Conservation genetics and evolutionary history of *Gleditsia caspica*: Inferences from allozyme diversity in populations from Azerbaijan

Andrew Schnabel^{1,*} & Konstantin V. Krutovskii²

¹Department of Biological Sciences, Indiana University South Bend, South Bend, IN 46634; ²Institute of Forest Genetics, USDA Forest Service, Pacific Southwest Research Station, Environmental Horticulture Department, University of California, Davis, CA 95616 (*Corresponding author: Fax: 574-237-6589; E-mail: aschnabe@iusb.edu)

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Abstract

Gleditsia caspica is an endemic tree species found in the endangered lowland Hyrcanian forests near the Caspian Sea in southeastern Azerbaijan and northwestern Iran. Phylogenetic analyses show that *G. caspica* is a derivative of *G. japonica*, a widely distributed species of eastern Asia. Using allozyme markers to investigate genetic diversity within two populations from Azerbaijan, we discovered that a population from a protected nature reserve was composed largely of first-generation hybrids between *G. caspica* and *G. triacanthos*, which is native to eastern North America but is planted as an ornamental shade tree in Azerbaijan. Hybrids exhibited higher levels of self-fertilization and lower seed production than either parental species. In the second population, composed of pure *G. caspica*, 11 of 31 loci scored were polymorphic, the average number of alleles per locus was 1.39, and gene diversity was 0.105. All diversity estimates were substantially lower than those we obtained from a small sample of *G. japonica* from South Korea and than estimates from more extensive samples of South Korean *G. japonica* and North American *G. triacanthos* published by other workers. We conclude that (i) in addition to ongoing threats from habitat conversion and fragmentation, *G. caspica* may also be threatened in Azerbaijan by hybridization with *G. triacanthos*; and (ii) low variability in *G. caspica* populations is most likely not a consequence of recent habitat loss and reductions in population size, but instead reflects a long history of range contraction and endemism following isolation from *G. japonica* during the Pliocene or Pleistocene.

Introduction

Genetic threats to rare and endangered species can appear in multiple guises. One well-documented threat comes from human-caused degradation and fragmentation of communities and whole ecosystems, resulting in smaller, more isolated populations that are subject to loss of genetic diversity, increased inbreeding, and ultimately, lowered population fitness (Ellstrand and Elam 1993; Reed and Frankham 2003). A second threat comes from hybridization with introduced, alien species, which may result in disruption of locally adapted genotypes and displacement of native genotypes by hybrids (Simberloff 1996; Vila et al. 2000). In several cases, hybridization has been shown

to produce taxa that are strongly invasive (Ellstrand and Schierenbeck 2000), which can have negative consequences for populations of parental species and other species within their communities.

The focus of our study was *Gleditsia caspica*, which is the most geographically isolated member of a genus of 13 species that largely inhabit eastern and southeastern Asia (Gordon 1966; Schnabel and Wendel 1998; Schnabel et al. 2003). Although most often treated as a separate species (Gordon 1966), *G. caspica* is considered by Paclt (1982) to be a subspecies of the widespread, eastern Asian species, *G. japonica*. Phylogenetic studies by Schnabel and Wendel (1998) and Schnabel et al. (2003) suggest that *G. caspica* and *G. japonica* are sister taxa and that

G. caspica is a derivative of *G. japonica*, possibly arising during the Pliocene. This *G. japonica*/*G. caspica* clade, which also includes the Chinese *G. delavayi*, forms a larger monophyletic group with the eastern North American species, *G. triacanthos* and *G. aquatica*.

Gleditsia caspica Desf. (Leguminosae) is endemic to the highly diverse and critically endangered Caspian Hyrcanian mixed forests that are found along the southern Caspian coastal plain and lower mountain slopes (to about 2500 m) in extreme southeastern Azerbaijan and northwestern Iran (Rechinger 1986; Sokolov 1986; Boulos et al. 1994). The total area of the Hyrcanian ecoregion covers only about 50,000 km², and within this region, *G. caspica* is native exclusively to the lowland forests, typically below 800 m elevation (Frey and Probst 1986; Boulos et al. 1994). The lowland Hyrcanian forests have suffered extensive degradation and fragmentation as a result of clearing for agriculture and grazing (Frey and Probst 1986). In addition to *G. caspica*, these forests are rich in other relic and endemic plant species (Akhani 1998; Olson et al. 2000).

In this study, we investigated allozyme diversity within populations of *G. caspica* from Azerbaijan, and compared those data to allozyme data in *G. japonica* and *G. triacanthos* both collected by us and taken from published sources (Huh et al. 1999; Schnabel and Hamrick 1990). Because the phylogenetic relationships of *G. japonica* are so well understood, we were able to interpret our data not only in terms of recent human-induced changes to *G. caspica* habitat, but also in terms of long-term evolutionary processes. These comparisons suggest that historical rather than recent human-related factors have had the greatest effect on current levels of allozyme diversity. Recent introductions of the alien *G. triacanthos*, however, pose a credible genetic threat to *G. caspica* through hybridization.

Methods

Collecting sites

We were able to locate only two populations in southern Azerbaijan (possibly partially due to travel restrictions we experienced near the Iranian border), and collections from within Iran were not possible. The first population was in the Girkan Nature Reserve, just outside the city of Lenkoran, Azerbaijan, and

consisted of about 75 individuals of mixed ages. We visited this population in May and September 1990. During the first visit, we collected reproductive data (presence of staminate vs. carpellate inflorescences; presence vs. absence of fruits from the previous year) and leaves for allozyme analyses from all 26 trees that were flowering. During the September visit, we collected additional leaf tissue for allozyme analysis from those same trees, and we also recorded data on number of fruits produced by each of the trees. To estimate number of seeds/pod produced by each tree, 1–23 pods were harvested from each of the 15 trees that had fruited in that year. A subsample of the seeds were germinated for use in mating system analyses.

The second population was located near the town of Astara, Azerbaijan, less than 1 km north of the Iranian border. We visited this population only in September 1990, at which time we collected mature leaves for allozyme analysis from 30 adult individuals. The sample represented the majority of individuals from this small and confined population. To estimate seed production per fruit and to obtain a larger sample for allozyme analyses, we collected 6–10 pods from each of the 13 trees that had fruited.

Sample sizes for allozyme analyses

Initial tests of allozyme diversity in *G. caspica* were conducted in 1990 at the N.I. Vavilov Institute of General Genetics, Moscow, Russia, using the collections of 26 adults from Girkan, 30 adults from Astara, and a small collection of nine individuals collected from the Central Botanical Garden, Kiev, Ukraine. The trees from Kiev had been grown from seeds collected at Girkan. As a comparison, we used leaves that were sampled from several cultivated *G. triacanthos* trees in Baku, Azerbaijan, and Moscow, Russia, where *G. triacanthos* is planted as an ornamental shade tree.

More extensive allozyme studies were conducted at the University of Georgia, Athens, USA, in 1992 and 1993. For these studies, allozyme diversity was estimated for a random sample of 48 *G. caspica* seeds from the fruits collected at Astara and for a sample of 55 *G. japonica* seeds (all from a single maternal tree) that were sent from South Korea by Dr. M. G. Chung (Gyeongsang National University, South Korea). For a mating system analysis of the Girkan population, we used an additional 186 seeds from the nine maternal trees that produced sufficient numbers of fruits.

Electrophoretic analyses

For analysis of adult leaf tissue, several leaflets from each tree were ground to a fine powder with the aid of liquid nitrogen, and the powder was then mixed immediately with a vegetative extraction buffer (Cheliak and Pitel 1984). The resulting slurry was placed in 1.5 ml microcentrifuge tubes and centrifuged at 6,000 rpm for 30 min. The supernatant solution was then stored at -70°C . Five enzyme systems (six putative loci) were resolved for all plants using a Tris-citrate buffer (pH 7.2; system 5 in Soltis et al. 1983): alcohol dehydrogenase (*Adh*), isocitrate dehydrogenase (*Idh2*), malate dehydrogenase (*Mdh2*), 6-phosphogluconate dehydrogenase (*Pgd2*, *Pgd3*), and shikimate dehydrogenase (*Skdh*). Staining recipes followed Wendel and Weeden (1989). Individuals of *G. caspica* and *G. triacanthos* were run simultaneously on the same gels to facilitate comparisons between species.

Seeds were germinated and grown to the 2–3 leaf stage before being used for enzyme extraction. All enzyme extraction and electrophoretic protocols were as described in Schnabel and Hamrick (1990). We ran several initial test gels containing all three species and some putative *G. caspica* $\times$ *G. triacanthos* hybrids (from the Girkan population; see Results), and we included standards of *G. triacanthos* in all of the *G. caspica* gel runs and standards of *G. caspica* and *G. triacanthos* in all of the *G. japonica* gel runs. For estimation of genetic variation in the Astara population, we used 15 enzyme systems, from which 30 putative loci were resolved: acid phosphatase (*Acp1*, *Acp2*, *Acp3*), aldolase (*Ald1*, *Ald2*), aspartate aminotransferase (*Aat2*), diaphorase (*Dia1*, *Dia2*), fluorescent esterase (*Fe1*, *Fe2*, *Fe3*), isocitrate dehydrogenase (*Idh2*), leucine aminopeptidase (*Lap1*, *Lap2*), malate dehydrogenase (*Mdh1*, *Mdh2*, *Mdh3*), malic enzyme (*Me2*), peroxidase (*Per1*, *Per3*), 6-phosphogluconate dehydrogenase (*Pgd1*, *Pgd2*, *Pgd3*), phosphoglucoisomerase (*Pgi1*, *Pgi2*), phosphoglucomutase (*Pgm1*, *Pgm2*), shikimate dehydrogenase (*Skdh*), and triose phosphate isomerase (*Tpi1*, *Tpi2*). Allozyme variation in *G. japonica* was determined for 23 putative loci. Some loci that were scored for *G. caspica* were not resolved for *G. japonica* and vice versa (see Table 2 for list of loci). For the mating system analysis, each seedling was scored for eight polymorphic loci (*Fe2*, *Fe3*, *Idh2*, *Pgd2*, *Pgd3*, *Pgi2*, *Pgm2*, *Skdh*).

Data analysis

Allozyme variability was quantified using several standard statistics (Hamrick and Godt 1996): the proportion of polymorphic loci (P), the mean number of alleles per locus (A), the mean number of alleles per polymorphic locus (A_P), observed heterozygosity (H_o), and Hardy-Weinberg expected heterozygosity (H_e ; gene diversity; Astara population only). Sampling variances for H_o and H_e were calculated according to Nei (1987) and Hedrick (2000). Deviations of observed genotype frequencies from Hardy-Weinberg expectations were assessed by means of χ^2 tests (Hedrick 2000). Levels of outcrossing for the Girkan population as a whole and for individual families were estimated using the MLTR program of Ritland (2002). Standard deviations were calculated using 500 bootstraps, where the progeny array was considered the unit of observation for population estimates.

Results

In the initial analysis of adult leaf tissue performed at the Vavilov Institute, only one (*Idh2*) of the six loci tested was polymorphic in the Astara population, and three loci (*Idh2*, *Pgd3*, *Skdh*) shared no alleles with the *G. triacanthos* cultivars (Table 1). The Girkan population, on the other hand, was polymorphic at five of six loci, each of which showed complete or nearly complete heterozygosity. Heterozygous individuals at Girkan always contained one allele typical of each *G. caspica* and *G. triacanthos* (i.e., based on comparisons of samples from the Astara population and cultivated *G. triacanthos*, as well as on known allele frequencies from more extensive sampling in *G. triacanthos* by Schnabel and Hamrick 1990). For example, 24 of 26 individuals were heterozygous for the *Pgd3-a* allele fixed in *G. triacanthos* samples and the *Pgd3-b* allele fixed in the *G. caspica* samples from Astara. The loci *Idh2* and *Skdh* exhibited essentially the same results as *Pgd3*. Only one individual sampled at Girkan differed consistently from the rest, and this individual showed genotypes homozygous for *G. triacanthos* alleles at *Idh2*, *Pgd2*, *Pgd3*, and *Skdh*. The samples from the Central Botanical Garden, Kiev, had combinations of genotypes not seen in either at Astara or Girkan or among the *G. triacanthos* cultivars.

All of the 26 trees we observed flowering at Girkan produced inflorescences that could be categorized as

Table 1. Allele frequencies, observed heterozygosities (H_o), and sample sizes (N) for six isozyme loci analyzed using adult leaf tissue from *Gleditsia caspica* and *G. triacanthos*

Locus	Species (population)			
	<i>G. caspica</i> (Astara)	<i>G. caspica</i> (Girkan)	<i>G. caspica</i> (Central Bot. Garden, Kiev)	<i>G. triacanthos</i> (cultivated)
<i>Adh</i>				
<i>a</i>	0.00	0.00	0.00	0.03
<i>b</i>	1.00	1.00	1.00	0.76
<i>c</i>	0.00	0.00	0.00	0.21
H_o	0.00	0.00	0.00	0.47
<i>N</i>	30	26	9	34
<i>Idh2</i>				
<i>b</i>	0.00	0.52	0.50	1.00
<i>c</i>	0.82	0.48	0.50	0.00
<i>d</i>	0.18	0.00	0.00	0.00
H_o	0.37	0.96	1.00	0.00
<i>N</i>	30	26	9	8
<i>Mdh2</i>				
<i>a</i>	1.00	0.50	0.50	0.38
<i>b</i>	0.00	0.50	0.50	0.62
H_o	0.00	1.00	1.00	0.50
<i>N</i>	30	26	9	8
<i>Pgd2</i>				
<i>a</i>	0.00	0.50	0.33	0.75
<i>b</i>	1.00	0.50	0.67	0.25
H_o	0.00	0.92	0.67	0.50
<i>N</i>	29	26	9	44
<i>Pgd3</i>				
<i>a</i>	0.00	0.50	1.00	1.00
<i>b</i>	1.00	0.50	0.00	0.00
H_o	0.00	0.92	0.00	0.00
<i>N</i>	29	26	9	8
<i>Skdh</i>				
<i>a</i>	0.00	0.52	0.50	1.00
<i>d</i>	1.00	0.48	0.50	0.00
H_o	0.00	0.96	1.00	0.00
<i>N</i>	30	26	9	8

either staminate or carpellate, with each tree producing only one kind of inflorescence. About 76% of the trees with morphologically staminate inflorescences produced some fruits. Many of the fruits at Girkan were smaller and contained more aborted seeds than in the Astara population. An average (± 1 SD) of 5.0 ± 3.0 seeds were produced per fruit at Girkan ($N = 14$ trees; 1–26 fruits sampled/tree), whereas 16.4 ± 3.9 seeds were produced per fruit at Astara ($N = 13$ trees; 6–10 fruits sampled/tree). This difference was highly

significant ($t = 8.4$, $df = 22$, $P < 0.001$; 3 trees, each with only one fruit, excluded from test).

The samples of seedlings analyzed at the University of Georgia showed that genetic diversity in the Astara population of *G. caspica* was substantially lower than diversity in the single open-pollinated family of *G. japonica* (Table 2). Of the 31 loci sampled in *G. caspica* (including *Adh* sampled only from adult leaf tissue), 11 (35%) were polymorphic. Only one of those polymorphic loci (*Lap1*) had greater than two alleles, resulting in low numbers of alleles per locus (mean $A \pm 1$ SD = 1.39 ± 0.56) and per polymorphic locus ($A_P = 2.09 \pm 0.30$). Consequently, estimates of gene diversity were also low (mean $H_e = 0.105 \pm 0.031$). Observed and expected heterozygosities did not differ from one another for any of the loci (χ^2 tests; results not shown). In the *G. japonica* sample, 14 of 23 loci (60.9%) were polymorphic, with mean $A = 1.78 \pm 0.80$ and mean $A_P = 2.29 \pm 0.61$. Although gene diversity was not calculated for this sample, mean observed heterozygosity was substantially greater than for *G. caspica* (Table 2).

These results were not biased by differences in the sets of loci sampled for each species (Table 2). Of the 21 loci we sampled in both species, seven were monomorphic in both samples, six were polymorphic in *G. japonica* but monomorphic in *G. caspica*, and no monomorphic locus in *G. japonica* was polymorphic in *G. caspica*. Of the eight loci that were polymorphic in both samples, one locus had fewer alleles in *G. caspica*, whereas all the others had the same number of alleles in both species.

Mating system analysis estimated an outcrossing rate of about 50% for the Girkan population as a whole (Table 3). The multilocus and average single-locus estimates did not differ substantially from one another. Differences in outcrossing rates among individual families were large, varying from complete selfing to complete outcrossing.

Discussion

Hybridization in the Girkan population and conservation implications

The genetic analyses conducted at the Vavilov Institute suggest that the Astara population has reduced variation relative to *G. triacanthos* but that the Girkan population is much more variable and shares alleles of both *G. caspica* and *G. triacanthos*. Nearly complete

Table 2. Summary of mean allozyme diversity statistics for the 31 loci sampled in *Gleditsia caspica* (Astara population) and 23 loci sampled in *G. japonica*. Because the *G. japonica* sample was taken from a single maternal family, no calculation of expected heterozygosity was made. Dashes indicate loci that were not scored for one or the other species. Sample sizes are 48 for *G. caspica* (except for *Adh*, where $N = 30$) and 55 for *G. japonica*. SD = Standard deviation

Locus	<i>G. caspica</i>			<i>G. japonica</i>	
	No. alleles	H_o	H_e	No. alleles	H_o
<i>Aat1</i>	—	—	—	1	0.000
<i>Aat2</i>	1	0.000	0.000	2	0.518
<i>Acp1</i>	1	0.000	0.000	1	0.000
<i>Acp2</i>	1	0.000	0.000	—	—
<i>Acp3</i>	1	0.000	0.000	—	—
<i>Adh</i>	1	0.000	0.000	—	—
<i>Ald1</i>	1	0.000	0.000	—	—
<i>Ald2</i>	1	0.000	0.000	—	—
<i>Dia1</i>	1	0.000	0.000	—	—
<i>Dia2</i>	2	0.062	0.061	2	0.036
<i>Fe1</i>	1	0.000	0.000	2	0.036
<i>Fe2</i>	2	0.500	0.500	4	0.654
<i>Fe3</i>	1	0.000	0.000	3	0.509
<i>Idh2</i>	2	0.354	0.342	2	0.636
<i>Lap1</i>	3	0.396	0.471	—	—
<i>Lap2</i>	2	0.333	0.305	—	—
<i>Mdh1</i>	2	0.396	0.474	2	0.054
<i>Mdh2</i>	1	0.000	0.000	1	0.000
<i>Mdh3</i>	1	0.000	0.000	1	0.000
<i>Me1</i>	—	—	—	1	0.000
<i>Me2</i>	1	0.000	0.000	2	0.309
<i>Per1</i>	2	0.146	0.135	—	—
<i>Per3</i>	1	0.000	0.000	1	0.000
<i>Pgd1</i>	2	0.021	0.021	2	0.273
<i>Pgd2</i>	1	0.000	0.000	2	0.454
<i>Pgd3</i>	1	0.000	0.000	—	—
<i>Pgi1</i>	1	0.000	0.000	1	0.000
<i>Pgi2</i>	1	0.000	0.000	1	0.000
<i>Pgm1</i>	2	0.354	0.318	2	0.127
<i>Pgm2</i>	2	0.479	0.404	2	0.254
<i>Skdh</i>	1	0.000	0.000	3	0.418
<i>Tpi1</i>	1	0.000	0.000	1	0.000
<i>Tpi2</i>	2	0.271	0.232	2	0.018
Mean $\pm$ 1 SD (all loci)	1.39 $\pm$ 0.56	0.107 $\pm$ 0.008	0.105 $\pm$ 0.031	1.78 $\pm$ 0.80	0.187 $\pm$ 0.011
Mean $\pm$ 1 SD (21 loci)	1.38 $\pm$ 0.50	0.116 $\pm$ 0.010	0.112 $\pm$ 0.032	1.86 $\pm$ 0.79	0.205 $\pm$ 0.012

heterozygosity for alleles typical of each species strongly indicates that most of the trees sampled at Girkan were first generation interspecific hybrids. Based on genotypes alone, only one of the individuals sampled (a female tree) was a pure species, but it was apparently *G. triacanthos*, not *G. caspica*.

The comparisons of fruit and seed production provided additional evidence for the presence of hybrids in the Girkan population. Under normal circumstances, pure *G. caspica* is expected to have 16–20 ovules/ovary (Gordon 1966), although viable seed production might be expected to be less than that due to abortion and insect predation, as is found in *G. triacanthos* (Mathwig 1971) and *G. japonica*

(Takakura 2002). The much lower seed production at Girkan than at Astara is consistent with the hypothesis that the first-generation hybrids at Girkan have lowered fertility compared to pure *G. caspica*.

This difference could be explained by changes in the breeding system of the hybrids compared to the parental species. Species of *Gleditsia* typically exhibit near dioecy (Gordon 1966; Robertson and Lee 1976), with individuals producing either functionally carpelate or functionally staminate inflorescences, and therefore, self-fertilization is not expected within their populations (but see Huh et al. (1999) for a possible counter-example). None of the loci we sampled from the Astara population exhibited deviations from

Table 3. Outcrossing estimates (t) for nine *Gleditsia* families from a single population in the Girkan Nature Reserve, Azerbaijan. Estimates are based on analysis of genotypes from eight polymorphic allozyme loci. Standard deviations (SD) were obtained from 500 bootstraps. *N* = sample size

Family (<i>N</i>)	Multilocus t (SD)	Single locus t (SD)
1 (22)	0.001 (0.330)	0.001 (0.316)
2 (23)	0.001 (0.281)	0.001 (0.273)
3 (16)	0.812 (0.369)	0.910 (0.385)
4 (22)	0.323 (0.317)	0.316 (0.320)
5 (26)	1.200 (0.250)	1.200 (0.267)
6 (23)	0.679 (0.173)	0.513 (0.187)
7 (9)	0.793 (0.481)	0.454 (0.272)
8 (23)	0.001 (0.329)	0.001 (0.321)
9 (21)	0.001 (0.365)	0.001 (0.372)
Population estimate (186)	0.534 (0.237)	0.475 (0.242)

Hardy-Weinberg expectations, which suggests to us that little self-fertilization occurs in pure *G. caspica* populations. Mating system analyses also indicate that *G. triacanthos* populations in North America are completely outcrossing (Schnabel and Hamrick 1990). The hybrid Girkan population, however, exhibited high levels of self-fertilization, with extreme variation among families. We note that our standard deviations and family outcrossing estimates are probably somewhat inflated due to sample sizes that are less than ideal (Ritland 2002), but whatever bias or inflation there may be in the estimates, they cannot completely offset the differences between families that have diametrically opposed outcrossing rates. It thus appears that the hybrids exhibit both an altered breeding system and lowered fertility relative to both of the parental species.

If the Girkan trees are truly F_1 hybrids, and the trees at the Central Botanical Garden, Kiev, were grown from seeds produced by Girkan trees, then the Kiev trees are effectively F_2 hybrid progeny and would be expected to show the mixture of genotypes that we observed. We also observed that these trees appeared to be producing large numbers of pods with many seeds and thus seemed to be fully fertile. As pointed out by Parks and Wendel (1990), this pattern of semi-fertile F_1 hybrids and fully fertile F_2 progeny has been observed in other woody genera when hybrids have formed between Asian and North American species (e.g., *Liriodendron*, *Magnolia*). Based on data from the fossil record (Herendeen and Dilcher 1992) and

from phylogenetic analyses (Schnabel and Wendel 1998; Schnabel et al. 2003), North American and Asian *Gleditsia* have been isolated for at least 5–10 million years. North American and Caspian populations must have been isolated for an even longer period of time. Our unexpected discovery of *Gleditsia* hybrids thus supports the general conclusion by Wen (1999), based on a review of all available studies at the time, that interspecific compatibility is not necessarily evidence of a recent divergence.

The fact that hybrid individuals were found in a protected nature reserve also has implications for the conservation of *G. caspica* populations in Azerbaijan and most likely in Iran, where *G. triacanthos* is also planted as an ornamental shade tree (Dr. H. Akhiani, University of Tehran, personal communication). The main objective of the Girkan Nature Reserve, which is the only protected area in the very southern part of Azerbaijan where *G. caspica* is native, is preservation of the unique lowland forest community of the Hyrcanian ecoregion (Gasnov 1990). The Hyrcanian forests in Azerbaijan and Iran, especially in the lowlands where *G. caspica* is native, are severely threatened by conversion to agricultural and pasture land (Frey and Probst 1986; Millington and Gokhelashvili 2000; Olson et al. 2000). Because the Girkan population appears to be composed of F_1 hybrids and some pure *G. triacanthos*, with no *G. caspica* that we could find in that part of the forest, it seems reasonable to assume that this hybrid population was planted by humans. Nonetheless, other *G. caspica* populations within the 3000-hectare reserve, if they exist, should probably be tested to determine if they are also of hybrid origin.

For two reasons, the threat from hybridization is a realistic concern. First, even though the Girkan population was not reproducing well, some seed production was occurring, and we observed a variety of age classes in the population. From the Kiev Botanical Garden data, we can infer that second and later generation hybrids are fully fertile, which indicates that *G. triacanthos* genes could spread from their initial location to pure *G. caspica* populations by either seed or pollen gene flow. In North America, *Gleditsia triacanthos* seeds are transported quickly through agricultural landscapes, because they are readily eaten by cattle (Blair 1990). We would expect the same process to occur in Azerbaijan. Pollen gene flow is also possible, because *Gleditsia* species have generalist pollination syndromes, and paternity analyses suggest that *G. triacanthos* pollen can be

transported at least several hundred meters (Schnabel and Hamrick 1995). Second, as documented by Ellstrand and Schierenbeck (2000), hybridization can stimulate the evolution of invasive plant populations. This concern applies to *G. caspica*, because *G. triacanthos*, although being widely promoted and tested as a useful species for temperate zone agroforestry management (Gold and Hanover 1993; Ainalis and Tsiouvaras 1998; Arredondo et al. 1998), often becomes invasive when planted outside its native range (Csurhes and Kriticos 1994; Ghera et al. 2002). Thus, it is not unreasonable to infer that a hybridized *G. caspica* might also develop invasive tendencies. Boulos et al. (1994) report that some areas of degraded, Caspian coastal plain have already been invaded by exotics that sometimes form a “spiny maquis”.

A further conservation concern raised by this study is the misidentification of *Gleditsia* species within botanical gardens and arboreta, as exemplified by the discovery of hybrid *Gleditsia* genotypes in the Kiev Botanical Garden. Genetic testing of these specimens is typically not done, and because many species of *Gleditsia* are morphologically similar to one another (Gordon 1966), misidentification is undoubtedly a problem (e.g., Schnabel and Wendel 1998). One of the goals of many arboreta and botanical gardens is the preservation and propagation of representatives from specific regions or habitats. For instance, the *Gleditsia* samples we obtained in Kiev were growing in a section of the garden that was supposed to contain representative species of lowland Hyrcanian forests. If other gardens or arboreta are also maintaining hybrid or otherwise misidentified *G. caspica* trees, then this aspect of an overall preservation strategy for the species will be in jeopardy.

Genetic diversity and evolutionary history of G. caspica

One concern for rare and endangered species is the loss of genetic diversity over time as populations become isolated from one another and much reduced in size. For any species, however, an adequate explanation of current diversity patterns requires consideration of both evolutionary history and contemporary population processes. Certain characteristics, such as breeding system and geographic range, are sometimes good predictors of levels of allozyme diversity in plants (Hamrick and Godt 1996). For example, wind-pollinated species with outcrossed or mixed

mating systems and large geographic ranges tend to harbor greater allozyme diversity within their populations than species with high levels of self-fertilization or limited distributions. These differences are often explained in terms of effective population sizes, where species with a history of large effective sizes harbor more variation than those with smaller effective sizes (Karron 1987; Hamrick and Godt 1996). Viewed within a broader evolutionary context, an endemic species may currently have low levels of genetic variation, because it belongs to a clade with low variation, or because it lost variation during speciation. In the latter case, the low variability could be a consequence of genetic bottlenecks experienced during speciation or of the influence of long-term endemism and small population sizes following speciation (e.g., Loveless and Hamrick 1988; Pleasants and Wendel 1989; Sherman-Broyles et al. 1992; Edwards and Sharitz 2000; Cheng et al. 2000; Maki et al. 2002).

Because of the hybrid nature of the Girkán plants, our analysis of allozyme diversity in *G. caspica* is limited to consideration of the Astara population. For two reasons, we would expect this population to be representative of other populations within its narrow range. First, *G. caspica* is a highly outcrossing species, and Hamrick and Godt (1996) show that outcrossing species maintain the great majority of their variation within populations. Second, Neel and Cummings (2003) show that for rare species with low F_{ST} values, estimates of gene diversity (H_e) within populations are very similar regardless of the number of populations sampled. We do, however, recognize the limitations of the data set, and do not argue that we have captured as full a picture of diversity in this species as would be necessary for setting conservation targets (Neel and Cummings 2003).

Nonetheless, our data convincingly show that *G. caspica* maintains lower levels of genetic diversity than does its progenitor, *G. japonica*, for which we had samples from only one progeny array. An even stronger case can be made by comparing the Astara data with the more extensive data set of Huh et al. (1999), who sampled 17 allozyme loci from 12 populations of *G. japonica* in South Korea. Mean diversity statistics from that study, which included 14 of the same loci we sampled, were $P = 53\%$ (range 47–59%), $A = 1.75$ (1.59–1.82), $A_P = 2.42$ (2.11–2.75), and $H_e = 0.247$ (0.193–0.289). These values are comparable to diversity estimates based on our small sample of *G. japonica*, and the range of each statistic does not overlap our low estimates for

the Astara population. Thus, even the least variable population sampled by Huh et al. (1999) appears to contain substantially more allozyme diversity than the Astara *G. caspica* population.

On a locus-by-locus basis, our data also suggest that *G. caspica* has only a subset of the alleles found in *G. japonica*. Of the 14 loci sampled both by us and by Huh et al. (1999), five were monomorphic in both species, four were polymorphic in *G. japonica* but not in *G. caspica*, and the remaining five loci (polymorphic in both species) all showed higher gene diversity estimates in *G. japonica* than in *G. caspica*. Average gene diversity for these latter five loci was 0.463 for *G. japonica* and 0.294 for *G. caspica*.

Two processes have probably combined to reduce levels of genetic diversity in *G. caspica* compared to its progenitor. First, fossil evidence suggests that *Gleditsia* was widespread throughout the Caucasus (Abkhazia, eastern Georgia, Armenia) during the Miocene and at least part of the Pliocene (Shakryl 1992), but no *Gleditsia* fossils have been found further west or north of this region. Thus, it is likely that *G. caspica* evolved from one or more populations on the western margin of the range of its *G. japonica*-like progenitor, and those populations may have contained only a subset of the total diversity present in the species. Second, genetic variation was probably lost over time due to the combination of natural selection and genetic drift as the range of *Gleditsia* contracted during the Pliocene and Pleistocene, leaving a currently highly restricted range that is isolated by thousands of kilometers from the nearest *G. japonica* populations. Paclt (1982) argues that several species from the warm-temperate forests of the southern Caucasus, including *G. caspica*, probably once had wider ranges stretching to the Himalayas and sometimes to eastern Asia, and that present-day discontinuous distributions within these groups resulted from repeated Pleistocene glaciations that confined populations to isolated refugia such as the southern Caucasus and the Korean peninsula. At least for legumes, evidence supports this hypothesis, insofar as the modern flora of the Caucasus harbors many fewer legume genera than are found in the fossil record from the Miocene and Pliocene (Shakryl 1992). *Gymnocladus*, for example, the sister genus of *Gleditsia*, has been found in Pliocene deposits from the Caucasus, but presently grows only in eastern/southeastern Asia and eastern North America.

The argument for loss of variation during speciation in *G. caspica* is strengthened by noting

that Schnabel and Hamrick (1990) found levels of allozyme diversity in *G. triacanthos* populations from North America that were very similar to the estimates of Huh et al. (1999) for *G. japonica*. *Gleditsia japonica* and *G. triacanthos* are the most widespread species in one of the most strongly supported monophyletic groups within *Gleditsia*, and each of these widespread species has one or two more narrowly distributed sister taxa on their respective continents (Schnabel et al. 2003). In North America, *G. triacanthos* is sister to *G. aquatica*, a species confined largely to floodplains and swamps of the lower Mississippi river valley and the southeastern coastal plain of the United States. In Asia, *G. japonica* or a *G. japonica*-like ancestor has apparently given rise not only to *G. caspica*, but also to at least one other species, *G. delavayi*, currently found in southern China (Gordon 1966; Li 1982, 1988). Judging by *G. japonica* and *G. triacanthos*, the ancestral condition within this clade appears to be one of high levels of genetic diversity. It would be interesting to sample populations of *G. delavayi* and *G. aquatica* to determine whether similar reductions in diversity have occurred during the evolution of these species. One might predict that reductions, if any, should be less pronounced than in *G. caspica*, because neither *G. delavayi* nor *G. aquatica* have experienced the geographic isolation and endemism that characterize the evolutionary history of *G. caspica*.

In conclusion, we have shown that a population of the endemic species, *G. caspica*, in Azerbaijan has substantially less genetic variation than populations of more widespread relatives from eastern Asia and North America. Even though *G. caspica* currently appears to be found mostly in small populations in habitats that have suffered from considerable degradation and fragmentation, the low level of variability we found appears most likely to be a consequence of an extended period of range contraction and endemism during the speciation process rather than of current population processes. These conclusions may change in the future, however, if hybridization with North American *G. triacanthos* continues and results in introgression of alien genes into native *G. caspica* populations.

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