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PATTERNS OF ASPARTATE AMINOTRANSFERASE VARIATION IN RELATION TO POPULATION SIZE, FOUNDER EFFECT, AND PHYTOGEOGRAPHIC HISTORY IN *Cypripedium calceolus*

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Abstract. Eight populations of *Cypripedium calceolus* L. were studied in different parts of Estonia. The heterozygosity in three aspartate aminotransferase loci was found to be high (0.40–0.53). In a recently established population where thanks to land rise the growth of forest plants has been possible during 200 years at most, the founder effect was confirmed by population genetic data. Geographically closer populations did not show a higher genetic similarity than more distant populations. An equally high level of genetic variability in isolated populations of a species of restricted distribution can be explained by wider occurrence of this species in previous times.

Key words: *Cypripedium calceolus*, distribution, founder effect, genetic distance, heterozygosity.

INTRODUCTION

The distribution of genetic variation within and among plant populations is influenced both by contemporary and historical factors (Dolan, 1994). The significance of population size for breeding structure, genetic diversity, and evolutionary dynamics is a central focus of concern in conservation biology. Where populations are small and isolated from one another, genetic drift exerts a dominant influence on the genetic structure of the population. Populations that have remained small for long periods are likely to be considerably less diverse than those that have become small only recently. The distribution and level of

genetic diversity within and among populations of rare plants probably depend on whether the species has always been rare or has become rare recently as a result of human influence. In 1963 Mayr coined the term "founder effect" to refer to the establishment of a new population by a few original founders that carry only a small fraction of the total genetic variation of the parental population (Barrett & Kohn, 1991).

Despite the tremendous diversity of the orchid family, very little is known about the level of genetic diversity within natural populations. Genetic variability has been studied only in some species, e.g., in several *Cypripedium* species (Case, 1993, 1994), *Gymnadenia conopsea* (Scacchi & De Angelis, 1989; Schlegel et al., 1989), *Epipactis* species (Scacchi et al., 1987), *Orchis* species (Schlegel et al., 1989; Scacchi et al., 1990; Rossi et al., 1992), *Cephalanthera* species (Scacchi et al., 1991), *Dactylorhiza* species (Schlegel et al., 1989; Hedrén, 1996), and *Leporella fimbriata* (Peakall & James, 1989). The object of this study, *Cypripedium calceolus* L., is a long-lived clonal orchid species with a quite wide Eurasian and North-American distribution (considering the species *sensu lato*) (Hultén & Fries, 1986). The individual clones of this species may persist for more than a hundred years (Kull, 1988).

The aim of the present study was to examine the genetic variability of aspartate aminotransferase in natural populations of *C. calceolus* in Estonia and to investigate the founder effect in a population of this plant species.

MATERIALS AND METHODS

The material was collected and analysed in 1987, 1988, 1991, 1994, and 1996. Eight populations were studied in different parts of Estonia (Fig. 1). The number of individuals sampled (Table 1) represents 10 to 100% of the clones in each population. A leaf tip was taken from each sampled plant (Kull, 1988). The samples of neighbouring plants of the same phenotype were excluded from calculations to avoid clonally reproduced ramets of the same genet. The identification of clones follows the experience of our previous study (Kull, 1988). AAT (EC 2.6.1.1) was chosen as the most variable enzyme with a good resolution (Kull, 1988).

Enzyme extracts were prepared by crushing small leaf pieces in a cold buffer containing 0.05 M tris-hydroxy-methyl-aminomethane (tris), 0.01 M EDTA, and 5 mM cysteine hydrochloride. After the removal of cell debris, 20–50 mg aliquots of a sucrose–Sephadex G-200 4:1 mixture were added to the enzyme extracts to increase their viscosity. The extracts were immediately subjected to electrophoresis in a polyacrylamide gel. After electrophoresis, the gels were incubated and stained in histochemical mixtures (Jaaska, 1981; Jaaska & Jaaska, 1986). The gene diversity statistics of Nei (1973) was used for statistical analysis.

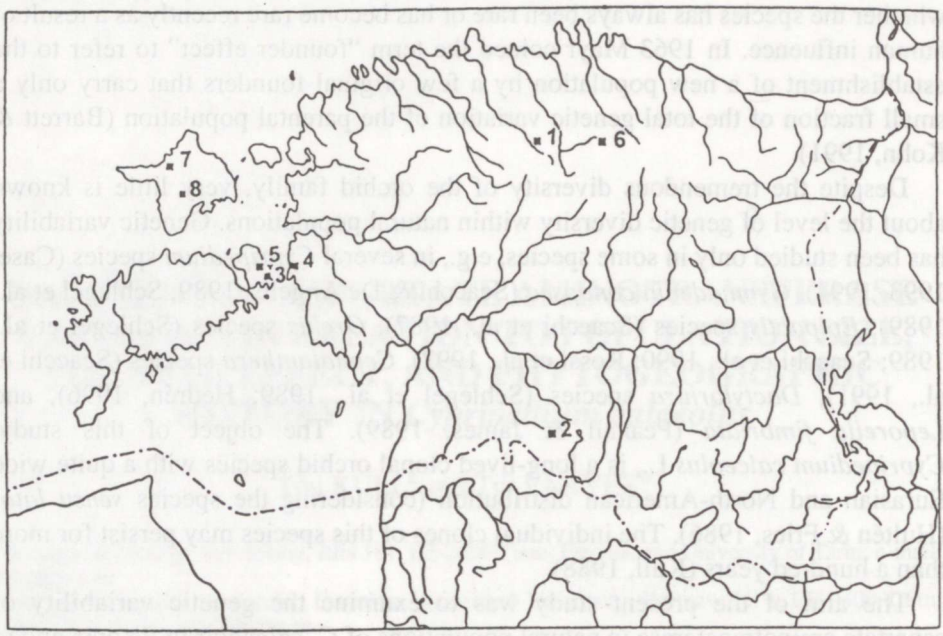

Fig. 1. Map of the studied populations. Numbers refer to populations listed in Table 1.

Table 1

Sample size and average heterozygosity of the investigated populations of *Cypripedium calceolus*

Population	No. of individuals sampled	Population size (No. of flowering shoots)	Average heterozygosity for 3 loci of AAT (± SE)
1. Ussisoo	71	200	0.45 (0.12)
2. Õisu	34	130	0.45 (0.18)
3. Simiste	77	2000	0.51 (0.03)
4. Laelatu	57	500	0.50 (0.07)
5. Võlla	33	100	0.51 (0.12)
6. Varangu	42	160	0.40 (0.07)
7. Kõrgessaare	49	800	0.16 (0.15)
8. Aruselja	14	50	0.53 (0.11)

RESULTS

The average heterozygosity was high in all three loci of AAT in the studied populations, ranging from 0.40 to 0.53. The only exception was Kõrgessaare (7), where the average heterozygosity was 0.16 (Table 1). In this population one allele in each locus was prevailing or had frequencies close to fixation: AAT-1a 100%, AAT-2d 71%, AAT-3a 99% (Table 2).

The level of variability was high in all populations, but allele frequencies fluctuated remarkably (Table 2). In most populations all three loci did not show regular deviations ($P < 0.05$) from Hardy–Weinberg expectations (Table 2), while in some populations there was significant deficiency of heterozygotes. There was no correlation between the level of heterozygosity and population size ($P = 0.05$). UPGMA dendrogram of genetic distances (Fig. 2) reveals that geographically closely located populations (3 and 5; cf. Fig. 1) did not cluster together genetically although the distances between them were only a few kilometres.

Table 2

Allele frequencies in eight different populations of *Cypripedium calceolus*. Deviations from Hardy–Weinberg expectations: [a] deficiency of heterozygotes, [b] deficiency of homozygotes

Locus & allele	Population*							
	1	2	3	4	5	6	7	8
AAT-1a	0.64	0.48	0.56	0.55	0.44	0.21	1.00	0.64
AAT-1b	0.36	0.52	0.44	0.45	0.56	0.79	0.00	0.36
χ^2	6.67[a]	0.29	5.75[a]	0.64	5.68[b]	0.01	–	–
AAT-2a	0.23	0.18	0.27	0.26	0.15	0.08	0.02	0.25
AAT-2b	0.52	0.29	0.57	0.41	0.24	0.63	0.05	0.39
AAT-2c	0.12	0.19	0.14	0.22	0.38	0.07	0.22	0.18
AAT-2d	0.13	0.34	0.02	0.11	0.23	0.22	0.71	0.18
χ^2	2.64	5.85	8.72	7.06	3.24	21.85[a]	5.94	–
AAT-3a	0.87	0.94	0.65	0.49	0.82	0.81	0.99	0.77
AAT-3b	0.13	0.06	0.35	0.51	0.18	0.19	0.01	0.23
χ^2	0.60	7.19	1.58	10.95[a]	0.01	2.34	–	–

* Numbers as in Table 1.
– Not counted because of small sample size.

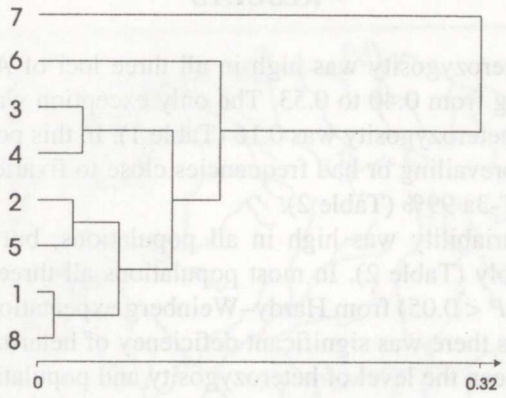

Fig. 2. Dendrogram of genetic distances of the populations. 1, Ussisoo; 2, Õisu; 3, Simiste; 4, Laelatu; 5, Võlla; 6, Varangu; 7, Kõrgessaare; 8, Aruselja.

DISCUSSION

Empirical data show that clonal plant species are in general as variable as others despite the low levels of sexual recombination (Ellstrand & Roose, 1987; Hamrick & Godt, 1989; Widén et al., 1994). Occasional sexual reproduction can probably maintain the level of genetic diversity similar to that of sexually reproducing species (Parker & Hamrick, 1992). Patchy genetic structure in natural plant populations often results from limited pollen or seed dispersal (Linhart et al., 1981). However, the geographic range and the breeding system are the best predictors of genetic parameters, endemic species distribute their variation much in the same way as widespread species (Hamrick & Godt, 1989). Some authors state that species with a limited range and few individuals exhibit low levels of genetic polymorphism (Karron et al., 1988). In our study of *C. calceolus* the population size had no impact on heterozygosity since smaller populations were not less polymorphic than larger ones.

Generative reproduction of *C. calceolus* is quite rare in Estonian populations. A considerable number of seedlings have been found only in two populations (Kull, 1995). According to our observations, this species, considered to have a typical cross-pollination system provided by bees, is still self-compatible.

In the American subtaxa of *C. calceolus* the level of allozyme variation is high, and the largest amount of variation (81%) is contained within individual populations (Case, 1993). The pattern of variation of three loci of AAT in our study was similar to that of American subtaxa: AAT-1 was monomorphic in all the examined populations, AAT-3 had a poor resolution, and AAT-2 was polyallelic (six alleles in America; Case, 1993). In North America the high level

of genetic variation in *C. calceolus* populations is accompanied with a high level of morphological variation, which is not the case in the European populations of the species. The Estonian populations of *C. calceolus* are also remarkable for their high average heterozygosity of AAT: the frequencies of different alleles in different populations are mosaic, and there is no correlation with the geographic distance. However, there occurs at least one population near the seashore on Hiiumaa Island (7) whose heterozygosity is low as compared to the other populations. This population lies at the edge of a coastal forest, close to the sea. The surface on the coast is plain, the site is 1–2 m above sea level. Considering the postglacial neotectonic uplift of the Earth's crust (at present on the average 2.4 mm per year in the region of Estonia), conditions for the growth of forest herbs in this coastal site have existed for probably no more than 200 years. Thus the population is comparatively young, and the low variability is accounted for by the founder effect, as it has been observed, for example, in isolated populations of *Sarracenia purpurea* (Schwaegerle & Schaal, 1979).

The other investigated sites may have been inhabited by *C. calceolus* for a much longer time, which is also supported by indirect evidence of the history of communities in these sites. Being a perennial clonal species, *C. calceolus* has clones that may live at least 100 years (Kull, 1988). This makes changes in population dynamics quite slow.

Our data on isoenzymes raise several questions. Why is the heterozygosity of AAT in most of the populations so high? Why is the pattern of variation mosaic, without geographical associations? Why does heterozygote deficiency occur in some populations and loci? If selection offers an advantage to heterozygotes, how can there exist populations with significant heterozygote deficiency (7)? Besides founder effect this could be explained by Wahlund effect, but lack of heterozygotes has been observed in different loci in different populations. The best explanation would probably be that the populations of *C. calceolus* in Estonia are remnants of larger and much more widespread populations. Indeed, there are many sites where the species was recorded at the beginning of the century and from where it has disappeared later. Also, wooded meadows, one of the common habitats for *C. calceolus* in Estonia, have almost disappeared during the last half-century. An indirect evidence of the previous wide distribution of the species in Estonia would be 32 different popular names used by local inhabitants to denote this species (Vilbaste, 1993).

The suggestion that clonal diversity should diminish over time needs further verification, although there exists limited evidence of this (Maddox et al., 1989).

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ASPARTAAT-AMINOTRANSFERAASI VARIEERUVUS KAUNIL KULDKINGAL (*Cypripedium calceolus*) SÕLTUVALT POPULATSIOONI SUURUSEST, ASUTAJAEFEKTIST JA AJALOOLISEST LEVIKUST

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On uuritud kaheksat kauni kuldkinga (*Cypripedium calceolus*) populatsiooni Eesti eri paigus. Aspartaat-aminotransferaasi kõigi kolme lookuse varieeruvus osutus väga kõrgeks. Jääajajärgse maakerke tõttu mere alt vabanenud Põhja-Hiiumaal, kus metsataimede kasvuks sobivad olud on eksisteerinud kõige enam 200 aastat, võib täheldada asutajaefekti. Geograafiliselt lähestikku paiknevad populatsioonid ei ole geneetiliselt sarnasemad kui kaugel paiknevad. Nii suurt polümorfismi piiratud levikuga liigi puhul võiks seletada populatsioonide kontakti ja laiema levikuga möödunud aegadel.