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Allozyme Variation in *Oxytropis retusa* Matsum. from the Kuril Archipelago

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Abstract *Oxytropis retusa* Matsum. (Fabaceae) is a rare endemic species inhabiting the Kuril Islands. Allozyme patterns of plants from the Paramushir and Chirpoi islands were studied to evaluate polymorphism indices. Starch gel electrophoresis was used to analyze the isozymes of 15 enzyme systems. The results suggest that multilocus systems encode seven of the studied enzymes, and that eight enzymes are under single-gene control. A total of 21 loci were revealed. Three were polymorphic: Fest-2, Gpt-1, and Pgi-2. The proportion of polymorphic loci (P) was 0.14, and the number of alleles per locus (A) was 1.19. These indicate a low polymorphism level commonly characteristic of endangered species.

Key words: *Oxytropis retusa,* Kuril Islands, starch gel electrophoresis, allozyme variation.

Oxytropis retusa Matsum. (Fabaceae), an endemic species of the Kurils, occurs on several islands from Paramushir to Urup (Fig. 1). This perennial herbaceous ornamental plant is an inherent element of the unique flora of the Kurils. However, it is infrequent throughout its geographic range, owing to its specific demands in habitat conditions. It is usually found on hillsides and rocks adjacent to the coast (Pavlova, 1989; personal observation). Its population density and the total number of plants are very low in those habitats. The species was included in the list of rare species of the Far East of Russia (Kharkevich and Kachura, 1981). To design a conservation strategy for this rare species, it is necessary to reveal its population dynamics, mating system, and genetic diversity in natural populations. We used allozyme analysis to estimate the level of genetic polymorphism in *O. retusa* from two islands, Paramushir and Chirpoi.

Materials and Methods

Mature plants were randomly collected from natural populations at a total of three sites on two islands, Paramushir and Chirpoi (Fig. 1). We collected six mature plants from each site on Paramushir and seven from Chirpoi. The plants were transferred to a laboratory greenhouse in Vladivostok in July 1997, where fresh young leaves were used for analysis. The following 15 enzymes were revealed and scored by using starch gel electrophoresis: alanine aminopeptidase (AAP), aldolase (ALD), alcohol dehydrogenase (ADH), diaphorase (DIA), fumarase (FUM), fluorescent esterase (FEST), fructose-1,6-diphosphatase (FDP), glucose-6-phosphate dehydrogenase (G6PD), glutamate dehydrogenase (GDH), glutamate-pyruvate transaminase (GPT), glycerate dehydrogenase (G2DH), leucine aminopeptidase (LAP), malic enzyme (ME), phosphoglucose isomerase (PGI), and sorbitol dehydrogenase (SDH). For extraction of soluble enzymes, 150–200 mg of fresh leaf tissue was placed in a chilled mortar with 0.25–0.3 mL of cold buffer. The extraction buffer used was 0.1 M sodium phosphate buffer (pH 7.5) with 0.001 M EDTA, 0.01 M ascorbic acid, 10% sucrose, 1% Tween 20, 1% β-mercaptoethanol, and 1% PVP-40. The starch gel concentration was 14%. AAP, DIA, FEST, FDP, FUM, GPT, G2DH, and SDH were revealed in the continuous Tris–citrate buffer (pH 6.2) of Wendel and Stuber (1984). ADH, ALD, G6PD, LAP, ME, and PGI were revealed in the continuous Tris–EDTA–borate buffer (pH 8.6) of Wendel and Stuber (1984). Staining procedures for ADH, ALD, FDP, FUM, G6PD, GDH, LAP, and PGI followed Shaw and Prasad (1970); those for DIA,

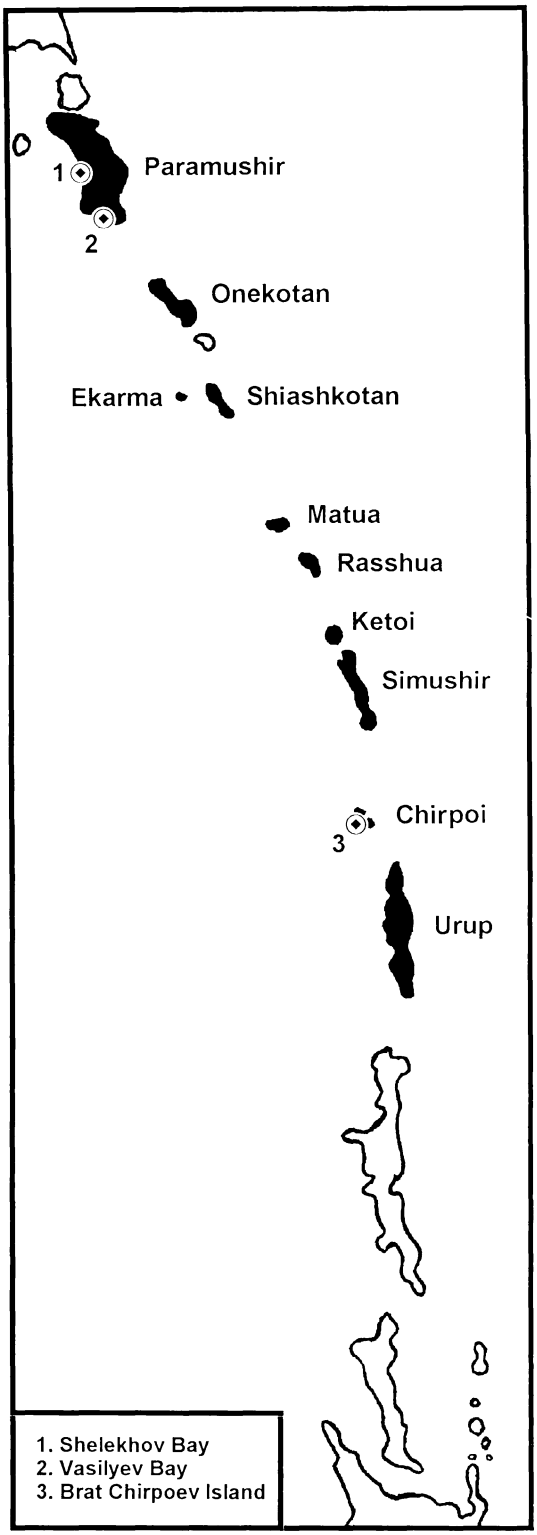

Fig. 1. Geographic distribution (filled area) of *Oxytropis retusa* Matsum. on the Kuril Archipelago and the sampling locations.

FEST, GPT, G2DH, ME, and SDH followed May (1992); those for AAP followed Conkle et al. (1982).

There is no available information on the inheritance of isozymes in *O. retusa* or other *Oxytropis* species. Genetic interpretation of isozyme data was based on the presence of banding patterns (i.e. one or two bands for codominant monomeric enzymes, and either one or three bands for codominant dimeric enzymes). If more than one putative loci were present for a single enzyme, loci were numbered sequentially, with the most anodally migrating isozyme designated as controlled by locus 1. Enzyme variants at an individual loci were given letters, with the fastest allozyme designated as controlled by allele A. The proportion of polymorphic loci (P) and the number of alleles per locus (A) were averaged for all loci. The genetic diversity (H*e*) was calculated for each locus and population as $He = 1 - \Sigma pi^2$, where pi is the frequency of the *i*th allele in each population. The mean H*e* value was obtained by averaging over the whole population.

Results and Discussion

A total of 21 loci encoding 15 enzymes were discovered in the leaf tissue of *O. retusa* (Table 1). All the observed enzyme bands migrated anodally. Electrophoresis results suggested that multilocus systems encode seven enzymes, and that eight enzymes are under single-gene control (Figs. 2, 3). At least three zones of activity were observed on FDP-zymograms. However, only one, the furthest migrated zone, was deeply stained. It was interpreted as a genetic locus; two other inconsistently stained zones were abandoned. Eighteen of the 21 scored loci were monomorphic in all individuals possessing a single enzyme band per locus. The three enzymes, FEST, GPT, and PGI, exhibited polymorphic banding patterns (Fig. 3).

Fluorescent esterase (FEST). We observed two zones of activity (Fig. 3). The fastest migrating zone was monomorphic for all individuals. The second zone was variable, and five phenotypes were revealed: three single-banded (fast, moderate, and slow) and two double-banded. These results suggest that Fest-2 is under single-locus control and that

Table 1. Enzyme investigated, their Enzyme Commission Number (E.C. No), abbreviation, numbers of scored loci, variable loci, and alleles revealed in *Oxytropis retusa* Matsum.

Enzyme	E.C. No	Abbreviation	Scored loci	Variable loci	Alleles
1. Alanine aminopeptidase	3.4.11.2	AAP	1	0	1
2. Aldolase	4.1.2.13	ALD	1	0	1
3. Alcohol dehydrogenase	1.1.1.1	ADH	1	0	1
4. Diaphorase	1.8.4.1	DIA	2	0	2
5. Fluorescent esterase	3.1.1.2	FEST	2	1	4
6. Fructose-1,6-diphosphatase	3.1.3.11	FDP	1	0	1
7. Fumarase	4.2.1.2	FUM	1	0	1
8. Glucose-6-phosphate dehydrogenase	1.1.1.42	G-6-PD	2	0	2
9. Glutamate dehydrogenase	1.4.1.2	GDH	2	0	2
10. Glutamate-pyruvate transaminase	2.6.1.2	GPT	2	1	3
11. Glycerate dehydrogenase	1.1.1.29	G-2-DH	1	0	1
12. Leucine aminopeptidase	3.4.11.1	LAP	1	0	1
13. Malic enzyme	1.1.1.40	ME	1	0	1
14. Phosphoglucose isomerase	5.3.1.9	PGI	2	1	3
15. Sorbitol dehydrogenase	1.1.1.14	SDH	1	0	1
			21	3	25

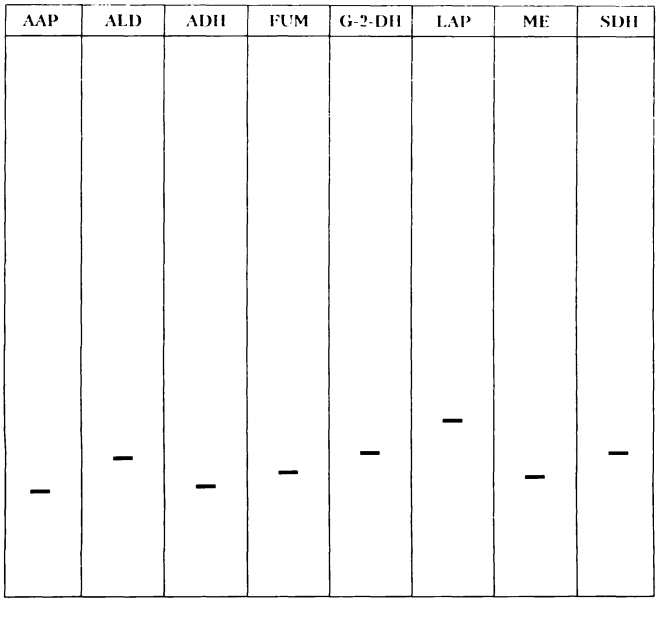

Fig. 2. Schematic representation of banding pattern in single-locus enzyme systems studied in *Oxytropis retusa* Matsum.

the existing variants correspond to three different alleles.

Glutamate-pyruvate transaminase (GPT). Electrophoretic evidence (Fig. 3) suggests the presence of two loci: Gpt-1 was variable with two alleles and Gpt-2 was monomorphic.

Phosphoglucose isomerase (PGI). There was variation in the second (slower) zone, with three phenotypes, two single-banded (one fast and one slow) and one triple-banded (Fig. 3). In the latter phenotype, a middle band of high staining intensity is expected to be an intralocus heterodimer. The analysis suggests that PGI in *O. retusa* is controlled by two genes. Pgi-1 was invariable in all individuals, and Pgi-2 revealed two variants of allelic nature.

Although several other enzymes were also

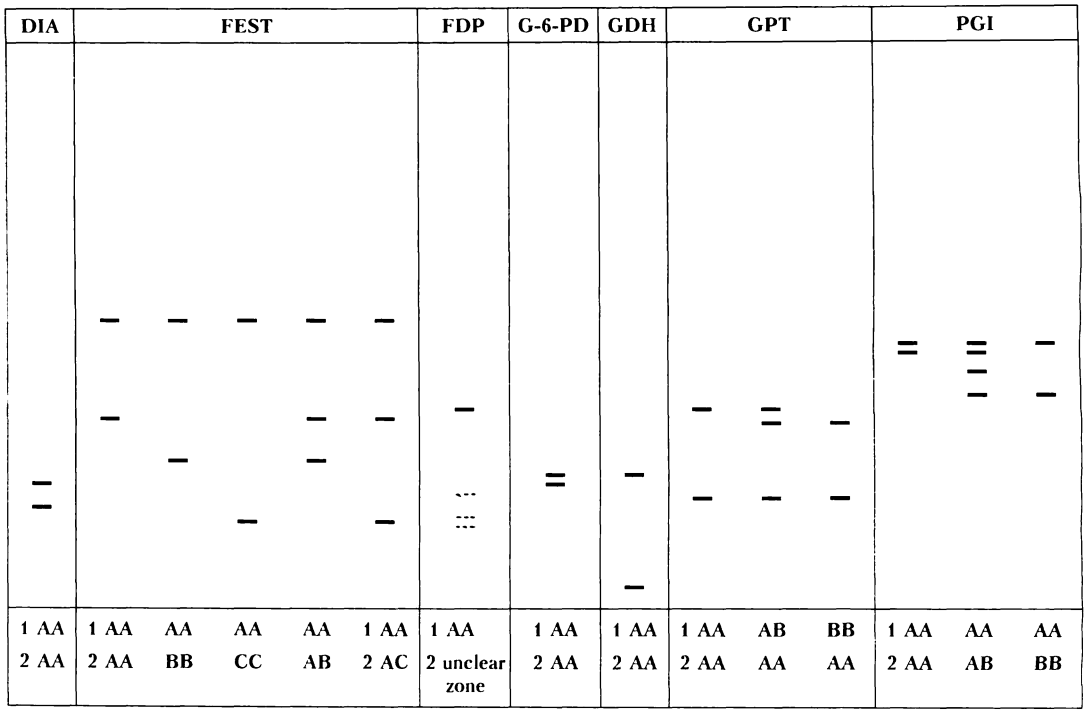

Fig. 3. Schematic representation of banding pattern and assignment of loci and alleles in multilocus enzyme systems studies in *Oxytropis retusa* Matsum.

examined, their banding patterns were uninterprable owing to faint or inconsistent staining.

For the 21 loci, the proportion of polymorphic loci (P) was 0.14, and the number of alleles per locus (A) was 1.19. Both parameters were equal for three populations (Shelekhov Bay, Vasilyev Bay, and Brat Chirpoev Island). He for the three populations were 0.072, 0.067, and 0.067 (mean 0.069). The established parameters of genetic polymorphism are probably minimal for this species, because our values are based on a relatively low number of loci within a small sample. In addition, we viewed each fixed zone of enzyme activity as a putative monomorphic locus in multibanded monomorphic enzyme systems. Thus, a real proportion of monomorphic loci can be slightly lower for island populations of *O. retusa*. However, all these remarks cannot significantly modify the general conclusion of very low polymorphism in this species.

The percentage of polymorphic loci is known to vary among legume species, thus resulting in high amplitude of allozyme diversity in the family Fabaceae. For example, in *Gleditsia triacanthos* L., P=0.62 and A=2.2 (Schnabel and Hamrick, 1990); in wild populations of *Phaseolus coccineus*, A=2.6−2.9 (Escalante et al., 1994); in wild populations of *Trifolium pratense* L., P=0.47−0.59 and A=1.5−1.8 (Semerikov and Belyaev, 1995). Usually, high P and A values are characteristic of species with a wide geographic range. In *Astragalus osterhouti* Jones, a rare species with a range restricted to four populations totaling approximately 3000 reproductive individuals in an area of 25 km^2, P=0.16 and A=1.25 (Karron et al., 1988). The genetic diversity of *O. retusa* measured by P and A indices is very close to that of *A. osterhouti*. This could be expected from the fact that *O. retusa* is an endemic of the Kurils. Another congeneric endemic from Russian Primorye, *O. chankaensis* Jurtz., was shown to possess similar characteristics of polymorphism, P=0.25 and A=1.25 (Kholina, in press). The low genetic diversity of *O. retusa* calculated as He reveals the typical connection between restricted status and genetic polymorphism (Hamrick and Godt, 1989). However, the

mechanism responsible for the low genetic variability in restricted species of *Oxytropis*, particularly *O. retusa*, is unclear. The levels of allozyme diversity in plant populations are a function of geographic range, mating system, life strategy, and taxonomic status (Hamrick, 1983; Hamrick and Godt, 1989). On the other hand, the low genetic polymorphism in *O. retusa* may be connected with the present population size and genetic drift. Moreover, the bottleneck effect may be a significant factor influencing the low genetic diversity of the species.

All allelic variants revealed at the three polymorphic loci for *O. retusa* were found in the three samples from the two islands. There were no significant differences for *He* among populations. This is an unexpected result, because the Paramushir and Chirpoi populations of *O. retusa* are separated by a group of oceanic islands (Matua, Rasshua, Ketoi, and Simushir, each inhabited by *O. retusa*) and three large straits, Bussol, Krusenstern' and Forth Kurilskiy'. However, more samples have to be examined to find the reasons responsible for this low difference between the two populations.

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千島列島産 *Oxytropis retusa* の酵素変異

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オヤマノエンドウ属の1種 *Oxytropis retusa* Matsum. は，千島列島固有の稀種である．本種の種内多型を評価するために，パラムシル島とチルポイ島から採集された標本を基に，アロザイムパターンを調べた．デンプンゲル電気泳動法を用いて，15酵素系のアイソザイムを分析した．その結果，多型的な遺伝子座が7つの酵素をコードし，単型的な遺伝子座が8酵素をコードしていることが示唆された．合計21の遺伝子座の存在が明らかにされた．そのうち，Fest-2, Gpt-1，および Pgi-2 の3遺伝子座が多型的であった．多型的遺伝子座の割合は，0.14であり，1遺伝子座あたりの対立遺伝子の数は1.19であった．これらの結果は，本種における多型性が低いことを示唆する．危急種においては，多型性が低いことが一般的に知られており，本種もその一例となるものと考えられる．