingyu, Beijing	213	39°39′24″N, 115°31′39″E	40	25
YS1	Yesanpo, Hebei	224	39°39′54″N, 115°27′40″E	50	25
YS2	Yesanpo, Hebei	264	39°39′59″N, 115°27′35″E	70	16
SH	Sihe, Beijing	280	39°42′58″N, 115°43′33″E	150	20
JYK	Jayukou, Beijing	251	39°48′17″N, 115°46′26″E	275	28

were large in the localities surveyed; however, most of these populations have now disappeared and only a few small ones remain (F.M. Zhang and J. López-Pujol, personal observation).

Allozyme electrophoresis has been widely used to assess genetic diversity within and among populations of plant species (Hamrick and Godt 1989; Hamrick and Godt 1996; Crawford 2000). Here we used allozyme markers to examine the genetic diversity and structure of *C. acerifolia* populations. We aimed (*i*) to determine the levels and distribution of genetic diversity within and among populations; (*ii*) to evaluate the degree of threat on the basis of IUCN criteria (IUCN 2001), and the current and potential threats; and (*iii*) to propose strategies for the preservation and management of this endemic species.

Materials and methods

Nine populations covering the entire distribution of *C. acerifolia* were sampled in August 2003. Seven populations were located in the Beijing Municipality, and two were in the Hebei Province (Table 1 and Fig. 1). We sampled 16–28 individuals from each population; no larger sample sizes could be obtained because of inaccesibility to the habitat of this species; it grows on vertical rocky walls. Young leaves from each individual were harvested, placed in plastic bags and transported to the laboratory in a portable refrigerator. Sampling was done with care (collecting just one leaf) to minimize potential damage to populations.

Genetic variability was assessed using standard methods for starch gel electrophoresis of allozymes (Soltis et al. 1983). Leaves were ground in refrigerated porcelain plates using a Tris–maleate extraction buffer with 4% w/v of PVP-40 (polyvinyl-pyrrolidone) and 0.1% v/v 2-mercaptoethanol (modified from Soltis et al. 1983). Experiments with a Tris–citric acid extraction buffer (the composition of which is described in López-Pujol et al. 2001) were also performed but gave poorer results. The extracts were absorbed into 2 × 6 mm paper wicks, which were stored at –80 °C until electrophoresis. Eighteen enzymes were tested using horizontal starch gels. Of these, 12 were satisfactorily resolved, resulting in 19 putative loci: *Aat-1*, *Acp-1*, *Acp-2*, *Amp-1*, *Dia-1*, *Dia-2*, *Dia-3*, *Dia-4*, *Dia-5*, *Dia-6*, *Hex*, *Idh*, *Mdh-1*, *Me-2*, *6Pgd*, *Pgi-1*, *Pgm-2*, *Skd-1*, and *Skd-2*. Aspartate aminotransferase (AAT, EC 2.6.1.1), aminopeptidase (AMP, EC 3.4.11.1), diaphorase (DIA, EC 1.6.99.-), and hexokinase (HEX, EC 2.7.1.1) were satisfactorily resolved on buffer system No. 6 of Soltis et al. (1983); malate dehydrogenase (MDH, EC 1.1.1.37) and phosphoglucoisomerase (PGI, EC 5.3.1.9) on buffer system No. 11 of Soltis et al. (1983); and acid phosphatase (ACP, EC 3.1.3.2), isocitrate dehydrogenase (IDH, EC 1.1.1.42), malic enzyme (ME, EC 1.1.1.40), phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), phosphoglucomutase (PGM, EC 5.4.2.2), and shikimate dehydrogenase (SKD, EC 1.1.1.25) on Clayton and Tretiak's (1972) buffer system. ADH and G6PD showed activity but were not scorable because of poor or inconsistent band resolution. We used 12.5% starch for buffer No. 6, while 14% starch was applied to buffer No. 11 and to Clayton and Tretiak's buffer (1972). Staining procedures followed the method described by Soltis et al. (1983) and Wendel and Weeden (1989), with slight modifications.

Loci were numbered consecutively and alleles at each locus were labelled alphabetically, beginning from the most anodal form in both cases. Interpretation of banding patterns was done on the basis of the quaternary structure of isozymes, subcellular localization and number of loci usually expressed in diploid plants (Gottlieb 1982; Soltis and Soltis 1989). Allele frequencies at each locus were calculated for each population. To estimate the levels of genetic diversity, the following statistics were calculated: P, the percentage of polymorphic loci when the most common allele had a frequency of <0.95; A, the mean number of alleles per locus; A_p: mean number of alleles per polymorphic locus; H_o, the observed heterozygosity; and H_e, the expected panmictic heterozygosity. The mean fixation index (F) for all polymorphic loci in each population was also calculated to compare genotype proportions with those expected under Hardy–Weinberg equilibrium. The chi-squared test (χ^2) was used to evaluate deviations of F from zero, with Levene's (1949) correction for small sample size. Population structure was analysed using Wright's (1965) F statistics: the inbreeding coefficient (F_{IS}), the fixation index (F_{ST}), and the overall inbreeding coefficient (F_{IT}). Gene flow was determined using Wright's (1951) equation: $Nm = (1 - F_{ST})/4\ F_{ST}$. A chi-squared test was also performed to assess the statistical significance of F_{ST} values for each locus. In addition, we calculated Nei's (1978) genetic identity (I) between pairs of populations, which was used to cluster the populations into a dendrogram following UPGMA (unweighted pair group method with averaging). Finally, the Mantel (1967) test was conducted between genetic differentiation and geographical distances between pairs of populations to determine a possi-

Table 2. Summary of genetic variation for 19 loci in the nine populations of *Clematis acerifolia*.

Population	P	A	A_p	H_o	H_e	F
SFS	31.6	1.31	2.00	0.058 (0.033)	0.080 (0.034)	0.275
LD1	26.3	1.31	2.00	0.047 (0.024)	0.082 (0.034)	0.427
LD2	21.1	1.37	2.25	0.063 (0.031)	0.091 (0.040)	0.308
PY1	21.1	1.26	2.00	0.011 (0.006)	0.048 (0.027)	0.771
PY2	15.8	1.21	2.33	0.026 (0.017)	0.061 (0.035)	0.574
YS1	15.8	1.26	2.33	0.054 (0.032)	0.078 (0.044)	0.308
YS2	15.8	1.21	2.33	0.056 (0.032)	0.077 (0.042)	0.273
SH	15.8	1.21	2.33	0.045 (0.027)	0.047 (0.027)	0.042
JYK	21.1	1.26	2.25	0.063 (0.033)	0.086 (0.041)	0.267
Mean	20.5	1.27	2.20	0.047	0.072	0.360
Standard deviation	5.5	0.05	0.15	0.018	0.016	0.209

Note: P, percentage of polymorphic loci; A, mean number of alleles per locus; A_p, mean number of alleles per polymorphic locus; H_o, observed heterozygosity; H_e, expected panmictic heterozygosity; F, mean fixation index. Standard error is given in parentheses.

ble "isolation-by-distance" pattern of differentiation (Wright 1943). BIOSYS-1 (Swofford and Selander 1989) was used for most of the calculations and for dendrogram construction. The NTSYS package (Rohlf 1994) was used to perform the Mantel test.

Results

We detected 28 alleles from the 19 interpretable loci. The number of alleles ranged from 23 (82.1%) in populations PY2, YS2, and SH to 26 (92.8%) in LD2. Only one distinct (population specific) allele (*Dia-1b* in JYK) was detected while no rare alleles (those with frequencies <0.05) were found. Most loci (11 of 19) were monomorphic across populations, and all polymorphic loci exhibited two alleles except for *Me-2*, which showed three.

The average genetic variability of *C. acerifolia* was low: P = 20.5%, A = 1.27, A_p = 2.20, and H_e = 0.072 (Table 2). Values of P ranged from 15.8 to 31.6%, A from 1.21 to 1.37, A_p from 2.00 to 2.33, and H_e from 0.047 to 0.091. The most variable population was LD2 (H_e = 0.091) and the least variable were SH and PY1 (H_e = 0.047 and H_e = 0.048, respectively). Values of the observed heterozygosity were lower than those of expected panmictic heterozygosity in all populations, with the mean fixation index being positive in all the populations (Table 2). Excess of homozygotes was also detected from F values for all the polymorphic loci in each population (Table 3). The chi-squared test (χ^2) showed that 19 of the 40 F values conformed to Hardy–Weinberg equilibrium ($p \geq 0.05$), while the remaining 21 were significantly greater than zero ($p < 0.05$) and positive, indicating an excess of homozygotes. Heterozygote deficiency within populations were also supported by the mean value of F_{IS} (0.333).

Genetic divergence among populations was established by computing the F_{ST} parameter, which measures differentiation among populations. The mean F_{ST} was considerably high (0.273) with respect to the mean F_{IT} (0.515), indicating that a significant fraction of the genetic variability of *C. acerifolia* is attributable to differences among populations (Table 4). The number of migrants per generation was consequently low (Nm = 0.66), which implies that the level of historical gene flow is limited among populations. Values for Nei's (1978) genetic identity (I) were high between pairs of populations (mean = 0.970, range: 0.941–0.999; Table 5). The correlation between the matrix of the pairwise F_{ST} values and geographical distances did not support a pattern of genetic differentiation of populations under the "isolation-by-distance" model, since the Mantel statistical showed a negative and marginally significant value (r = –0.326, one-tailed p = 0.037 after 9999 permutations). In fact, the highest pairwise I value (0.999) was between populations separated by nearly 10 km (YS2 and JYK), while one of the lowest (0.947) corresponded to populations separated by only 0.060 km (LD1 and LD2; data not shown). This lack of significance is clearly reflected in the UPGMA dendrogram (Fig. 2).

Discussion

The association between lack of genetic variation and rare and endemic species was established several decades ago (Stebbins 1942). Most compilations of plant allozyme data (e.g., Hamrick and Godt 1989; Hamrick et al. 1991; Karron 1991; Gitzendanner and Soltis 2000) show a pattern of low genetic diversity in rare and endemic species. Scarce diversity in the latter has been attributed to small population sizes, isolation of populations, and adaptation to a uniform habitat (Barrett and Kohn 1991; Ellstrand and Elam 1993). Nevertheless, the literature is also replete with rare taxa that maintain unexpectedly high levels of diversity (Gottlieb et al. 1985; Ranker 1994; Young and Brown 1996; Ge et al. 1999; Williamson and Werth 1999; Neel and Ellstrand 2001). These observations indicate that, in addition to the factors mentioned above, the levels of genetic variation may be affected by several life-history traits (e.g., breeding system, seed dispersal mechanism, and life form), population history (e.g., occurrence of bottlenecks and founder events), and type of speciation (Hamrick and Godt 1989; Booy et al. 2000; Dodd and Helenurm 2002).

Clematis acerifolia shows a low level of genetic diversity (P = 20.5%, A = 1.27, and H_e = 0.072) compared with long-lived perennial herbaceous or endemic species (Hamrick and Godt 1989). This result may be due to several reasons. On the one hand, habitat requirements for this species are highly specific (vertical rocky walls), suggesting that the low levels

Table 3. Values of fixation index (F) for all polymorphic loci in the nine populations of *Clematis acerifolia*.

Locus	SFS	LD1	LD2	PY1	PY2	YS1	YS2	SH	JYK
Aat-1	1.000***	0.657**	0.642**	—	0.554**	—	—	—	—
Acp-2	0.827***	0.640**	0.519*	0.880***	—	0.400*	0.150ns	—	0.432*
Dia-1	—	—	—	—	—	—	—	—	0.627***
Idh	–0.053ns	–0.027ns	—	—	—	—	—	—	—
Mdh-1	—	—	1.000***	1.000***	—	—	—	—	—
Me-2	0.167ns	0.124ns	0.030ns	1.000***	0.913***	0.619***	0.549**	0.057ns	0.100ns
Pgm-2	–0.333ns	–0.226ns	0.162ns	–0.026ns	–0.150ns	–0.167ns	–0.082ns	–0.250ns	0.067ns
Skd-2	0.636**	1.000***	–0.048ns	0.441*	—	–0.020ns	—	0.444*	—

Note: Conformance to Hardy–Weinberg equilibrium was tested using chi-square analysis: ns, $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

of diversity detected in this species are a consequence of its rarity. In this case, the species may present a degree of adaptation to the lack of genetic variability and could represent its "natural" genetic situation (cf. Huenneke 1991). Nevertheless, population history is probably the current factor with the greatest influence on genetic variation. Populations of *C. acerifolia* are subjected to severe levels of habitat fragmentation, which began a few decades ago. On the basis of IUCN criteria used to estimate the distribution area of a species (see IUCN 2001), the extent of occurrence of *C. acerifolia* is about 400 km^2; however, the area of occupancy is much lower (less than 3 km^2), and probably 20-fold smaller than fifty years ago. Most present-day populations are very small, typically with fewer than 100 individuals and in some cases with only a handful of individuals (F.M. Zhang and J. López-Pujol, personal observations). The reduced size of these populations may be a result of habitat fragmentation caused by several human activities, such as tourism, road construction, and mining. This fragmentation theoretically leads to a decrease in population size and an increase in both spatial isolation and edge-to-area ratio of habitat remnants (Opdam et al. 1994). These changes are expected to decrease the genetic variation of remnants through increased genetic drift and inbreeding, and potentially to increase genetic divergence among populations as a result of reduced gene flow (Young et al. 1996).

The significant deficiency of heterozygotes within populations of this species cannot be explained only by chance, and there are two main potential causes for this phenomenon: (*i*) a breeding system mainly consisting of selfing and (*ii*) genetic substructuring of populations (Wahlund effect). The pollination mechanism and the mating system of *C. acerifolia* are largely unknown; thus the deficiency of heterozygotes cannot be attributed to inbreeding. Nevertheless, the observation that about 50% of the polymorphic loci showed a significant excess of homozygotes cannot be explained by autogamy alone since we would thus expect heterozygote deficiences at all loci. Rather, this finding indicates that populations may be composed of minor units within which mating is nearly random, but between which mating may be infrequent (Heywood 1991). If there is a genetic substructure within populations (Wahlund effect), polymorphic loci with even distribution of alleles (a minority of cases) approximate Hardy–Weinberg values, but loci for which allele distribution is uneven show heterozygote deficits (Mayes et al. 1998; Williamson and Werth 1999; Batista et al. 2001; Otero-Arnaiz et al. 2005).

Table 4. Estimates of F-statistics for all polymorphic loci in the nine populations of *Clematis acerifolia*.

Locus	F_{IS}	F_{IT}	F_{ST}
Aat-1	0.635	0.820	0.507***
Acp-2	0.540	0.626	0.187***
Dia-1	0.627	0.661	0.093***
Idh	–0.044	–0.008	0.035ns
Mdh-1	1.000	1.000	0.038ns
Me-2	0.383	0.581	0.321***
Pgm-2	–0.095	0.136	0.211***
Skd-2	0.509	0.533	0.050*
Mean	0.333	0.515	0.273

Note: F_{IS}, inbreeding coefficient; F_{ST}, fixation index; and F_{IT}, overall inbreeding coefficient. Statistical significance of F_{ST} values was tested using chi-square analysis: ns, $p \geq 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

This structure may be caused by limited pollinator activity or restricted seed dispersal. *Clematis acerifolia* seeds are probably dispersed by gravity, although spider webs (common in cliffs) could act as seed receptacles and thereby limit their dispersion, as observed in other chasmophytes, such as a number of *Petrocoptis* species (López-Pujol et al. 2001). This structure in subpopulations may be enhanced by habitat fragmentation, which is probably responsible for the subdivision of ancient large populations into the small units observed presently. Evidence of this phenomenon has been found in most of the localities surveyed, where human activities have severe effects on the landscape.

The value of genetic divergence among *C. acerifolia* populations (F_{ST} = 0.273) was higher than that obtained from several taxa subjected to fragmentation (Raijmann et al. 1994; Luijten et al. 2000; Neel and Ellstrand 2001; Lienert et al. 2002). This finding, in addition to the inferred low value of gene flow (Nm = 0.66), may infer that fragmentation has increased the genetic differentiation of populations. However, *C. acerifolia* is a perennial plant with a long life span. Therefore, most indivuals might predate the onset of habitat fragmentation (about 50 years ago). If this were the case, current anthropogenic impact may have contributed to enhancing a pre-existing population genetic structure and isolation of populations.

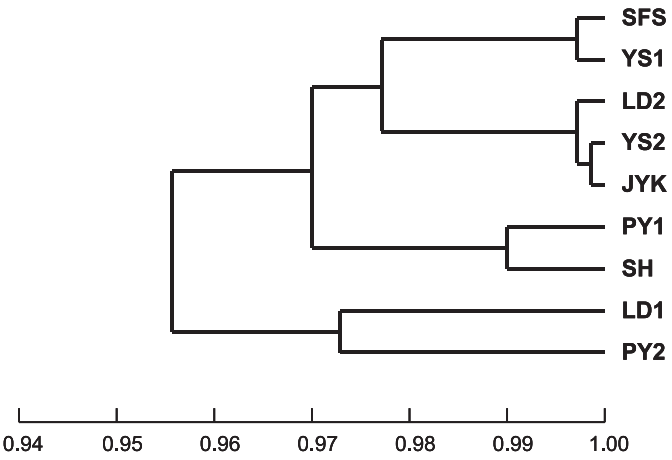
Similarly, the absence of rare alleles within populations, the maximum of two alleles shown by all but one polymor-

Table 5. Matrix of geographic distances (above diagonal) and Wright's (1965) genetic differentiation (F_{ST}, below diagonal) between populations of *Clematis acerifolia*.

Population	SFS	LD1	LD2	PY1	PY2	YS1	YS2	SH	JYK
SFS	—	5.454	5.421	8.233	8.145	9.937	9.969	3.054	4.769
LD1	0.182	—	0.060	2.782	2.691	4.492	4.526	3.303	6.425
LD2	0.181	0.232	—	2.813	2.726	4.521	4.555	3.249	6.366
PY1	0.275	0.310	0.212	—	0.225	1.713	1.749	5.838	8.492
PY2	0.286	0.163	0.245	0.248	—	1.839	1.878	5.810	8.531
YS1	0.029	0.187	0.109	0.213	0.234	—	0.051	7.449	9.868
YS2	0.155	0.232	0.039	0.120	0.196	0.070	—	7.473	9.878
SH	0.179	0.239	0.222	0.098	0.198	0.119	0.112	—	3.402
JYK	0.155	0.234	0.020	0.193	0.222	0.072	0.018	0.168	—

Note: Geographic distances are expressed in kilometres.

Fig. 2. Dendrogram resulting from UPGMA analysis of *Clematis acerifolia* populations based on pairwise values of Nei's (1978) genetic identity (SD = 0.965%; cophenetic correlation = 0.824).



phic locus, and the monomorphic nature of most of the loci (11 of 19) may be features that were present before the current loss of habitat, although habitat fragmentation would have enhanced the erosion of genetic variation. The absence of rare alleles within populations is probably due to gene flow events in the past. Alternatively, these alleles may not have been dectected in our survey because of the small sample sizes used. Episodes of population bottlenecks in the past might also account for this absence. The recent extinction of intervening populations (in recent decades) may explain the lack of a significant correlation between geographic and genetic differentiation of populations, but also the low levels of genetic variability detected through the loss of unique alleles present in the extinct populations.

On the basis of our field observations, we conclude that *C. acerifolia* individuals present high longevity. Recruitment rates are probably low; only a very limited seedling establishment was observed, probably because of the low availability of suitable habitats in rock. Only a few seeds remaining in fissures with a certain degree of humidity survive; nevertheless, most fall onto the soil, where they have no recruitment opportunity. Moreover, seeds do not show long-distance dispersal capacity. All these traits confer strong stability to populations and very slow population growth rates, which, together, lower the importance of recruitment for population persistence. However, not only do these characteristics not allow demographic recovery after a stochastic event or habitat disturbance produced by human activities but they also limit new colonization episodes. However, the life-history and autoecologic characteristics of *C. acerifolia* (long life span, high habitat specificity, low recruitment rates, and unlikely long-distance seed dispersal), whose dynamics are of remnant type (Eriksson 1996; García 2003), may buffer this species against the negative impacts of population bottlenecking (genetic drift and inbreeding) because they preclude large demographic fluctuations that lead to strong population stability.

Low levels of diversity and high genetic differentiation among populations are features typically related to narrow geographical range and population isolation, high habitat specificity, and short-distance seed dispersal (Loveless and Hamrick 1984). Plants inhabitating rocky environments, such as *Borderea chouardii* (Segarra-Moragues and Catalán 2002), *Hippocrepis valentina* (González-Candelas and Montolío 2000), *Silene hifacensis* (Prentice et al. 2003), and *Antirrhinum valentinum* (Mateu-Andrés and Segarra-Moragues 2000), among others, usually share these traits. However, population history may lead to a distinct situation, as occurs in *Borderea pyrenaica* (G_{ST} = 0.035; Segarra-Moragues and Catalán 2002), *Petrocoptis montsicciana, Petrocoptis pardoi* (H_e = 0.239 and H_e = 0192, respectively; López-Pujol et al. 2001) and *Antirrhinum microphyllum* (H_e = 0.204 and F_{ST} = 0.056; Torres et al. 2003).

Fragmentation may also produce the same pattern of genetic depauperation within populations and high intrapopulation differentiation. China, whose landscape has been subjected to enormous transformation and fragmentation, has several examples, such as *Mosla hangchouensis* (A = 1.25, H_e = 0.082 and F_{ST} = 0.222; Zhou et al. 1998), *Ophiopogon xylorrhizus* (A = 1.35, H_e = 0.091, and F_{ST} = 0.181; Ge et al. 1997), and *Goodyera procera* (A = 1.22, H_e = 0.073, and G_{ST} = 0.520; Wong and Sun 1999). Unfortunately, congeneric comparisons, which are highly suitable because the phylogenetic effects on genetic diversity patterns can be controlled (see Gitzendanner and Soltis 2000), cannot be made because of the lack of data on genetic diversity within other species of *Clematis*.

Inbreeding can lead to short-term decreases in individual fitness; the random loss of alleles in genetic drift reduces plant capacity to achieve long-term survival after habitat changes (Young et al. 1996). These processes, exacerbated by habitat fragmentation, make populations more sensitive

to local extinction, since they show reduced adaptability to biotic and abiotic environmental alterations (Barrett and Kohn 1991) and limited resistance to pests and diseases (Frankham 1995). The estimates of low levels of genetic diversity within *C. acerifolia* indicate that conservation measures are required to maintain current population sizes and to limit habitat fragmentation and destruction to prevent further loss of genetic diversity within this species. The current populations of *C. acerifolia* are small, they are therefore inevitably affected by demographic, genetic, and environmental stochasticity, in addition to catastrophes (despite their demographic stability), which may accelerate the extinction of entire populations. However, human activities have a significantly negative impact on the survival of this species. For example, the construction of infrastructures associated with tourist activities (road works, holiday resorts) have negative repercussions on populations in the Yesanpo area (e.g., YS1 and YS2) through fragmentation of natural habitats, while the drastic landscape transformation in the Baihua mountains probably accounts for the disappearance of the original herbarium localities. The largest population (JYK) is currently under two direct threats because of its location in a mining basin: the fragmentation of the population caused by extraction activities and pollution (coal dust particles) deposited on leaves (F. M. Zhang and J. López-Pujol, personal observation). In the locus classicus, Shangfangshan mountain, only a very small population remains (SFS) in spite of the fact that this area held many populations in the past (He et al. 1984).

Although *C. acerifolia* can be considered "critically endangered" (CR) following the IUCN criteria (B2ab(i,ii,iii,iv,v); IUCN 2001), it has not been included in the first volume of the China Plant Red Data Book (Fu 1992). Therefore, we recommend its inclusion in one of the forthcoming volumes, to achieve legal protection by Chinese laws. Habitat protection is the best in situ conservation measure because it allows the maintenance of current population sizes, gene flow, and interactions between the species and its ecosystem, without detaining evolutionary processes (Falk and Holsinger 1991). However, this protection is generally limited to a few selected populations. According to the formula proposed by Ceska et al. (1997), $1 - (F_{ST})^n$, the preservation of the largest population that contains a private allele (JYK) and the most diverse population (LD2) would allow the preservation of 92.5% of the genetic diversity in *C. acerifolia*. However, if we add LD1, the third highest population in H_e and geographically close to LD2, 98.0% of the total variation and 100% of the detected alleles could be conserved. Reintroductions of *C. acerifolia* in extinct localities and (or) reinforcement of current populations may be necessary to ensure population viability in the long-term. Nevertheless, these strategies require additional data on the autoecology, demography, pollination biology, mating system and seed dispersal of this species. Furthermore, the collection of *C. acerifolia* seeds for the long-term conservation of this species in a germplasm bank is the main ex situ policy. Although there are several formulas to calculate the number of seeds and populations to be collected (CPC 1991; Hamrick et al. 1991), given the limited distribution area of *C. acerifolia*, the low number of populations, and their significant genetic structure, seed collection must be performed at all sites to ensure the preservation of all genetic variability.

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