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PLANT GENETICS

Allozyme Variation of the Relict Plant *Aristolochia manshuriensis* Kom. (Aristolochiaceae)

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Abstract—Allozyme variation of a rare relict plant, birthwort *Aristolochia manshuriensis* Kom., was examined. The main parameters of genetic variation in natural populations of *A. manshuriensis* from the Anan'evka, Nezhinka, and Malaya Borisovka river basins (Primorskii Krai) were inferred from analysis of nine enzyme systems, presumably encoded by 18 loci. At 99% polymorphism criterion, 24.4% of *A. manshuriensis* loci were shown to be polymorphic. The mean number of alleles per locus was 1.24; the mean observed and expected heterozygosities, 0.12 and 0.10, respectively.

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INTRODUCTION

Relict plant species have witnessed unique processes related to global climatic changes and transformations of the continents. The Russian Far East underwent a massive Pleistocene climatic cooling, remaining free of a continuous solid ice cover, and thereby retaining many plant species that had originated in earlier epochs [1]. These preserved, mostly heat-loving plants, to date are generally low in abundance and have strongly fragmented ranges. The condition of populations of such plants is often exacerbated by human impact, which further reduces their size [2]. This generally results in decreasing genetic diversity and increasing negative effects of gene drift and inbreeding, which can lead to the complete extinction of the species [3, 4]. For instance, many Far Eastern plant species, which that were common even in the early 20th century, became rare and endangered under increasing anthropogenic influence.

The birthwort *Aristolochia manshuriensis* Kom. belongs to the ancient angiosperm family Aristolochiaceae. It is a relict wood liana, endemic to the Manchurian floristic region [5, 6]. Kurentsova [7] believes *A. manshuriensis* to be an element of the Turgai flora; Popov [8] assigns the members of the genus *Aristolochia* to the Gingko flora, which is among the oldest angiosperm floras. The main part of the species range covers North Korea and northeastern China. In Russia, natural *A. manshuriensis* populations occur only in the southern Primorye, reaching the northern boundary of the species. Some authors [5, 9] suggest continuity and long duration of the *A. manshuriensis* occurrence in southern Primorye, which is indirectly supported by the cenotypic relationships of this species [10]. Indeed, the relict butterfly *Papilio alcinous*, which uses *A. manshuriensis* leaves as a sole food source, occurs only in *A. manshuriensis* habitats in the south of Primorye [5].

In southern Primorye, known habitats of *A. manshuriensis* are located in the valleys of Borisovka, Nezhinka, Anan'evka rivers and their tributaries, divided by ridges [5, 9, 10]. The species is listed in the Red Book of the Russian Federation as endangered [11]. Natural *A. manshuriensis* populations are in a depressed state, self-reproduction of the species is insignificant [7, 9].A key role in reducing the population size of the species is played by anthropogenic factor, primarily fires and uncontrolled collecting of the plant, caused by high medicinal value of its extract [12]. Today the issue of conservation and restoring the natural *A. manshuriensis* populations is extremely urgent. Its resolution will allow to preserve not only the species and the natural biotops, but a valuable medicinal resource [12, 13].

To prevent rare species from extinction, a basic level of their genetic variation should be maintained to serve as a primary source for adaptive change [14–17]. Because of this, examination of genetic parameters is central in projects on rare species conservation [18, 19]. Data on allozyme polymorphism in *A. manshuriensis*, as well as in other *Aristolochia* species, lack in literature. In this connection, the aim of the present study was selecting markers and estimating the level of allozyme variation in *A. manshuriensis* inhabiting the Russian part of the species range and requiring development of a conservation strategy.

Table 1. Buffer systems and conditions of electrophoretic separation of the enzymes

Buffer system	Electrode buffer	Gel buffer	Regime and duration of separation	Enzymes
TC	0.223 M Tris, 0.086 M citric acid, pH 6.2	Electrode buffer diluted 1 : 22	15 mA, 105 V, 14 h; 13 mA, 95 V, 15 h	ACP, ME
TC	0.14 M Tris, 0.04 M citric acid, pH 7.8	Electrode buffer diluted 1 : 2.5	80 mA, 180 V, 14 h	FE, GPT, IDH, MDH
TEB	0.18 M Tris, 0.10 M boric acid, 0.004 M EDTA, pH 8.6	Electrode buffer diluted 1 : 25	17 mA, 180 V, 16 h	AAT, FE, GPI, GPT, PGM

MATERIALS AND METHODS

For isozyme analysis, we used stored in liquid nitrogen leaves of 191 *A. manshuriensis* plants from three natural populations located in the Nezhinka (57 plants), Anan'evka (27 plants), and Malaya Borisovka (107 plants) river basins (Nadezhdinskii, Khasanskii, and Ussuriiskii districts of Primorye) (Fig. 1). Within populations, *A. manshuriensis* usually occurs in river floodlands, being restricted by a certain height. The plant distribution is nonuniform, populations consisting of patches of dense monodominant groups. Sometimes, individual plants occur at a distance of 0.5 km and more from each other. This is particularly characteristic of population Borisovka River and its tributary Malaya Borisovka, where the patchy population structure is weakly expressed. For allozyme analysis, we collected leave samples from randomly chosen plants from such monodominant groups. Since both seed reproduction [9, 10] and vegetative reproduction [5, 7] had been described in natural *A. manshuriensis* populations, a distance of at least 15 m was taken between sampling sites; a distance between groups within each population ranged from 300 m to 4 km.

The leaves were homogenized in 50 μl of 0.1 M phosphate extraction buffer (pH 7.4), containing 10 mM ascorbic acid, 1 mM EDTA, 1% detergent Triton X-100 and 1% β-mercaptoethanol. Electrophoresis was run in 13% starch gel in three buffer systems: Tris-citrate (pH 6.2), Tris-citrate (pH 7.8), and Tris-EDTA-borate (pH 8.6). The buffer systems and electrophoresis conditions are described in Table 1. The following enzyme systems were examined: aspartate aminotransferase (AAT, E.C. 2.6.1.1), glutamate pyruvate transaminase (GPT, E.C. 2.6.1.2), (GPI, E.C. 5.3.1.9), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), acid phosphatase (ACP, E.C. 3.1.3.2), malate dehydrogenase (MDH, E.C. 1.1.1.37), malic enzyme (ME, E.C. 1.1.1.40), fluorescent esterase (FE, E.C. 3.1.1.2), and phosphoglucomutase (PGM, E.C. 2.7.5.1). After electrophoretic fractionation, the enzymes were visualized using standard procedures [20] with slight modifications. Enzymes, loci, and alleles were designated following accepted nomenclature [21], i.e., loci and alleles were numerated in order of decreasing electrophoretic mobilities of the bands of the corresponding enzymes.

Polymorphism parameters were calculated by means of conventional procedures [22]. The proportion of polymorphic loci was estimated according to the 95% criterion (a locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95) and to the 99% criterion (scoring loci with the frequencies of the common alleles not exceeding 0.99). Population subdivision was analyzed using Wright's *F*-statistics [23]: the coefficient of inbreeding of an individual relative to the population (F_{IS}), the coefficient of inbreeding of an individual relative to the total species (F_{IT}), and the subdivision index, or the coefficient of inbreeding of a population relative to the species (F_{ST}). These parameters were estimated for each locus and on average over all loci.

RESULTS

Electrophoretic analysis of nine *A. manshuriensis* enzymes revealed 23 different electrophoretic variants, presumably encoded by 18 loci (Figs. 2, 3). Two alleles were found in all polymorphic loci (Fig. 3). Nearly all allelic variants were readily interpreted on the basis of the data on their quaternary structure and principle of codominant inheritance. For only three enzymes the patterns were problematic to interpret.

Glucosephospate isomerase (GPI) showed two activity zones on the gel (Figs. 2, 3) The fast fraction GPI-1, seen as a broad diffuse band, was not taken into account. This fraction is characteristic of many plants species and is thought to result from the expression of a plastid gene [24]. The bottom zone was polymorphic and detected as three or five enzyme fractions. This zone was interpreted as locus *Gpi* with two alleles, each of which was detected as three fractions, and heterozygotes, as five fractions.

Isocitrate dehydrogenase (IDH) has two activity zones (Fig. 2). The fast zone (IDH-1) was represented by one enzyme fraction, the slow one (IDH-2) by three fractions in all plants samples examined. Both zones lacked variation and were scored as the products of monomorphic loci *Idh-1* and *Idh-2*, respectively.

Malate dehydrogenase (MDH) was detected on the electrophoregrams as four activity zones, which were interpreted as being the products of monomorphic genes *Mdh-1, Mdh-2, Mdh-3*, and *Mdh-4*. The fastest

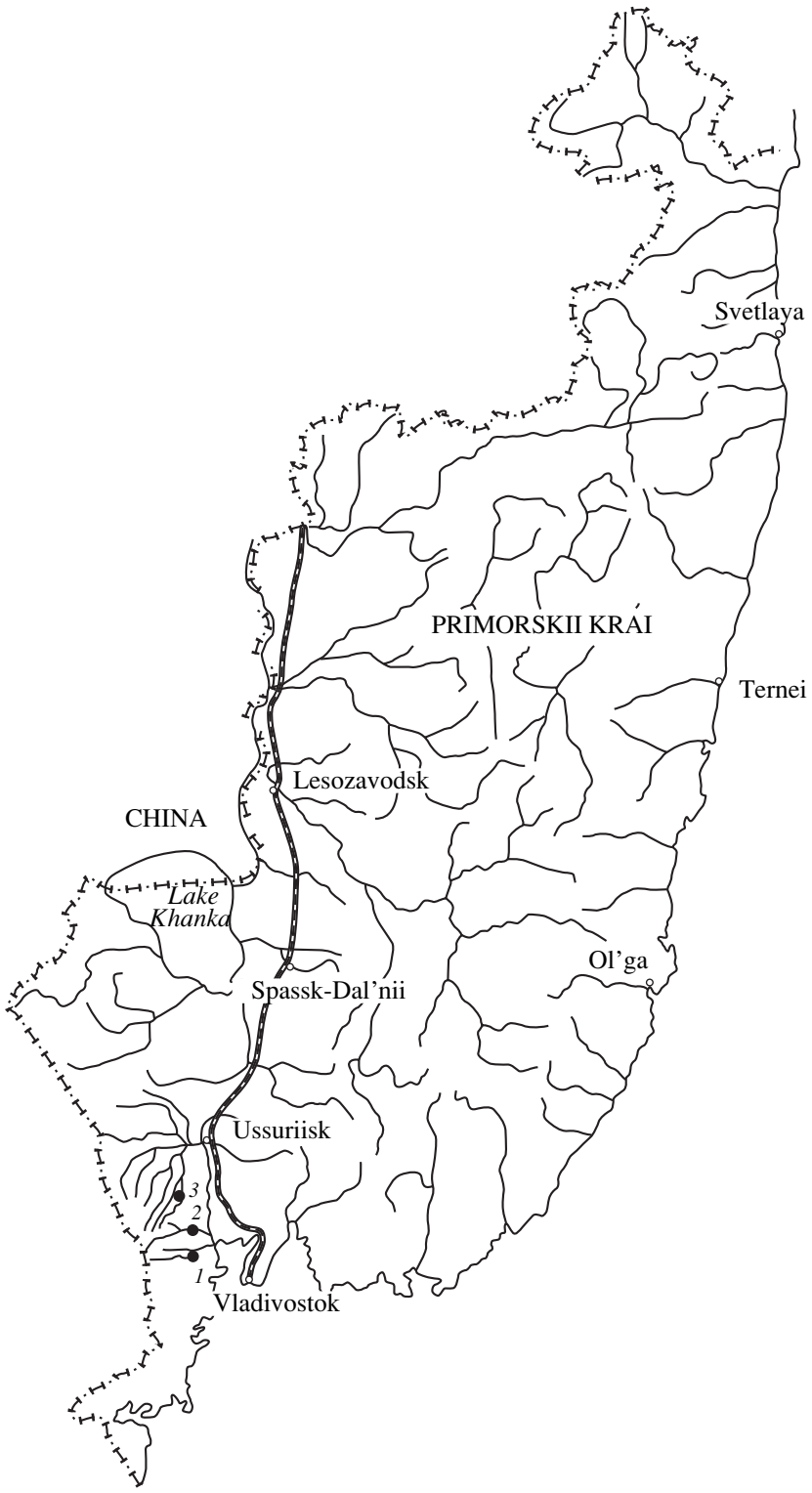

Fig. 1. Sampling sites of *A. manshuriensis* plants. Numerals designate populations: *1*, Nezhinka River basin; *2*, Anan'evka River basin; *3*, Malaya Borisovka River basin.

zone had seven fractions, and each of the slower zones, one (Fig. 2).

Multiple enzyme fractions within a zone may be explained either by posttranslational modifications of the protein products of one gene [20] or by duplication of some genes or the whole genome during evolution [24]. In most plant species studied, the isozyme composition of some enzymes, including GPI, IDH, and

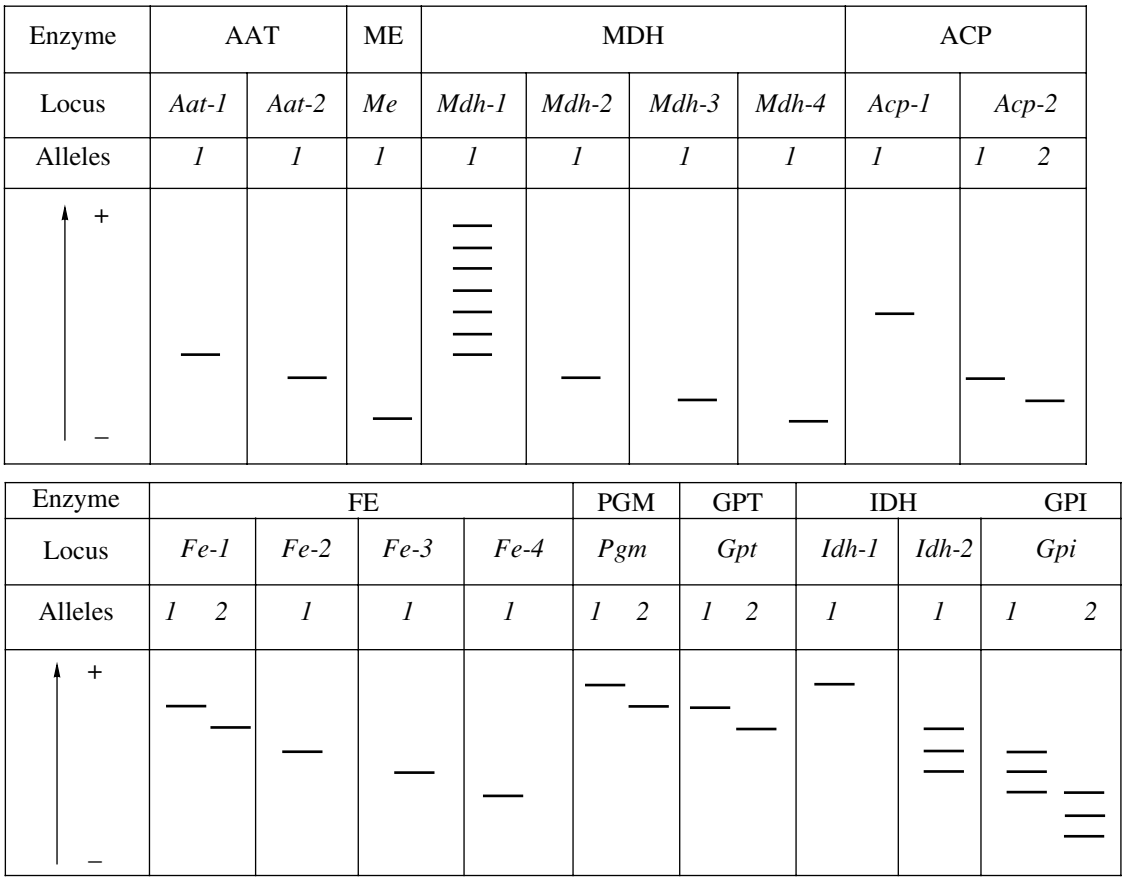

Fig. 2. Scheme of electrophoretic enzyme variants in leaves of *A. manshuriensis*.

MDH, has at least two activity zones, which correspond to the cytoplasmic and the plastid fraction [20, 24]; the products of nuclear genes of one enzyme often combine in the cytosole, forming intermediate hybrid zones [20]. The multiple forms of the *A. manshuriensis* enzymes examined may also result from duplications, but elucidation of these issues was beyond the scope of our study.

The allele frequencies in the populations studied are presented in Table 2. The *A. manshuriensis* populations examined did not differ in the allele composition, but differed in their frequencies. Among the polymorphic loci detected, only one, *Acp-2*, was highly variable in all populations. One more locus, *Pgm*, showed high variation only in one population, Malaya Borisovka. Interestingly, different alleles of this locus prevailed in thee other two populations, whereas at all of the remaining loci, the same alleles are common in all of the three populations (Table 2). Only one allele out of all found in *A. manshuriensis, Gpi*^{1}, was rare for population Malaya Borisovka, i.e., occurred in it at a frequency less than 0.05. No rare alleles were found for the species as a whole.

Heterogeneity of allele frequencies was found at loci *Gpi, Pgm*, and *Gpt* (Table 2). The among-population differences in allele frequencies of loci *Fe-1* and *Acp-2* were nonsignificant, but the general heterogeneity test for all marker genes detected significant differences among the populations ($P < 0.01$, Table 2).

In general, polymorphism in the *A. manshuriensis* populations proved to be low. Averaged over the populations, the proportion of polymorphic loci P was 24.1%; the number of alleles per locus A was 1.24 (Table 3). The observed heterozygosity varied insignificantly in the *A. manshuriensis* populations, being on average 12%. In total for the species, this estimate virtually coincides with the expected heterozygosity value, calculated from the Hardy–Weinberg proportions (0.10, Table 3).

The population-genetic structure of *A. manshuriensis* was analyzed using Wright's *F*-statistics (Table 4). The coefficient of inbreeding of the individual relative to the population F_{IS} estimated for different loci, varied from –0.9065 to 0.3546; the average value for all loci was –0.0385, showing a slight excess of heterozygotes at the population level. The estimates of the inbreeding coefficient of an individual relative the total species, F_{IT}, ranged from –0.9062 for locus *Acp-2* to 0.4619 for locus *Gpi*, while the average value for all loci showed an approximately 1.6% heterozygote deficiency in the total species (0.0156, Table 4). The subdivision index

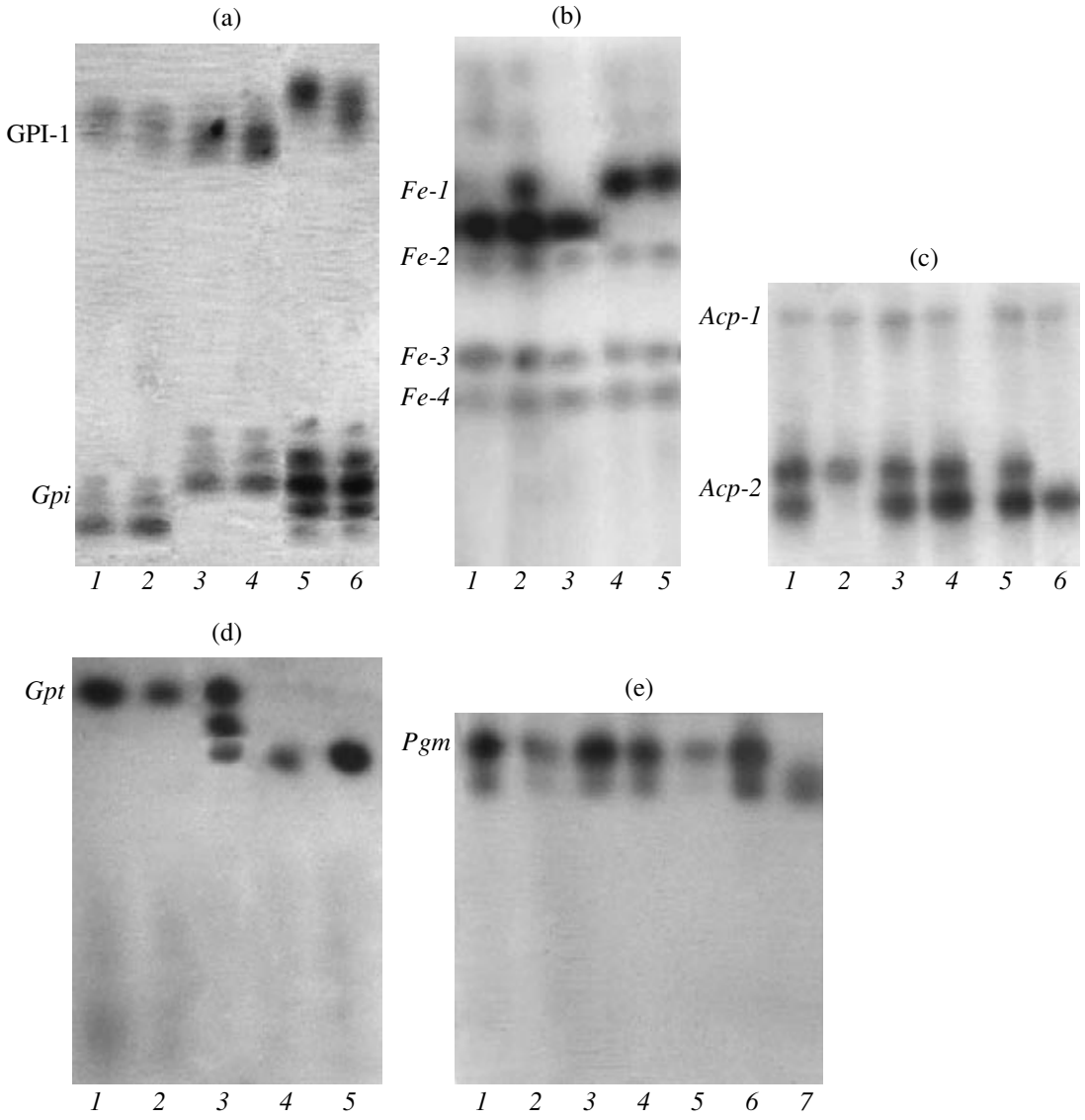

Fig. 3. Zymograms of polymorphic enzymes from leaves of *A. manshuriensis*. (a) *Gpi*, plant genotypes: *1, 2*—homozygotes $Gpi^{2/2}$; *3, 4*—homozygotes $Gpi^{1/1}$; *5, 6*—heterozygotes $Gpi^{1/2}$; (b) *Fe-1*, plant genotypes: *1, 3*—homozygotes $Fe\text{-}1^{2/2}$; *4, 5*—homozygotes $Fe\text{-}1^{1/1}$; *2*—heterozygote $Fe\text{-}1^{1/2}$; (c) *Acp-2*, plant genotypes: *2*—homozygote $Acp\text{-}2^{1/1}$; *6*—homozygote $Acp\text{-}2^{2/2}$; *1, 3, 4, 5*—heterozygotes $Acp\text{-}2^{1/2}$; (d) *Gpt*, plant genotypes: *1, 2*—homozygotes $Gpt^{1/1}$; *4, 5*—homozygotes $Gpt^{2/2}$; *3*—heterozygote $Gpt^{1/2}$; (e) *Pgm*, plant genotypes: *5*—homozygote $Pgm^{1/1}$; *7*—homozygote $Pgm^{2/2}$; *1, 2, 3, 4, 6*—heterozygotes $Pgm^{1/2}$.

F_{ST} varied from 0.0001 to 0.1662, being 0.0714 on average for all loci. This means that only 7.1% of the total variation is accounted for by the interpopulation covariation component, whereas the most part of the variability is maintained within populations. Thus, the results of test for heterogeneity and the F_{ST} value suggest some differentiation among the *A. manshuriensis* populations.

DISCUSSION

In the populations of *A. manshuriensis*, a low level of polymorphism was found as compared to the average estimates for plants (Table 5). The proportion of polymorphic loci and the number of alleles per locus in *A. manshuriensis* at the species level were substantially lower than in such plant groups, as woody, rare, and endemic plants. At the population level, polymorphism parameters in *A. manshuriensis* were only a little lower than the corresponding average values in populations of rare and endemic plants. The expected heterozygosity in this species at the population and species level (0.10 and 0.11, respectively) was slightly lower than the values characteristic of plants in general (0.11 and 0.15) and for woody plants in particular (0.148 and 0.177). However, it coincides with the averages for endemics at

Table 2. Allele frequencies at polymorphic loci in samples from natural *A. manshuriensis* populations and test for heterogeneity of the samples

Locus	Allele	Population			Mean weighted frequency for three populations
		Nezh.	An.	Mal. Bor.	
	N	57	27	107	191
Gpi	*1*	0.6052	0.7407	0.9532	0.8193
	2	0.3948	0.2593	0.0468	0.1807
Test for heterogeneity: $\chi^2 = 63.52$; *d.f.* = 2; $P < 0.01$					
Pgm	*1*	0.2807	0.7037	0.5000	0.4635
	2	0.7193	0.2963	0.5000	0.5367
Test for heterogeneity: $\chi^2 = 28.99498$; *d.f.* = 2; $P < 0.01$					
Fe-1	*1*	0.2719	0.2963	0.3878	0.3403
	2	0.7281	0.7037	0.6122	0.6597
Test for heterogeneity: $\chi^2 = 5.6429$; *d.f.* = 2; $P > 0.05$					
Gpt	*1*	0.6404	0.8519	0.9345	0.8351
	2	0.3596	0.1481	0.0655	0.1649
Test for heterogeneity: $\chi^2 = 46.6430$; *d.f.* = 2; $P < 0.01$					
Acp-2	*1*	0.5175	0.5000	0.5046	0.5078
	2	0.4825	0.5000	0.4954	0.4922
Test for heterogeneity: $\chi^2 = 0.0640$; *d.f.* = 2; $P > 0.05$					
Test for heterogeneity: $\chi^2 = 144.8648$; *d.f.* = 10; $P < 0.01$					

Note: *N* is the number of plants examined.

Table 3. Genetic diversity parameters in natural *A. manshuriensis* populations

Population	*N*	H_o	H_e	*A*	P_{95}	P_{99}
Nezhinka River basin	57	0.13	0.12	1.28	27.78	27.78
Anan'evka River basin	27	0.11	0.10	1.22	22.22	22.22
Malaya Borisovka River basin	107	0.11	0.09	1.23	22.22	23.33
Total for the species	191	0.12	0.10	1.24	24.07	24.44

Note: *N*, the number of plants examined; P_{95}, P_{99}, %, polymorphism at the 95 and 99% criterion; H_o, observed heterozygosity; H_e, expected heterozygosity; *A*, the number of alleles per locus.

the species level (0.110) and is even a little higher than the corresponding value at the population level (0.076). The observed heterozygosity in the *A. manshuriensis* populations is higher than the average estimate for rare plant populations (Table 5).

Note that Table 5 lists average polymorphism indices of rare and endemic plants, whereas some members of these groups may exhibit extremely low or very high values of these parameters. For instance, very low allozyme variation was reported for such narrow endemic species as *Clarkia franciskana* (*P* = 7.7% [25]), *Oenothera organensis* (*P* = 6.7% [26]), *Lisianthius habuensis* (*P* = 8.3% [27]), *Pinus torreyana* (*P* = 3.4%, *He* = 0 [28]), *Panax ginseng* (*P* = 7.6%, *He* = 2.2% [29]). However, some species with extremely restricted ranges are characterized by very high poly-

Table 4. Wright's *F*-statistics in the *A. manshuriensis* populations

Locus	F_{IS}	F_{IT}	F_{ST}
Gpi	0.3546	0.4619	0.1662
Pgm	–0.1335	–0.0584	0.0662
Fe-1	0.1962	0.2067	0.0130
Gpt	0.2961	0.3744	0.1112
Acp-2	–0.9065	–0.9062	0.0001
Over all loci	–0.0385	0.0156	0.0713

Note: F_{IS}, coefficient of inbreeding of an individual relative to the population; F_{IT}, coefficient of inbreeding of an individual relative to the total species; F_{ST}, population subdivision index.

Table 5. Polymorphism parameters in *A. manshuriensis* compared to corresponding mean parameters for different plant groups

Species	P_p	P_s	A_p	A_s	H_o	H_T	H_e	H_{es}	G_{ST} (F_{ST})	Reference
	%									
Aristolochia manshuriensis	24.1	27.78	1.24	1.28	0.12	0.12	0.10	0.11	0.0713	Our data
Plants	35	51	1.52	1.97			0.113	0.150		[41]
Woody	49.3	65.0	1.76	2.22			0.148	0.177	0.088	[15, 41]
Rare	29.9	36.7	1.53	1.94	0.095	0.219				[40]
Endemic	29.2	43.8	1.43	1.88				0.076	0.110	[15]
Cross-pollinating species:										[41]
by wind	49.7	66.1	1.79	2.40	0.259	0.293	0.148	0.162	0.099	
by insects	35.9	50.1	1.54	1.99	0.243	0.310	0.124	0.167	0.197	

Note: P_p, the proportion of polymorphic loci at the population level; P_s, the proportion of polymorphic loci at the species level; A_p, the number of alleles per locus at the population level; A_s, the number of alleles per locus at the species level; H_o, the observed heterozygosity at the population level; H_T, the observed heterozygosity at the species level; H_e, the expected heterozygosity at the population level; H_{es}, the expected heterozygosity at the species level.

morphism, for instance *Layia discoidea* (P = 90.5% [30]), *Limnanthes vinculans* (P = 41.2% [31]), *Allium aaseae* (P = 56.9% [32]), *Eucalyptus argutifolia* (P = 45.9% [33]) and *Oxytropis chankaensis* (P = 48.6% [34]).

Earlier, it was noted that rarity of a species is explained by different reasons in each individual case [19], which may reflect on variation of this species [15, 35]. It seems that rarity of *A. manshuriensis* is primarily explained by the history of formation of its populations located at the northern border of its range. Many relict species, especially at range margins, survive at the limit of their adaptive potential, being subject to section, on the one hand, and gene drift because of low population number, on the other. For example, Soltis et al. [36] have shown that populations of the relict plant *Bensoniella oregona* virtually completely lacked allozyme polymorphism, which the authors explain by their existence on a geologically ancient territory in the form of small populations, the bottleneck effect, and inbreeding associated with the mating system of this species. Conversely, strikingly high allozyme variability in another paleoendemic, *Dedeckera eurekensis* (P = 88.9, *He* = 33.2), found by Wiens et al. [37], they explain by selection favoring multilocus heterozygotes, and its rarity and poor reproductive success, by cross pollination and genetic load.

It is noteworthy that the proportion of polymorphic loci in *A. manshuriensis* at the species level is almost twice lower than on average in plants, whereas heterozygosity is nearly equal to the average value for plants (Table 5). The relatively high level of heterozygosity in *A. manshuriensis* populations is probably maintained through the mating system of the species, which is narrowly specialized to cross-pollination by flies of the genus *Pedoplata* [38]. It is thought that high heterozygosity is essential for plants to adapt to changing environments [4]. It is possible that in *A. manshuriensis*, as well as in the aforementioned *Dedeckera eurekensis* [37], outbreeding resulted in powerful vegetative growth and abundant flowering of this relict liana, which, however, has a low percentage of fruit formation [38].

Interestingly, the lowest heterozygosity in *A. manshuriensis* was found in the largest and most thriving population of the Malaya Borisovka River (Table 3). This may be explained by the northernmost position of this population that has a difficult access (Fig. 1). The highest polymorphism level was recorded in the Nezhinka River population, which, similarly to the Anan'evka River population, is represented only by plants in the juvenile stage and growth of cut down or burnt lianas (no fruit-bearing plants were found in these two populations).

The differences in polymorphism parameters between the populations of the Nezhinka and Anan'evka rivers, which are similar in structure and the level of anthropogenic impact, can hardly be explained by different directions of selection. It is also highly unlikely that these differences are caused by inbreeding, because *A. manshuriensis* is highly adapted to cross-pollination: selfing is virtually impossible in this species, owing to a specific flower structure (gynostemium) and temporal discordance between the male and female flowering phases [38].

Analysis using Wright's *F*-statistics also showed the absence of inbreeding in the *A. manshuriensis* populations. The F_{IS} index revealed 3.8% excess of heterozygotes in the *A. manshuriensis* populations, while 1.6% deficiency of heterozygotes was found at the species level (F_{IT} = 0.0156, Table 4). The small deficiency of heterozygotes in *A. manshuriensis* may be related to sibbing and geitonogamy, effected by insects, which cannot be excluded in this species, because no studies has been undertaken to elucidate this issue.

It is most likely that the differences in polymorphism parameters in the *A. manshuriensis* populations

are explained by stochastic processes, such as gene drift and bottleneck effect, which always reduce genetic diversity in small and fragmented populations with decreasing size [3, 4, 14, 16, 39]. This is indirectly shown by a low number of alleles per locus ($A = 1.24$, Table 3), because gene drift primarily reduces allele diversity, and, consequently, the proportion of polymorphic loci, whereas inbreeding results in a reduction in heterozygosity [16].

The test for heterogeneity showed differentiation among the *A. manshuriensis* populations from Primorye (Table 4). The F_{ST} value proved to be low (0.0713, Table 4), but comparable with the mean F_{ST} in woody plants (Table 5). This indicates low differentiation among the *A. manshuriensis* populations and the existence of gene flow among them currently or in the recent past. Gene flow and, consequently, low F_{ST} are characteristic for cross-pollinating species; species pollinated by insects usually exhibit higher F_{ST} values than wind-pollinating species (Table 5). In the *A. manshuriensis* populations, F_{ST} is rather low and comparable with that in wind-pollinating plants (Table 5). This may be explained by the fact that gene flow among the *A. manshuriensis* populations may involve not only pollen transfer, but also seed dispersal, because in this species seeds are adapted to be born by water flows. Another possible explanation of low differentiation observed in the *A. manshuriensis* populations is the recent integrity of its range and closer connection among the populations in the recent past, because the F_{ST} value in long-lived species often reflects the gene exchange level, which was characteristic for them at the time of their formation [16].

Thus, the relict *A. manshuriensis* populations from southern Primorye are characterized by low genetic diversity. Because of small size and fragmentation of the *A. manshuriensis* populations, as well as their isolation from one another, an essential contribution to the reduction in their variation is likely made by gene drift, including that caused by anthropogenic impact. The results of this study are of significant importance for developing strategies of conservation of this valuable relict species in Primorye, while the allele variants detected can be used as markers in monitoring and species conservation measures. Evaluation of the contribution of individual genetic processes in the formation of the *A. manshuriensis* population requires further research.

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REFERENCES

1. Krestov, P.V. and Verkholat, V.P., *Redkie rastitel'nye soobshchestva Primor'ya i Priamur'ya* (Rare Plant Communities of Primorye and Priamur'e), Vladivostok: DVO RAN, 2003.
2. Saunders, D.A., Hobbs, R.J., and Margules, C.R., Biological Consequences of Ecosystem Fragmentation: A Review, *Conserv. Biol.*, 1991, vol. 5, pp. 18–32.
3. Levin, D.A., Consequences of Stochastic Elements in Plant Migration, *Am. Nat.*, 1988, vol. 132, pp. 643–651.
4. Ellstrand, N.C. and Elam, D.R., Population Genetic Consequences of Small Population Size: Implications for Plant Conservation, *Annu. Rev. Ecol. Syst.,* 1993, vol. 24, pp. 217–242.
5. Kurentsova, G.E. and Valova, Z.G., *Aristolochia manshuriensis* Kom. and *Pinus funebris* Kom. as Constituents of Plant Cover in Southwestern Primorye, *Komarovskie Chteniya*, 1969, issue 17, pp. 51–61.
6. Kitagawa, M., *Neo-Lineamenta Florae Manshuricae. Hirchberg,* Hichberg: J. Cramer, 1979.
7. Kurentsova, G.E., *Reliktovye rasteniya Primor'ya* (Relic Plants of Primorye), Leningrad: Nauka, 1968.
8. Popov, M.G., *Filogeniya, florogenetika, florogeografiya, sistematika* (Phylogeny, Florogenetics, Florogeography, and Systematics), Kiev: Naukova Dumka, 1968, part 1.
9. Slizik, L.N., Rare and Valuable Species of Arborescent Lianas of Primorskii Kray, Resources of Their Conservation and Reproduction, *Aktual'nye voprosy okhrany prirody na Dal'nem Vostoke* (Current Problems of Conservation in the Far East), Konovalenko, V.G. and Smirnov, O.A., Eds., Vladivostok: DVNTs Akad. Nauk SSSR, 1978, pp. 47–55.
10. Slizik, L.N., Specific Features of Seasonal Growth Rhythm in Some Relic Arborescent Lianas of the Primorye Flora, *Redkie i ischezayushchie drevesnye rasteniya yuga Dal'nego Vostoka (biologiya, ekologiya, kariologiya)* (Rare and Disappearing Arborescent Plants in the South of Far East Region (Biology, Ecology, Karyology)), Smirnova, O.A., Ed., Vladivostok: DVNTs Akad. Nauk SSSR, 1978, pp. 105–112.
11. *Krasnaya kniga RSFSR. Rasteniya* (Red Book of Russian Federation: Plants), Moscow: Rosagropromizdat, 1988.
12. Bulgakov, V.P. and Zhuravlev, Yu.N., Production of *Aristolochia manshuriensis* Kom. Callus Culture Tissues, *Rastitel'nye Resursy*, 1989, vol. 25, no. 2, pp. 266–270.
13. *Rastitel'nye resursy Rossii i sopredel'nykh gosudarstv. Sem. Aristolochiaceae* (Plant Resources of Russia and Adjacent Countries), Budantsev, L.N., Ed., St. Petersburg, 1996, pp. 103–104.
14. Altukhov, Yu.P., *Geneticheskie protsessy v populyatsiyakh* (Genetic Processes in Populations), Moscow: Akademkniga, 2003.
15. Hamrick, J.L and Godt, M.J.W., Allozyme Diversity in Plant Species, *Plant Population Genetics, Breeding, and Genetic Resources*, Brown, A.H.D.., Clegg, M.T., Kahler, A.L., and Weir, B.S., Eds., Sunderland: Sinauer, 1989, pp. 43–63.

16. Oostermeijer, J.G.B., Is Genetic Variation Important for the Viability of Wild Plant Population?, *Schriftenr. Vegetationskunde. Bundesamt fur Naturschutz, Bonn*, 2000, no. 32, pp. 23–30.
17. Oostermeijer, J.G.B., Luijten, S.H., and Nijs, J.C.M., Integrating Demographic and Genetic Approaches in Plant Conservation, *Biol. Conserv.*, 2003, vol. 113, pp. 389–398.
18. Soule, M.E., Thresholds for Survival: Maintaining Fitness and Evolutionary Potential, *Conservation Biology: an Evolutionary–Ecological Perspective*, Soule, M.E. and Wilcox, B.A., Eds., Sunderland: Sinaeur, 1980, pp. 151–169.
19. Zhuravlev, Yu.N., Koren', O.G., Muzarok, T.I., et al., Molecular Markers for Rare Plant Species Conservation in the Far East Region, *Russ. J. Plant Physiol.*, 1999, vol. 46, no. 6, pp. 838–848.
20. Levites, E.V., *Genetika izofermentov rastenii* (Genetics of Plant Isozymes), Novosibirsk: Nauka, 1986.
21. Prakash, S., Lewontin, R.C., and Hubby, T.L., A Molecular Approaches to the Study of Genic Heterozygosity in Natural Populations: IV. Patterns of Genic Variation in Central, Marginal and Isolated Populations of *Drosophila pseudoobscura*, *Genetics,* 1969, vol. 61, pp. 841–858.
22. Zhivotovskii, L.A., Statistic Methods Analysis Gene Frequencies in Natural Populations, *Itogi Nauki Tekh., Ser. Obshch. Genet.*, 1983, vol. 8, pp. 76–104.
23. Wright, S., The Genetical Structure of Populations, *Ann. Eugen.,* 1951, vol. 15, pp. 323–354.
24. Gottlieb, L.D., Conservation and Duplication of Isozymes in Plants, *Science*, 1982, vol. 216, pp. 373–380.
25. Gottlieb, L.D., Enzyme Differentiation and Phylogeny in *Clarkia franciscana, Clarkia rubicunda*, and *Clarkia amoena*, *Evolution*, 1973, vol. 27, pp. 205–214.
26. Levin, D.A., Ritter, K., and Ellstrand, N.C., Protein Polymorphism in the Narrow Endemic *Oenothera organensis*, *Evolution*, 1979, vol. 33, pp. 534–542.
27. Systma, K.J. and Schaal, B.A., Genetic Variation, Differentiation, and Evolution in a Species Complex of Tropical Shrubs Based on Isozymic Data, *Evolution,* 1985, vol. 39, pp. 582–593.
28. Ledig, F.T. and Conkle, M.T., Gene Diversity and Genetic Structure in a Narrow Endemic, Torrey Pine (*Pinus torreyana* Parry ex. Carr), *Evolution*, 1983, vol. 37, pp. 79–85.
29. Koren, O.G., Potenko, V.V., and Zhuravlev, Yu.N., Inheritance and Variation of Allozymes in *Panax ginseng* C.A. Meyer (Araliaceae), *Int. J. Plant Sci.*, 2003, vol. 164, no. 1, pp. 189–195.
30. Gottlieb, L.D., Warwick, S.I., and Ford, V.S., Morphological and Electrophoretic Divergence Between *Layia Discoidea* and *L. grandulosa*, *Syst. Bot.*, 1985, vol. 10, pp. 484–495.
31. Kesseli, R.V. and Jain, S.K., New Variation and Biosystematic Patterns Detected by Allozyme and Morphological Comparison in *Limnanthes* sect. Reflexae (Limnanthaceae), *Plant Syst. Evol.*, 1984, vol. 147, pp. 133–165.
32. Smith, J.F. and Pham, T.V., Genetic Diversity of the Narrow Endemic *Allium aaseae* (Alliaceae), *Am. J. Bot.,* 1996, vol. 83, pp. 717–726.
33. Kennington, W.J. and James, S.H., Allozyme and Morphometric Variation in Two Closely Relation Mallee Species from Western Australia, *Eucalyptus argutifolia* and *E. obtusiflora* (Myrtaceae), *Austral. J. Bot.*, 1998, vol. 46, pp. 173–186.
34. Kholina, A.B., Koren, O.G., and Zhuravlev, Yu.N., High Polymorphism and Autotetraploid Origin of the Rare Endemic Species *Oxytropis chankaensis* Jurtz. (Fabaseae) Inferred from Allozyme Data, *Russ. J. Genet.,* 2004, vol. 40, no. 4, pp. 393–400.
35. Nevo, E., Beiles, A., and Ben-Shlomo, P., The Evolutionary Significance of Genetic Diversity: Ecological, Demographic and Life History Correlates, *Lect. Notes Biomath.*, 1984, vol. 53, pp. 13–213.
36. Soltis, P.S., Soltis, D.E., and Tucker, T.L., Allozyme Variability Is Absent in the Narrow Endemic *Bensoniella oregona* (Saxifragaceae), *Conservation Biol.*, 1992, vol. 6, no. 1, pp. 131–134.
37. Wiens, D., Nickrent, D.L., Davern, C.I., et al., Developmental Failure and Loss of Reproductive Capacity in the Rare Palaeoendemic Shrub *Dedeckera eurekensis*, *Nature*, 1989, vol. 338, pp. 65–67.
38. Nakonechnaya, O.V., Koren', O.G., Nesterova, S.V., et al., Reproductive Biology of *Aristolochia manshuriensis* Kom. (Aristolochiaseae) in the Conditions of Introduction, *Rastitel'nye Resursy*, 2005, vol. 41, no. 3, pp. 14–25.
39. Barrett, S.C.H. and Kohn, J.R., Genetic and Evolutionary Consequences of Small Population Size Plants: Implication for Conservation, *Genetics and Conservation of Rare Plants,* Falk, D.A. and Holsinger, K.E., Eds., New York: Oxford Univ. Press, 1991, pp. 3–30.
40. Gitzendanner, M.A. and Soltis, P.S., Patterns of Genetic Variation in Rare and Widespread Plant Cogeners, *Am. J. Bot.*, 2000, vol. 87, no. 6, pp. 783–792.
41. Hamrick, J.L., Godt, M.J., and Sherman-Broyles, S.L., Factors Influencing Levels of Genetic Diversity in Woody Plant Species, *New Forests*, 1992, vol. 6, pp. 95–124.