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No Correlation Between Heterozygosity and Vegetative Fitness in the Narrow Endemic and Critically Endangered *Clematis acerifolia* (Ranunculaceae)

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Abstract The relationship between heretozygosity and vegetative fitness was explored in the narrow endemic and threatened *Clematis acerifolia* (Ranunculaceae), both at individual and population levels. The relationships between fitness, habitat factors, and population size were also analyzed. Allozyme electrophoresis was used to quantify the levels of heterozygosity of nearly 200 surveyed individuals belonging to the nine extant populations of this species. Six parameters of vegetative fitness were measured: plant height, shrub diameter, length of the largest leaf, width of the largest leaf, mean number of leaves/stem, and total number of stems. The percentage of tree cover (light availability) was measured as an indicator of habitat quality. A principal component analysis reduced the original fitness variables to two uncorrelated principal components. None of these correlated significantly with both heterozygosity and population size, in contrast to the expected result. Nevertheless, one of the principal components showed a positive relationship with light availability, which may indicate that habitat quality may have significant effects on the performance of this species. Thus, to ensure the viability of this endangered species, maintenance of adequate habitat quality (by avoiding further fragmentation) may be more important than maximizing genetic diversity within populations.

Keywords Heterozygosity · Fitness · Allozymes · Endangered · *Clematis acerifolia*

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Introduction

A large number of studies, both in animals and plants, show a positive relationship between individual heterozygosity and several components of fitness (reviewed in Mitton and Grant 1984; Allendorf and Leary 1986; Mitton 1994), although exceptions to this correlation have been reported (Savolainen and Hedrick 1995; Booy et al. 2000). Three main hypotheses have been proposed to explain this correlation, although additional models have also been formulated (Mitton and Grant 1984; Zouros and Foltz 1987; Mitton 1989, 1994; Booy et al. 2000; Thelen and Allendorf 2001; Hansson and Westerberg 2002). The overdominance hypothesis relies on the suggestion that heterozygous individuals for the loci studied have an intrinsically higher fitness than homozygotes, which can only be explained if we assume that isozyme markers are not neutral (Mitton and Grant 1984; Savolainen and Hedrick 1995; Booy et al. 2000). One mechanism to account for this is that heterozygotes at allozyme loci may be more efficient biochemically than homozygotes because they can produce different forms of the enzymes (Thelen and Allendorf 2001; Hansson and Westerberg 2002). In contrast, the inbreeding or dominance hypothesis is based on highly heterozygous individuals having higher fitness than predominantly homozygous individuals because in heterozygotes deleterious recessive alleles are masked by dominant alleles. This implies that isozyme loci are selectively neutral and act only as markers for the overall levels of heterozygosity (Kimura 1983; Nei 1987). The third hypothesis, associative overdominance, suggests that the allozyme loci are selectively neutral but in linkage disequilibrium with loci that have direct effects on components of fitness; this can lead to an apparent heterozygous advantage at allozyme loci due to their acting as convenient markers (Ohta 1971; Thelen and Allendorf 2001).

Nevertheless, other factors have also been postulated to exert strong effects on plant performance, apart from heterozygosity. Several demographic processes (e.g., pollinator limitation, low plant density) and habitat quality deterioration (e.g., eutrophication) have been related to a decrease in several fitness-related parameters (Campbell and Halama 1993; Fischer and Matthies 1997; Oostermeijer et al. 1998; Bosch and Waser 1999; Vergeer et al. 2003). Population size is another factor largely related to plant fitness; an increasing number of studies have reported a significant positive correlation between population size and performance (e.g., Oostermeijer et al. 1994; Boerrigter 1995; Kéry et al. 2000; Luijten et al. 2000; Vergeer et al. 2003; Hensen et al. 2005).

Relationships between genetic diversity (particularly heterozygosity) and fitness should be extensively explored because they have strong implications for the extinction risk of the species (Oostermeijer et al. 2003; Reed and Frankham 2003; Frankham 2005). This is particularly important if the concerned taxon is a narrow endemic or is subjected to severe habitat fragmentation or other kind of threat because of small population size (Ellstrand and Elam 1993; Gaston et al. 2000; Spielman et al. 2004). Since heterozygosity, population size, and fitness are expected to be intercorrelated, individuals in small populations would tend to exhibit depleted levels of performance, with a subsequent increased risk of local extinction.

Here we explore the relationship between both individual and population heterozygosity (surveyed by means of starch gel electrophoresis of allozymes) and several vegetative fitness-related traits in the threatened and narrow endemic Chinese *Clematis acerifolia*. In addition, the relationships between fitness and light availability (as one indicator of habitat quality) and population size are explored to determine if these factors have some influence on the performance of this species. Data obtained here may shed light on the understanding of the relationships between heterozygosity and fitness, and provide useful guidelines for the management and conservation of the endangered *C. acerifolia*.

Materials and Methods

Study Species

Clematis acerifolia Maximowicz (Ranunculaceae) is a narrow endemic of northeastern China, restricted to the southwest part of Beijing municipality and its adjacent area in Hebei Province, along the Baihua and Shangfang mountains. It is a small 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). Although information about pollination mechanisms, breeding system, and seed dispersal for this species is unknown, its genetic diversity and population structure have recently been studied in detail (López-Pujol et al. 2005). It blooms from April to May, and fruits are set from May to June. Suitable habitats for *C. acerifolia* are vertical cliffs that contain fissures in the rock and have a certain degree of humidity, located indistinctly in shrubby, open or closed wooded hillsides in low mountainous areas. It is likely one of the most threatened species of northeast China, classified by López-Pujol et al. (2005) as critically endangered, according to the most recent IUCN (2001) criteria. In recent decades it has suffered significant fragmentation and reduction of its original area, and it is confined at present to a few populations (we were able to detect only nine localities in the field), most of them containing fewer than 100 individuals.

Sampling Design and Fitness Measures

To assess the relationship between heterozygosity and fitness in *C. acerifolia*, we used the same populations and individuals as those studied in the previous genetic diversity survey (López-Pujol et al. 2005). In total, 196 individuals from 9 populations were collected for electrophoresis analysis in August 2003 (Table 1). A young leaf was sampled for each individual, placed in a Zip-lock plastic bag, and transported to the laboratory in a portable refrigerator.

A range of morphological vegetative fitness parameters was measured in situ for all the sampled individuals: the height of the plant; the shrub diameter, measured as the maximum width of the plant; the length of the largest leaf; the width of the largest leaf; the mean number of leaves per stem; and the total number of (hanging) stems.

Table 1 Nine populations of *Clematis acerifolia* sampled for this study

Locality (Pop. Code)	Latitude, longitude	Population size	Sample size	Heterozygosity[a]			Light availability (%)
				H_o	H_e	Average individual	
Shangfangshan, Beijing (SFS)	39°40′33″N, 115°49′29″E	80	20	0.058	0.080	0.054 (0.008)	80
Liudu, Beijing (LD1)	39°39′50″N, 115°37′32″E	60	20	0.047	0.082	0.046 (0.008)	90
Liudu, Beijing (LD2)	39°39′55″N, 115°37′36″E	50	22	0.063	0.091	0.060 (0.009)	79
Pingyu, Beijing (PY1)	39°39′44″N, 115°31′25″E	30	20	0.011	0.048	0.010 (0.005)	60
Pingyu, Beijing (PY2)	39°39′24″N, 115°31′39″E	40	25	0.026	0.061	0.025 (0.007)	85
Yesanpo, Hebei (YS1)	39°39′54″N, 115°27′40″E	50	25	0.054	0.078	0.053 (0.009)	95
Yesanpo, Hebei (YS2)	39°39′59″N, 115°27′35″E	70	16	0.056	0.077	0.053 (0.012)	90
Sihe, Beijing (SH)	39°42′58″N, 115°43′33″E	150	20	0.045	0.047	0.045 (0.010)	85
Jayukou, Beijing (JYK)	39°48′17″N, 115°46′26″E	275	28	0.063	0.086	0.060 (0.008)	67.5
Mean	–	89.44	–	0.047	0.072	0.045	81.28
Standard deviation	–	77.88	–	0.018	0.016	0.017	11.28

[a] H_o, observed heterozygosity. H_e, expected panmictic heterozygosity. Standard error is given in parentheses

In addition to these fitness parameters, we measured a widely used indicator of habitat quality at each sampled population, the percentage of tree and large-size shrub cover (estimated visually), as a proper indicator of light availability (cf. Paschke et al. 2002; Noel et al. 2007). For the fragmented populations, this parameter was measured for each fragment and then all the values were averaged. Population sizes were obtained mainly by estimation and not by direct count, due to the inaccessibility and lack of visibility of many sites.

Electrophoresis

Standard methods for starch gel electrophoresis of allozymes (Soltis et al. 1983) were used to detect levels of individual heterozygosity at sampled individuals. 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). The extracts were absorbed into 2 × 6 mm paper wicks, which were stored at −80°C until electrophoresis. Eighteen enzymes were tested, 12 of which were satisfactorily resolved, scoring for 19 putative loci. Of these, only 8 were polymorphic: *Aat-1*, *Acp-2*, *Dia-1*, *Idh*, *Mdh-1*,

Me-2, *Pgm-2*, and *Skd-2*. The remaining ones (*Acp-1*, *Amp-1*, *Dia-2*, *Dia-3*, *Dia-4*, *Dia-5*, *Dia-6*, *Hex*, *6Pgd*, *Pgi-1*, and *Skd-1*) were monomorphic across sampled individuals.

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 (1972) buffer. Staining procedures followed the method described by Soltis et al. (1983) and Wendel and Weeden (1989), with slight modifications.

Interpretation of banding patterns was performed on the basis of the quaternary structure of isozymes, subcellular localization, and number of loci usually expressed in diploid plants, according to standard principles (Wendel and Weeden 1989). Individual heterozygosity was measured as the number of allozyme loci for which the sampled individuals were heterozygous divided by the total number of loci (19) surveyed in these individuals. At the population level, mean heterozygosity was calculated by averaging the individual heterozygosities for each population.

Statistical Analysis of Fitness Parameters

The range of values of the measured plant fitness parameters for all the individuals within the 9 studied populations are presented in Fig. 1. Levels of statistical significance among populations for each of the fitness parameters (but also for individual heterozygosity) were determined by performing separate one-way ANOVAs (Fig. 1).

Prior to the analysis of the relationship between individual heterozygosity and fitness-related traits, data obtained for fitness parameters were standardized. Pearson's correlation coefficients were calculated among the plant parameters measured. Because most parameters were significantly intercorrelated, a principal component analysis (PCA) was conducted to obtain a small number of uncorrelated linear combinations of original variables (the principal components) that account for most of the variability of the data. Only the components with an eigen value ≥ 1 were selected. Subsequently, Pearson's correlation coefficients were calculated between the scores of individual plants ($N = 196$) on each of the principal components and individual heterozygosity. The relationships between fitness-related traits (represented by the principal components) and heterozygosity within the individuals for each of the nine examined populations were also addressed by means of linear correlations.

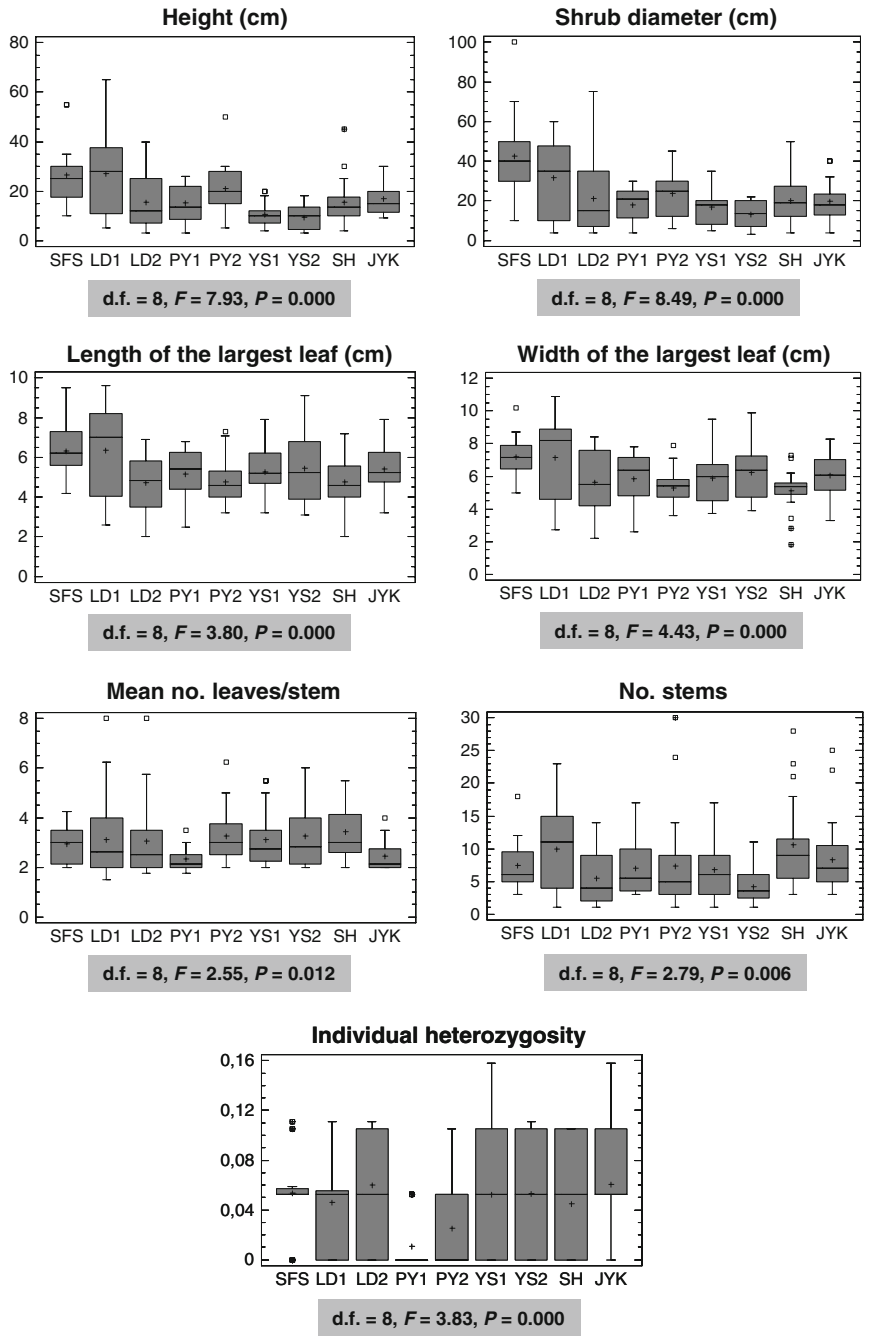

Fig. 1 Differences in plant fitness parameters among individuals within populations and among populations of *Clematis acerifolia*. ANOVA values between populations are listed under each parameter. Population codes as in Table 1

To test the relationship between heterozygosity and fitness at the population level ($N = 9$), Pearson's correlation coefficients were calculated between the average PC (principal component) scores of pooled individual plants for each population and several estimations of heterozygosity: H_o (observed heterozygosity), H_e (expected panmictic hererozygosity), and the average individual heterozygosity (the latter calculated as explained above). The other suggested relationships at the population level, i.e., between fitness and light availability, and between fitness and population size, were also studied by means of linear regressions. Finally, the relationship between population size and the three estimators of heterozygosity was assessed using the same procedure.

Results

Relationship Between Heterozygosity and Fitness

High and significant Pearson's correlation coefficients among most fitness parameters were found (see Table 2). Only the mean number of leaves/stem showed a weak correlation or even an absent one with the other measured fitness parameters (Table 2). Principal component analysis reduced the six original variables to two uncorrelated principal components with an eigen value ≥ 1, which together accounted for 77.8% of the total variability of the original data (data not shown). As expected by the values obtained by the Pearson's correlation coefficients (see Table 2), component 1 was highly correlated with all the fitness parameters except for the mean number of leaves per stem, which was obviously correlated with component 2 (data not shown).

Neither of the components ($r = 0.044$, $P = 0.543$ between PC1 and heterozygosity; and $r = -0.021$, $P = 0.769$ between PC2 and heterozygosity) were significantly correlated with heterozygosity at individual level (Table 3). The linear correlations performed between the two PCs and individual heterozygosities at each population also showed an overall lack of statistical significance, with a single exception (PY2 population; Table 3). Finally, simple regressions between the mean

Table 2 Pearson's correlation coefficients between plant fitness parameters in *Clematis acerifolia*

	Height	Shrub diameter	Length of largest leaf	Width of largest leaf	Mean no. leaves/stem	No. stems
Height	–					
Shrub diameter	0.749***	–				
Length of largest leaf	0.621***	0.679***	–			
Width of largest leaf	0.615***	0.692***	0.907***	–		
Mean no. leaves/stem	0.209**	0.192**	0.059	0.049	–	
No. stems	0.621***	0.649***	0.474***	0.472***	0.116	–

Note: Number of samples = 196; ** $P < 0.01$, *** $P < 0.001$

Table 3 Pearson's correlation coefficients between principal component scores and heterozygosity within populations of *Clematis acerifolia*

All individuals ($N = 196$)	Population (number of samples)								
	SFS (20)	LD1 (20)	LD2 (22)	PY1 (20)	PY2 (25)	YS1 (25)	YS2 (16)	SH (20)	JYK (28)
PC1 scores									
r 0.044	−0.336	−0.285	−0.091	0.155	*0.600*	0.044	−0.211	0.185	0.325
P 0.543	0.160	0.222	0.686	0.515	*0.001*	0.833	0.433	0.434	0.092
PC2 scores									
r −0.021	−0.074	0.096	0.001	−0.169	0.014	0.370	−0.194	−0.227	−0.108
P 0.769	0.762	−0.686	0.996	0.476	0.948	0.068	0.472	0.336	0.583

Note: Statistically significant cases are in italics. Population codes as in Table 1

heterozygosity (using whichever of the three heterozygosity measures described in Materials and Methods) and the average PC scores at the population level ($N = 9$) also showed a total lack of correlation (Table 4).

Relationship Between Light Availability and Fitness

A simple regression analysis between light availability for each population and average PC scores gave some interesting results (Table 4). Although there was no correlation for PC1 ($r = 0.080$, $P = 0.837$), a positive but only marginally significant correlation was found between light availability and PC2 ($r = 0.637$, $P = 0.065$), a component mainly related to the mean number of leaves per stem. A linear regression performed between the mean number of leaves per stem and light availability shows a significant correlation ($r = 0.870$; $P = 0.002$). Therefore, individuals of *C. acerifolia* tended to exhibit more leaves per stem if the site where they grew received more sunshine.

Table 4 Pearson's correlation coefficients between principal component scores and heterozygosity, light availability and population size at the population level in nine populations of *Clematis acerifolia*

	Heterozygosity[a]			Light availability	Population size
	H_o	H_e	Average individual		
PC1 scores					
r	0.132	0.252	0.121	0.080	0.004
P	0.735	0.512	0.757	0.837	0.992
PC2 scores					
r	0.039	−0.247	0.074	0.637	−0.190
P	0.921	0.521	0.849	0.065	0.624

[a] H_o, observed heterozygosity. H_e, expected panmictic heterozygosity

Relationships of Population Size, Heterozygosity, and Fitness

None of the regressions between population size and the average scores for each of the principal components showed any correlation (Table 4). In addition, no significant correlations were found between population size and the measures of heterozygosity ($r = 0.444$, $P = 0.231$ for average individual heterozygosity; $r = 0.439$, $P = 0.237$ for H_o; and $r = 0.158$, $P = 0.684$ for H_e).

Discussion

In general terms, no relationship has been found between the measured fitness-related traits and heterozygosity, at both individual and population levels, in *Clematis acerifolia*. This indicates that individuals with high allozyme heterozygosity show no difference in fitness-measured traits compared to those with low heterozygosity. Likewise, the populations with higher levels of heterozygosity do not necessarily exhibit better performance. This pattern apparently contradicts the theoretical expectation of positive correlation between heterozygosity and fitness (Hansson and Westerberg 2002; Reed and Frankham 2003), which has been reported for several perennial plant taxa from western Europe, such as *Swertia perennis* for vegetative fitness (Lienert et al. 2002) and *Gentiana pneumonanthe* (Oostermeijer et al. 1994), *Cochlearia bavarica* (Paschke et al. 2002), and *Succisa pratensis* (Vergeer et al. 2003) for generative fitness.

The possibility of a positive correlation between heterozygosity and fitness in *Clematis acerifolia* cannot be ruled out, however, because of the following considerations about the experimental conditions of our study. First, given that the plants have been studied directly in the field, there can be a source of environmental variation that affects our phenotypic measurements; i.e., the variability of the measured fitness parameters can rely on phenotypic plasticity rather than having a genetic basis. If the study had been done under greenhouse conditions, growth of the individuals under equal (environmental) conditions would have better reflected heterozygote advantage. Second, almost all the measured individuals were probably long-lived individuals, because they exhibited a wide and strong lignified basal stem; in contrast, seedlings or juveniles were notably absent in the field (J. López-Pujol and F.-M. Zhang, personal observation). Therefore, we could have measured the heterozygosity after selection has acted within populations (i.e., most inbred individuals were probably eliminated from populations at earlier stages, leaving mainly highly heterozygous old survivors), thus avoiding a positive correlation between heterozygosity and fitness (see Hansson and Westerberg 2002). It is interesting that the only population that showed a significant correlation between fitness (PC1) and individual heterozygosity is PY2. In this locality, many individuals of *C. acerifolia* were younger (with their stems less lignified) than those in the other populations studied (J. López-Pujol and F.-M. Zhang, personal observation). Therefore, we can assume that this population is relatively new (perhaps the result of a colonization event from PY1, probably a much older locality) and that natural selection has not had enough time to obscure the correlation signal.

An additional experimental weakness of our study that may have precluded the finding of a positive correlation is the low proportion of polymorphic loci found in *C. acerifolia* (all the analyzed individuals ranged from 0 to 3 heterozygous loci), limiting us from reaching strong conclusions. The use of hypervariable markers, such as microsatellites, and the combination of several markers representing different regions of the genome may help to solve this flaw. Despite this, individuals in our analysis showed a relatively wide range of heterozygosity within populations, and differences among populations were statistically significant (Fig. 1). On the other hand, the inclusion of reproductive parameters as fitness traits (such as seed set, germination rate, or seedling mortality) in further studies is highly desirable because it could reveal a different relationship between fitness and heterozygosity.

A number of studies have shown no relationship between heterozygosity and fitness, notably in several species of the genus *Pinus*. For example, no correlation was found between heterozygosity and growth rate in *Pinus contorta* (Knowles and Mitton 1980) and *P. ponderosa* (Linhart and Mitton 1985), and no relationships were found between several vegetative and reproductive fitness-related traits and heterozygosity in *P. sylvestris* (Savolainen and Hedrick 1995). A positive association between heterozygosity and vegetative and generative fitness was also absent in *Lychnis viscaria* (Lammi et al. 1999), in *Swainsona recta* (Buza et al. 2000), and in *Delphinium bolosii* (Orellana et al. 2007), whereas only a weak correlation was stated between vegetative fitness traits and heterozygosity in *Swertia perennis* (Lienert et al. 2002). Moreover, meta-analyses of published correlation coefficients among multilocus heterozygosity and two fitness traits (growth rate and fluctuating asymmetry) have suggested that the strength of these relationships is generally weak in natural populations (Britten 1996). Instead of following a general pattern, the presence or lack of these associations seems to vary according to the species studied (Lammi et al. 1999).

Ecological factors determining the habitat quality of a species (e.g., vegetation coverage, light availability, or soil mineral concentrations) may have a major influence on fitness in natural populations of plant species, as demonstrated in several species including *Arnica montana* (Fennema 1990), *Gentiana pneumonanthe* (Oostermeijer et al. 1998), *Cochlearia bavarica* (Paschke et al. 2002), *Succisa pratensis* (Vergeer et al. 2003), and *Ranunculus nodiflorus* (Noel et al. 2007). In fact, variability in habitat quality can have a much greater impact on fitness than heterozygosity. In *G. pneumonanthe*, habitat characteristics had a strong influence on reproductive fitness, while heterozygosity (genetic diversity) did not show a significant effect (Oostermeijer et al. 1998). In *R. nodiflorus*, all the variability found in fitness parameters was attributable to environmental variation instead of genetic (microsatellite) diversity, which was completely lacking in the populations studied (Noel et al. 2007). The only indicator of habitat quality measured in our study, light availability, is related to the mean number of leaves per stem in *Clematis acerifolia*, but not to the other fitness parameters. A detailed survey of the habitat conditions (soil composition, ground vegetation cover, and so on) may allow us to assess the real contribution of habitat quality (environmental factors) to the performance of individuals of *C. acerifolia*.

A positive relationship between population size and fitness has been reported for several species, e.g., *G. pneumonanthe* (Oostermeijer et al. 1994), *Cochlearia bavarica* (Paschke et al. 2002), *Succisa pratensis* (Vergeer et al. 2003), and *Pulsatilla vulgaris* (Hensen et al. 2005), among others. This correlation is expected because of the close relationship between population size and heterozygosity (Young et al. 1996; Frankham 1996; Oostermeijer et al. 2003), rather than a direct effect on fitness (see Vergeer et al. 2003, and references therein). In our study, however, we did not find a correlation between population size and fitness in *Clematis acerifolia*, which is not surprising, given the lack of any relationship between population size and heterozygosity on the one hand and between heterozygosity and fitness-related traits on the other (see Results).

In conclusion, heterozygosity (i.e., genetic factors) seems not to have a determinant influence on the fitness-related traits studied in *C. acerifolia*. Rather, environmental factors (e.g., habitat quality) are probably responsible for the variation in fitness traits in this plant species. Accordingly, any future management plan aimed at ensuring the viability of this endangered species should be focused more on maintaining (and eventually restoring) habitat quality than on maximizing genetic diversity within populations, which is the classical conservation recommendation (e.g., Frankham 2005). This represents a great challenge, however, because of the current fragmentation and deterioration of the habitat (see López-Pujol et al. 2005, for more details).

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References

Allendorf FW, Leary RF (1986) Heterozygosity and fitness in natural populations of animals. In: Soulé M (ed) Conservation biology: the science of scarcity and diversity. Sinauer, Sunderland, pp 57–76

Boerrigter EJM (1995) On the perspectives of populations of the rare plant species *Phyteuma nigra*. Ph.D. Thesis, State University of Groningen, The Netherlands

Booy G, Hendriks RJJ, Smulders MJM, van Groenendael JM, Vosman B (2000) Genetic diversity and the survival of populations. Plant Biol 2:379–395

Bosch M, Waser NM (1999) Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (Ranunculaceae). Am J Bot 86:871–879

Britten H B (1996) Meta-analyses of the association between multilocus heterozygosity and fitness. Evolution 50:2158–2164

Buza L, Young A, Thrall P (2000) Genetic erosion, inbreeding and reduced fitness in fragmented populations of the endangered tetraploid pea *Swainsona recta*. Biol Conserv 93:177–186

Campbell DR, Halama JJ (1993) Resource and pollen limitations to lifetime seed production in a natural plant population. Ecology 74:1043–1051

Clayton JW, Tretiak DN (1972) Amino-citrate buffers for pH control in starch gel electrophoresis. J Fish Res Board Can 29:1169–1172

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: Implications for plant conservation. Annu Rev Ecol Syst 24:217–242

Fennema F (1990) Effects of exposure to atmospheric SO_2, NH_3 and $(NH_4)_2SO_4$ on survival and extinction of *Arnica montana* L. and *Viola canina* L, Report Research Institute for Nature Management 90/14, Arnhem, The Netherlands

Fischer M, Matthies D (1997) Mating structure and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). Am J Bot 84:1685–1692

Frankham R (1996) Relationships of genetic variation to population size in wildlife. Conserv Biol 10:1500–1508

Frankham R (2005) Genetics and extinction. Biol Conserv 126:131–140

Gaston KJ, Blackburn TM, Greenwood JJD, Gregory RD, Quinn RM, Lawton JH (2000) Abundance-occupancy relationships. J Appl Ecol 37:39–59

Gong WZ, Long YY, Li MX (1985) Karyotype studies on *Clematis* from Beijing, China. J Wuhan Bot Res 3:371–379

Hansson B, Westerberg L (2002) On the correlation between heterozygosity and fitness in natural populations. Mol Ecol 11:2467–2474

Hensen I, Oberprieler C, Wesche K (2005) Genetic structure, population size, and seed production of *Pulsatilla vulgaris* Mill. (Ranunculaceae) in Central Germany. Flora 200:3–14

IUCN (2001) IUCN Red List Categories and Criteria: Version 3.1, UCN Species Survival Commission, Gland and Cambridge

Kéry M, Matthies D, Spillmann H-H (2000) Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. J Ecol 88:17–30

Kimura M (1983) The neutral theory of molecular evolution. Cambridge University Press, Cambridge

Knowles P, Mitton JB (1980) Genetic heterozygosity and radial growth variability in *Pinus contorta*. Silvae Genet 29:114–118

Lammi A, Siikamäki P, Mustajärvi K (1999) Genetic diversity, population size, and fitness in central and peripheral populations of a rare plant *Lychnis viscaria*. Conserv Biol 13:1069–1078

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

Linhart Y, Mitton JB (1985) Relationships among reproduction, growth rates, and protein heterozygosity in ponderosa pine. Am J Bot 72:181–184

López-Pujol J, Zhang F-M, Ge S (2005) Population genetics and conservation of the critically endangered *Clematis acerifolia* (Ranunculaceae). Can J Bot 83:1248–1256

Luijten SH, Dierick A, Oostermeijer JGB, Raijmann LEL, den Nijs HCM (2000) Population size, genetic variation, and reproductive success in a rapidly declining, self-incompatible perennial (*Arnica montana*) in The Netherlands. Conserv Biol 14:1776–1787

Mitton JB (1989) Physiological and demographic variation associated with allozyme variation. In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Dioscorides Press, Portland, pp 127–145

Mitton JB (1994) Molecular approaches to population biology. Annu Rev Ecol Syst 25:45–69

Mitton JB, Grant MC (1984) Associations among protein heterozygosity, growth rate, and developmental homeostasis. Annu Rev Ecol Syst 15:479–499

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Noel F, Machon N, Porcher E (2007) No genetic diversity at molecular markers and strong phenotypic plasticity in populations of *Ranunculus nodiflorus*, an endangered plant species in France. Ann Bot 99:1203–1212

Ohta R (1971) Associative overdominance caused by linked detrimental mutations. Genet Res 18:277–286

Oostermeijer JGB, van Eijck MW, den Nijs JCM (1994) Offspring fitness in relation to population size and genetic variation in the rare perennial plant species *Gentiana pneumonanthe* (Gentianaceae). Oecologia 97:289–296

Oostermeijer JGB, Luijten SH, Krenová ZV, den Nijs JCM (1998) Relationships between population and habitat characteristics and reproduction of the rare *Gentiana pneumonanthe* L. Conserv Biol 12:1042–1053

Oostermeijer JGB, Luijten SH, den Nijs JCM (2003) Integrating demographic and genetic approaches in plant conservation. Biol Conserv 113:389–398

Orellana MR, López-Pujol J, Blanché C, Bosch M (2007) Relationships between heterozygosity and fitness in the Iberian threatened larkspur *Delphinium bolosii* (Ranunculaceae). Flora 202:161–168

Paschke M, Abs C, Schmid B (2002) Relationship between population size, allozyme variation, and plant performance in the narrow endemic *Cochlearia bavarica*. Conserv Genet 3:131–144

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. Conserv Biol 17:230–237

Savolainen O, Hedrick P (1995) Heterozygosity and fitness: no association in scots pine. Genetics 140:755–766

Soltis DE, Haufler H, Darrow D, Gastony J (1983) Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers and staining schedules. Am Fern J 73:9–27

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. Proc Natl Acad Sci USA 101:15261–15264

Thelen GC, Allendorf FW (2001) Heterozygosity-fitness correlations in rainbow trout: effects of allozyme loci or associative overdominance? Evolution 55:1180–1187

Vergeer P, Rengelink R, Copal A, Ouborg NJ (2003) The interacting effects of genetic variation, habitat quality and population size on performance of *Succisa pratensis*. J Ecol 91:18–26

Wang W-T (2002) A revision of *Clematis* sect. Cheiropsis (Ranunculaceae). Acta Phytotaxon Sin 40:193–241

Wendel F, Weeden NF (1989) Visualization and interpretation of plant isozymes. In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Dioscorides Press, Portland, pp 5–45

Yang Q-E (2002) Cytology of ten species in *Anemone*, one in *Anemoclema* and six in *Clematis* (Trib. Anemoneae, Ranunculaceae) from China. Acta Phytotaxon Sin 40:396–405

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. Trends Ecol Evol 11:413–418

Zouros E, Foltz DW (1987) The use of allelic isozyme variation for the study of heterosis. In: Rattazzi MC, Scandalios JG, Whitt GS (eds) Isozymes: current topics in biological and medical research, vol 13, Alan R. Liss Publisher, New York, pp 1–59