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PLANT GENETICS

Genetic Diversity of a Rare Species *Aristolochia contorta* Bunge (Aristolochiaceae) in Primorsky Krai

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Abstract—The herbaceous vine, twisted birthwort *Aristolochia contorta* Bunge, is a rare species listed in the Red Data Book of Primorsky krai (2008). On the northern boundary of its range (south of the Russian Far East), the species is represented by small isolated populations confined to the river drainages. Using allozyme analysis, genetic variation of nine natural populations of *A. contorta* (247 accessions), which represented the main part of the species range in Russia, was examined. The values of genetic variation indices ($P = 22.7\%$; $A = 1.28$; $H_o = 0.129$; $H_e = 0.101$) were low and comparable with the data obtained for other rare plants. The proportion of unique genotypes (G/N) and Simpson's genotypic diversity index (D) ranged from 0.32 to 0.64 and from 0.60 to 0.98, respectively. This means that *A. contorta* is characterized by sexual and asexual reproduction. Moreover, the ratio between these types of reproduction varied among the populations. Complete absence of inbreeding and excess of heterozygotes ($F_{IS} = -0.282$), which was low probable in case of free mating, was observed. Evidently, clonal growth and (or) apomixis enables the species to maintain certain level of heterozygosity despite of small population sizes and non-regular gene exchange.

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INTRODUCTION

Twisted birthwort *Aristolochia contorta* Bunge ($2n = 14$, [1]) is a herbaceous vine, which on the territory of Russia grows in the south of Primorsky krai [2] and southwest of Khabarovsk krai (Jewish Autonomous Oblast) [3]. This is where the northern boundary of the species distribution range lies. The range also encompasses several provinces of China [4], Korea [5], and Japan [6]. However, the data on the species distribution across these regions are scarce. It can be suggested that in this part of the range, *A. contorta* is also rarely found due to commercial land development and sensitivity of this species to the landscape changes. In the Russian Far East, small natural populations of *A. contorta* are strongly fragmented, suppressed, and their number is decreasing due to intensifying anthropogenic load [7]. The rhizomes and fruits of *A. contorta* are used in Tibet medicine. In particular, fruits are used for treatment of tumors [8]. The main hazard for the species existence is the destruction of habitats due to economic activity, annual forest fires, and uncontrolled plant harvesting. The species is recorded in the "Red Book of the Primorsky krai" [9] as vulnerable species, and in the "Red Book of Jewish Autonomous Oblast" [3] as threatened species. However, during monitoring of the Primorye flora state, A. E. Kozhevnikov et al. [10] established that *A. contorta*, along with ginseng *Panax ginseng* and Manchurian birthwort *Aristolochia manshuriensis* should be included into the group of species with the high risk of extinction, the "threatened species". The species is badly in need of conservation and restoration of natural populations as the potential source of medicinal preparations, and as the unique components of biocoenosis, since the plant of interest is the key member of the ecosystem, being the only source of feed for relict butterfly *Sericinus montela* Gray [11]. No data on genetic variation of *A. contorta* are currently available. The absence of such information makes it impossible to evaluate the level of variation and its distribution pattern across the Russian part of the range, while it is necessary for the conservation of the species genetic resources.

Peripheral position of the *A. contorta* populations can affect the gene pool state, since it is known that edge populations are characterized by intensification of genetic processes, enhancement of the selection influence, and not seldom changes of the reproductive strategy towards the dominance of either sexual, or asexual reproduction [12–14]. In our earlier studies, the first data on the genetic diversity and population structure of a number of rare plant species from the south of Primorye were obtained [15–19]. Relict species, especially the elements of more thermophilic subtropical flora (*Aristolochia manshuriensis* and the members of the family Araliaceae), which in Primorye are growing at the northern boundaries of their ranges, have adapted to survive in extreme conditions. However, these species are characterized by low competitive ability, and in case of the environmental changes they become the most vulnerable. Decrease of the

Table 1. Populations examined, sample sizes, and habitat characteristics

River drainage	Sampling site	Symbol	N_{pop} (population size)	N_s (sample size)	Habitat
Sukhodol	settlement of Romanovka	RM	35	14	Brush–grass thicket near railway
Petrovka	township of Bol'shoy Kamen'	BK	60	19	Bottomland alder–willow forest near highway
Razdol'naya	settlement of Terekhovka, Ussuriisk raion	UR	150	36	Bottomland alder–willow and bird cherry–willow forest
	settlement Fadeevka, settlement Novo-Georgievka, Oktyabr'sky raion	OR	350	59	The same
Borisovka	settlement Borisovka	BR	110	26	"
Artemovka	settlement Shtykovo	ShT	100	27	Brush–grass thicket near railway
Shkotovka	settlement Shkotovo	ShK	50	25	The same
	Middle course of Shkotovka River, 20 km eastward from the township of Shkotovo	ST	150	22	Bottomland forest, the region of waste disposa
Ptich'e Lake	Ten km to the northeast from the township of Khasan	PT	100	19	Oak woodland at the foot of the slope, forbs

population number, taking place as a result of anthropogenic influence, leads to the loss of diversity upon the influence of the gene drift [15–19]. The present study is the continuation of these investigations.

Using allozyme analysis, in the present study genetic diversity of the *A. contorta* populations from the northern boundary of the range was examined. The tasks of the study included analysis of the genetic control of electrophoretic diversity of the enzymes of *A. contorta* and evaluation of the level of the population variation at the structural genes identified.

MATERIALS AND METHODS

A. contorta is rhizomatous perennial plant. It is in flower from July to August. The flowers are hermaphrodite and protogynous. The high degree of pollen defectiveness was obsereved (24.5%) [20]. The seeds germinate in September; wing-shaped formations of the seeds provide their dispersal by wind. At the same time, a part of seeds fall into water, and they are taken away by the current, especially during the period of heavy autumn rains [21]. Taking into considerations that in vines the cases of vegetative reproduction with the help of rhizomes were described [22, 23], it was suggested that this type of reproduction could be also typical of *A. contorta*. This suggestion was confirmed upon digging out of the parts of plant root system (Nakonechnaya, personal observation).

Isoenzymes were examined using frozen in liquid nitrogen leaves of 247 accessions of *A. contorta* from nine natural populations (Table 1 and Fig. 1). The plants in the populations usually grow in groups, which can be located about 100 m one from another. The leaves for the analysis were collected from the plants located at a distance of at least 10 m one from another. The leaves were homogenized in 200 μl of extracting solution (1% PVP-40; 1% sucrose; 1% β-mercaptoethanol in distilled water). Electrophoresis was run in horizontal 13% starch gel with addition of 10% sucrose in three buffer systems, including Tris–citrate (pH 6.2), Tris–cirate (pH 7.8), and Tris–EDTA–borate (pH 8.6) systems [24]. Composition of buffer systems and fractionation regime was described earlier [16]. Histochemical staining of enzymatic activity was carried out according to a standard method [24]. Alleles were designated according to their electrophoretic mobility relative to the most common variant, the mobility of which was recognized as 1.00. The indices of polymorphism (P), mean allele number per locus (A), average observed (H_o), and expected (H_e) heterozygosity were calculated using standard techniques [24, 25]. Statistical treatment of the data was performed using the TFPGA software program [26]. Genotypic diversity was estimated as the G/N ratio, where G, the number of different genotypes and N, sample size [27], and with the help of the modified Simpson's index (D), used for clonal plants [27, 28]: $D = 1 - [\Sigma n_i(n_i - 1)]/[N(N - 1)]$, where n_i, the number of plants carrying i phenotype analyzed and N, the total number of plants examined. This value shows the probability that two randomly rejected ramets in the population of N plants would have different multilocus genotypes.

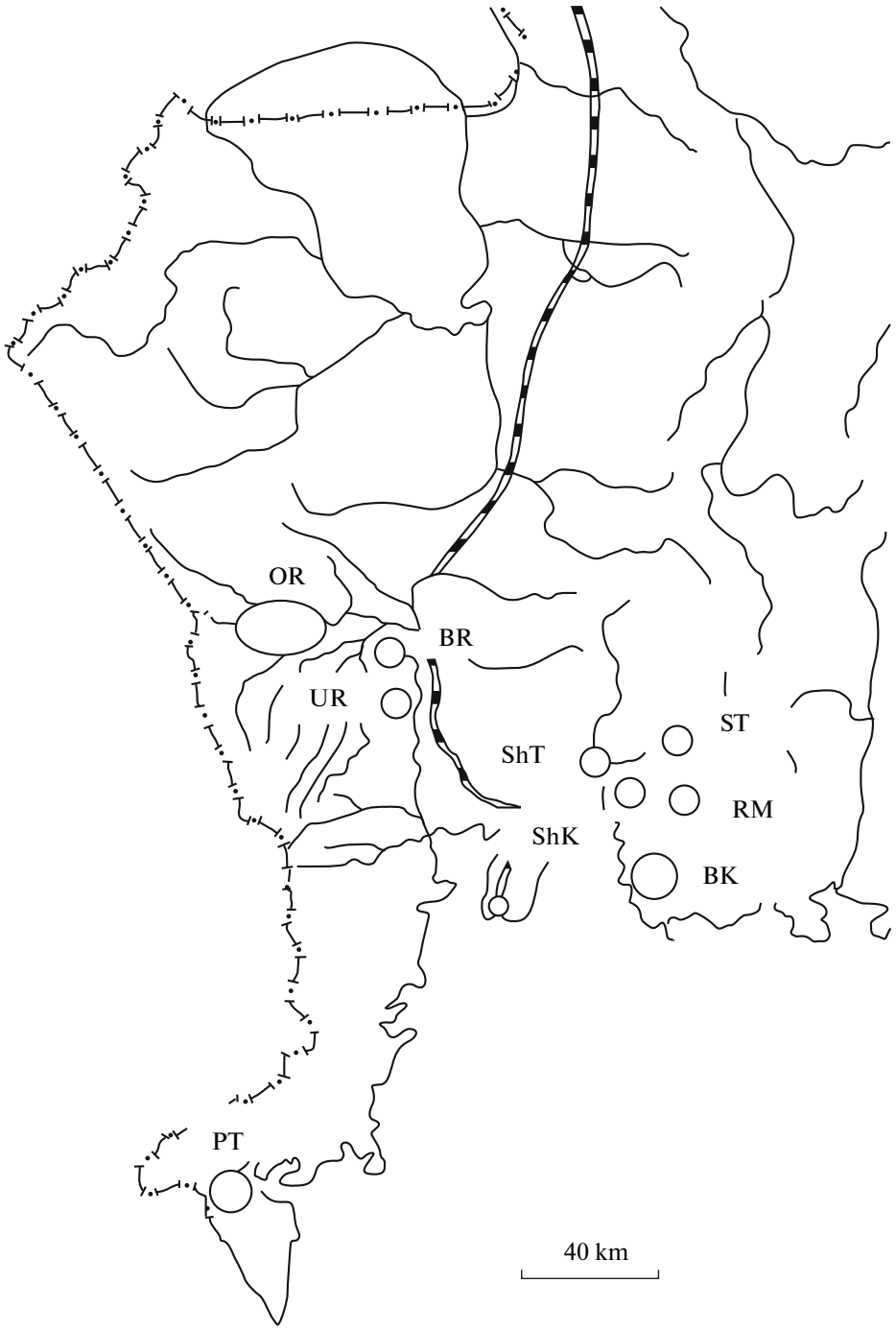

Fig. 1. Sampling sites of the *Aristolochia contorta* plants (symbol decoding is presented in Table 1).

RESULTS AND DISCUSSION

During the investigation, a total of 15 enzymatic systems were examined, with 13 of these stably identified. A total of 30 allelic variants, presumably encoded by 22 loci were identified (Table 2, Figs. 2 and 3). Next, descriptions of the phenotypes and genetic interpretation for each of the enzymatic system are presented.

ADH is represented on electrophoregrams by one activity zone, under control of one monomorphic *Adh* gene. AAT was represented by two zones, among which the faster-migrating zone was presumably encoded by the *Aat-1* locus with three alleles. The AAT-2 zone was unstably identified, and was not scored. The GPI staining identified four activity zones. The fastest band was represented by the products of the *Gpi-1* locus. The three slower-migrating

Table 2. Enzymatic systems tested and the number of polymorphic loci and alleles identified in the leaves of *Aristolochia contorta*

No.	Enzyme	Symbol	EC number	Interpreted loci	Polymorphic loci	Alleles
1	Alcohol dehydrogenase	ADH	1.1.1.1	1	0	1
2	Aspartate aminotransferase	AAT	2.6.1.1.	1	1	3
3	Glutamate-pyruvate transaminase	GPT	2.6.1.2.	1	0	1
4	Glucosephosphate isomerase	GPI	5.3.1.9.	4	0	4
5	Isocitrate dehydrogenase	IDH	1.1.1.42.	2	0	2
6	Acid phosphatase	ACP	3.1.3.2.	1	0	1
7	Colorimetric esterase	EST	3.1.1.1	1	0	1
8	Leucine aminopeptidase	LAP	3.4.11.1	1	1	4
9	Malate dehydrogenase	MDH	1.1.1.37.	3	0	3
10	Malic enzyme	ME	1.1.1.40.	1	0	1
11	Fluorescent esterase	FE	3.1.1.2.	2	2	4
12	Phosphoglucomutase	PGM	2.7.5.1.	2	0	2
13	6-Phosphogluconate dehydrogenase	6-PGD	1.1.1.44	2	1	3
	Total			22	5	30

zones, represented by the products of the three loci, *Gpi-2, Gpi-3,* and *Gpi-4*, were monomorphic. Moreover, the GPI-1 and GPI-3 zones were identified as double bands, and the GPI-2 zone, as triple band. GPT was identified as two activity zones. In the analysis, faster migrating zone, under control of monomorphic *Gpt-1* locus was scored. IDH was identified as two zones. The fast IDH-1 zone was represented by one band of the enzyme, while slower-migrating IDH-2 zone, by two bands. Both of the zones were not variable, and they were scored as the products of the *Idh-1* and *Idh-2* monomorphic loci, respectively. In ACP, three zones of the activity were identified, and the middle zone, ACP-2, presumably encoded by the monomorphic *Acp-2* locus was incuded in the analysis. Three zones of activity were present in EST. Among them, the slowly-migrating zone, EST-3, appearing in the cathode region and controlled by monomorphic *Est-3* locus, was scored. In LAP, one activity zone was identified, presumably encoded by the polymorphic *Lap* locus with four alleles. MDH was identified in the form of three activity zones. Moreover, the fastest zone was represented by three bands, the second fast zone, by two bands, and the slowest zone, by one band. These zones were interpreted as the products of the *Mdh-1*, *Mdh-2*, and *Mdh-4* monomorphic genes, respectively. In *A. manshuriensis* MDH consisted of four activity zones, which were interpreted as the products of monomorphic *Mdh-1, Mdh-2, Mdh-3,* and *Mdh-4* genes [20]. Furthermore, the fastest activity zone consisted of seven bands, while each of the other zones consisted of a single band. The mobility of all but one MDH bands identified in two *Mdh-3* species was identical. The exclusion was *Mdh-3*, which was absent from *A. contorta*. ME was represented by one activity zone, under control of the *Me* gene. In FE, four activity zones were identified. Two of these zones, FE-2 and FE-3, controlled by polymorphic *Fe-2* and *Fe-3* loci with two alleles were included in the analysis. PGM was represented by two activity zones, under control of two monomorphic *Pgm-1* and *Pgm-2* loci. 6-PGD was represented by three activity zones. The fastest 6-PGD-1 zone is controlled by monomorphic *6-Pgd-1* locus, and the slow-migrating zone is controlled by polymorphic *6-Pgd-2* locus with two alleles. The polymorphism of this activity zone can be judged from the products of interlocus interactions, which are represented in the middle activity zone between the products of the *6-Pgd-1* and *6-Pgd-2* loci. Among the enzymes examined, those controlled by the *Gpi-1, Gpi-2, Gpi-3, Idh-2, Mdh-1*, and *6-Pgd-1* loci, were identified as multiple bands. It is known that multiple enzyme bands within one zone can be associated with duplication of individual genes [29]. It cannot be excluded that multiple forms of the enzymes tested in *A. contorta* resulted from the interaction between the duplicated genes. Taken together, for 13 enzymatic systems from the leaves of *A. contorta*, a total of 17 monomorphic and five polymorphic genes were identified. All polymorphic loci were found to be highly variable (observed heterozygosity was about 35%): *Aat-1*, 0.449; *Fe-2*, 0.522; *Fe-3*, 0.979; *6-Pgd-2*, 0.425; *Lap*, 0.462. Each polymorphic loci contained from two to four alleles, and the mean number of alleles per population constituted 28 (Table 3). In nine populations, 24 alleles were identified (80%), including 17 alleles of monomorphic loci. Two alleles were found only in three populations: $Aat\text{-}1^{0.75}$, in ShK, ST PT, and $Lap^{0.00}$, in UR, OR, and ShT. Maximum number of alleles (29) was detected in populations OR and ShK.

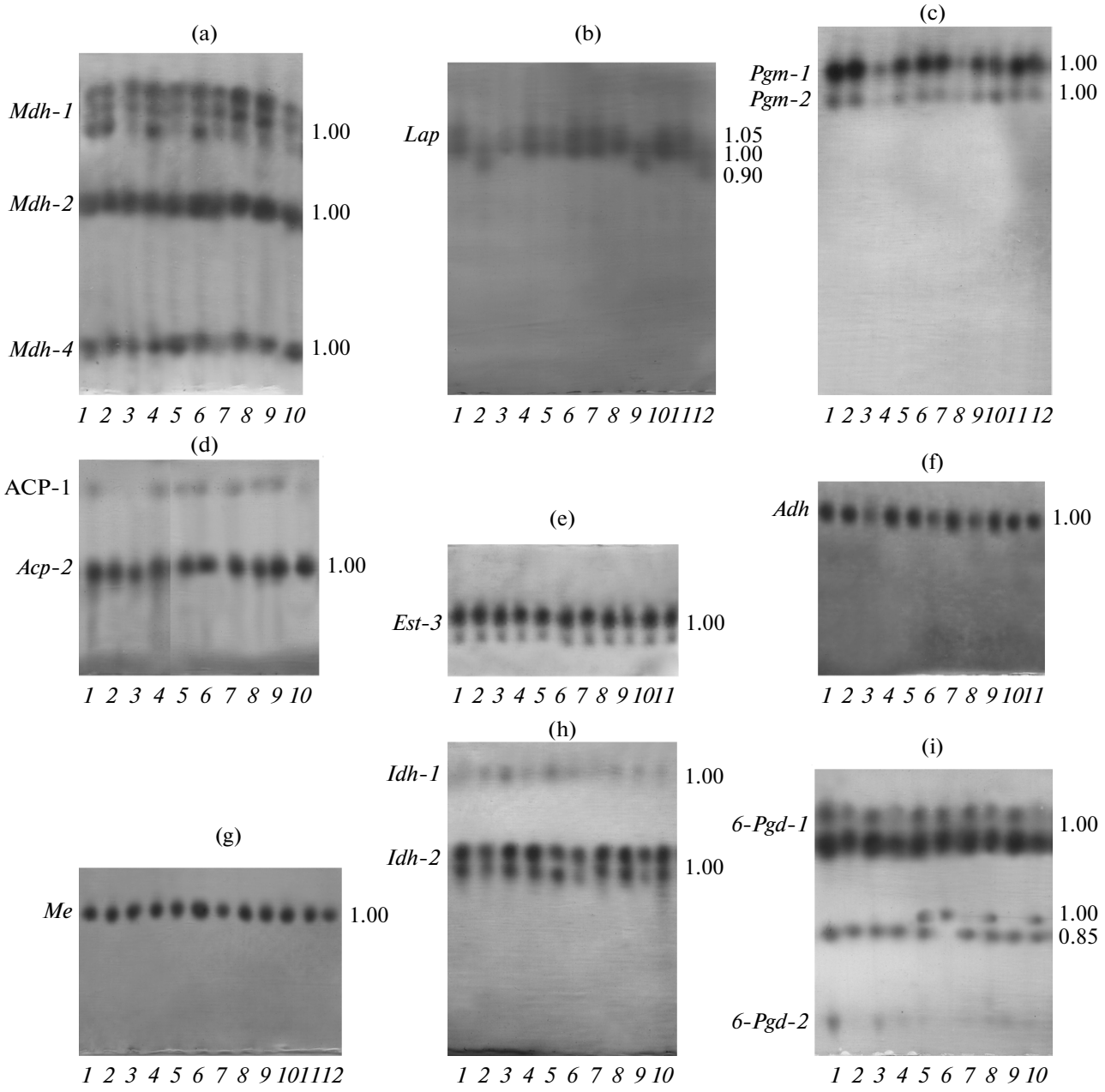

Fig. 2. Electrophoregram of nine enzymes of *Aristolochia contorta*. a, 1 to 10, *Mdh-1*$^{1.00}$/*Mdh-2*$^{1.00}$/*Mdh-4*$^{1.00}$; b, 1 and 3 to 8, 10, 11, *Lap*$^{1.00/1.05}$; 2, 9, 12, *Lap*$^{0.90/1.00}$; c, 1 to 12, *Pgm-1*$^{1.00}$/*Pgm-2*$^{1.00}$; d, 1 to 10, *Acp-2*$^{1.00}$; e, 1 to 11, *Est-3*$^{1.00}$; f, 1 to 11, *Adh*$^{1.00}$; g, 1 to 12, *Me*$^{1.00}$; h, 1 to 10, *Idh-1*$^{1.00}$/*Idh-2*$^{1.00}$; i, 1 to 4, 7, 9, *6-Pgd-1*$^{1.00}$/*6-Pgd-2*$^{0.85}$; 5, 8, 10, *6-Pgd-1*$^{1.00}$/*6-Pgd-2*$^{0.85/1.00}$; 6, *6-Pgd-1*$^{1.00}$/*6-Pgd-2*$^{1.00}$.

Based on allele frequencies at 22 loci, the main genetic variation indices were calculated (Table 4). Maximum index values were observed in population BR ($P_{99} = 22.7\%$; $A = 1.27$; $H_o = 0.177$; $H_e = 0.115$), and the minimum values, in population BK ($P_{99} = 18.2\%$; $A = 1.23$; $H_o = 0.086$; $H_e = 0.062$). The mean values over nine populations ($P_{99} = 21.2\%$; $A = 1.28$; $H_o = 0.129$; $H_e = 0.101$) were comparable with the values obtained for *A. manshuriensis* ($P = 24.1\%$, $A = 1.24$, $H_o = 0.12$, $H_e = 0.10$) [16], and close to the mean values obtained for the populations of rare species ($P = 29.9\%$; $A = 1.53$; $H_e = 0.095$) [30], the species with small ranges ($P = 30.6\%$, $A = 1.45$, $H_e = 0.105$) [31], and the species capable of sexual and asexual reproduction ($P = 29.4\%$; $A = 1.47$; $H_e = 0.103$) [31]. The diversity level, expressed as H_e, was close to that of the species characterized by mixed mating system (the presence of sexual and asexual reproduction) and dispersal of seeds by wind ($H_e = 0.118$) [32]. The low polymorphism level, described for the populations of

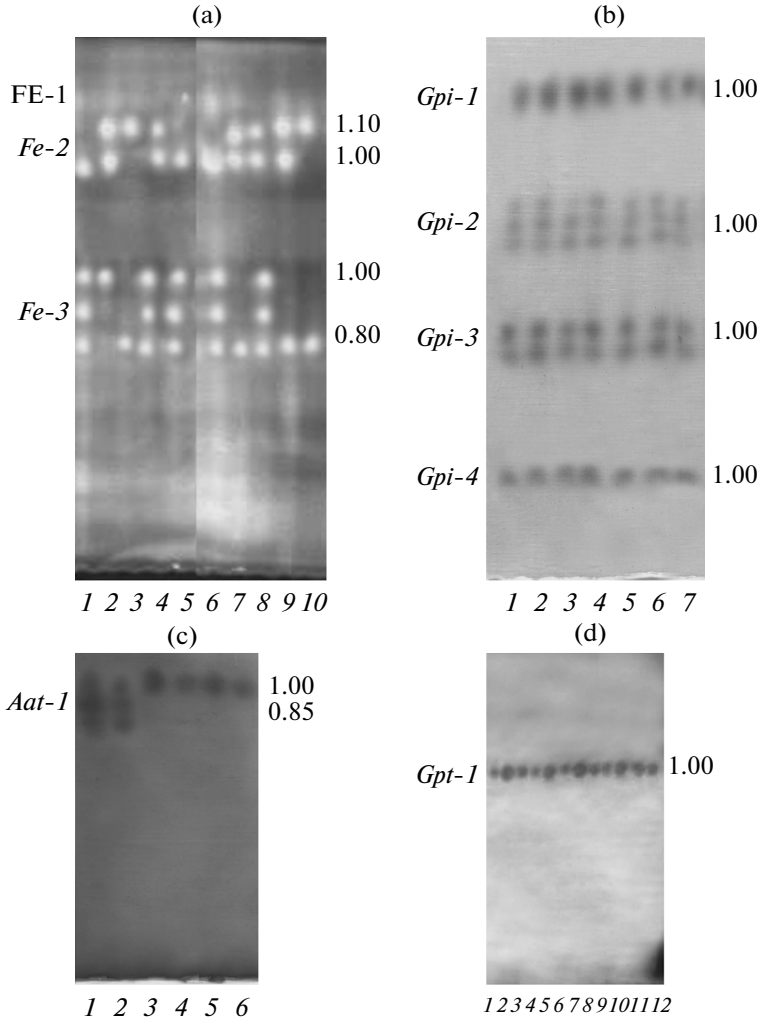

Fig. 3. Electrophoregram of four enzymes of *Aristolochia contorta*. a, 1, 5, 6, $Fe\text{-}2^{1.00}/Fe\text{-}3^{0.80/1.00}$; 2, $Fe\text{-}2^{1.00/1.10}/Fe\text{-}3^{1.00}$; 3, 10, $Fe\text{-}2^{1.10}/Fe\text{-}3^{0.80}$; 4, 8, $Fe\text{-}2^{1.00/1.10}/Fe\text{-}3^{0.80/1.00}$; 7, 9, $Fe\text{-}2^{1.00/1.10}/Fe\text{-}3^{0.80}$; b, 1 to 7, $Gpi\text{-}1^{1.00}/Gpi\text{-}2^{1.00}/Gpi\text{-}3^{1.00}/Gpi\text{-}4^{1.00}$; c, 1, 2, $Aat\text{-}1^{0.85/1.00}$; 3 to 6, $Aat\text{-}1^{1.00}$; d, 1 to12, $Gpt\text{-}1^{1.00}$.

A. contorta, is definitely determined by narrow ecological association of the species, weak competition ability, and considerable population fragmentation, which provides manifestation of the consequences of gene drift. However, it seems likely, that the level of genetic diversity observed was to a considerable degree based by the specific features of the species reproduction biology. It is known that the reproduction system is one of the most important features responsible for the level and the distribution pattern of allozyme polymorphism among the populations [31, 32]. For the populations existing in stressful conditions (including those at the periphery of the main range) the change of preferential type of reproduction was reported in a number of studies [12–14, 33, 34]. In the conditions of the disappearance of appropriate pollinators, decreasing of pollen fertility, and absence of conditions for normal seed ripening, the plants with the mixed mating system quite often turn to asexual reproduction. Interestingly, it was suggested that low genetic diversity indices obtained for the two vine species (with the same life form as in *A. contorta*), *Bryonia alba* [35] and *Ancistrocladus korupensis* [36], could be partly associated with the apomixis, typical of these species. It was currently established that apomixis is rather widely distributed among the plants [37, 38]. Taking into consideration that primary data on the reproductive biology of *A. contorta* indirectly pointed to possible existence of apomixis (high degree of pollen defectiveness along with weak seed setting [20]), it can be suggested that this process can contribute to the formation of the polymorphism level in *A. contorta*. The apomictic species are characterized by the levels of observed heterozygosity exceeding those of expected heterozygosity [36], which was described for *A. contorta*. There is no doubt that accurate conclusion on the presence of apomixis in the species of interest, as well as characterization of the certain type of apo-

Table 3. Allele frequencies at the polymorphic loci of *Aristolochia contorta* and the test for heterogeneity

Locus	Allele	Population								
		RM	BK	UR	OR	BR	ShT	ShK	ST	PT
Aat-1	0.75	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.614	0.053
	0.85	0.786	0.868	0.306	0.458	0.462	0.370	0.540	0.000	0.158
	1.00	0.214	0.132	0.694	0.542	0.538	0.630	0.380	0.386	0.789
Test for heterogeneity: $\chi^2 = 63.08$; $d.f. = 8$; $p < 0.01$										
Fe-2	1.00	0.464	1.000	0.583	0.551	0.500	0.648	0.700	0.932	0.211
	1.10	0.536	0.000	0.417	0.449	0.500	0.352	0.300	0.068	0.789
Test for heterogeneity: $\chi^2 = 78.29$; $d.f. = 8$; $p < 0.01$										
Fe-3	0.80	0.214	0.026	0.597	0.322	0.308	0.352	0.280	0.523	0.632
	1.00	0.786	0.974	0.403	0.678	0.692	0.648	0.720	0.477	0.368
Test for heterogeneity: $\chi^2 = 54.35$; $d.f. = 8$; $p < 0.01$										
Lap	0.00	0.000	0.000	0.083	0.051	0.000	0.037	0.000	0.000	0.000
	0.90	0.357	0.053	0.000	0.068	0.154	0.407	0.180	0.091	0.105
	1.00	0.500	0.500	0.445	0.449	0.500	0.500	0.500	0.500	0.500
	1.05	0.143	0.447	0.472	0.432	0.346	0.056	0.320	0.409	0.395
Test for heterogeneity: $\chi^2 = 1.30$; $d.f. = 8$; $p > 0.05$										
6-Pgd-2	0.85	0.679	0.500	0.750	0.424	0.673	0.000	0.720	0.318	0.000
	1.00	0.321	0.500	0.250	0.576	0.327	1.000	0.280	0.682	1.000
Test for heterogeneity: $\chi^2 = 135.70$; $d.f. = 8$; $p < 0.01$										
Test for heterogeneity over all loci: $\chi^2 = 332.72$; $d.f. = 40$; $p < 0.01$										

Table 4. Allozyme polymorphism parameters in the populations of *Aristolochia contorta*

Population	P_{95}, %	P_{99}, %	H_o	H_e	A	n_e
RM	22.7	22.7	0.091	0.104	1.27	1.116
BK	13.6	18.2	0.086	0.062	1.23	1.066
UR	22.7	22.7	0.119	0.105	1.27	1.113
OR	22.7	22.7	0.144	0.114	1.32	1.129
BR	22.7	22.7	0.177	0.115	1.27	1.130
ShT	18.2	18.2	0.135	0.089	1.27	1.110
ShK	22.7	22.7	0.133	0.111	1.32	1.125
ST	22.7	22.7	0.118	0.098	1.32	1.109
PT	18.2	18.2	0.108	0.081	1.27	1.088
Population average	20.7	21.2	0.129	0.101	1.28	1.111
Species average	22.7	22.7	0.128	0.119	1.36	1.135

Note: P_{95} and P_{99}, polymorphism with consideration of 95- and 99% criterion; H_o, observed heterozygosity; H_e, expected heterozygosity; A, number of alleles per locus; n_e effective number of alleles per locus.

mixis, can be made only based on cytoembryological investigations.

Analysis of clonal (genotypic) diversity showed the following. In 247 accessions examined a total of 93 multilocus genotypes were described. Among these, 69 genotypes (74.2%) were unique (or "local", according to (28, 29]), i.e., were found only in one population. Twenty genotypes were found in two pop-

Table 5. Genotypic diversity in the populations of *Aristolochia contorta*

Population	*N*	*G*	*G/N*	*N/G*	*D*	Number of unique genotypes (in %)
RM	14	9	0.64	1.6	0.91	3 (33.3)
BK	19	6	0.32	3.2	0.60	3 (50.0)
UR	36	15	0.42	2.4	0.92	9 (60.0)
OR	59	37	0.63	1.6	0.98	22 (59.5)
BR	26	14	0.54	1.9	0.94	3 (21.4)
ShT	27	9	0.33	3.0	0.82	7 (77.8)
ShK	25	15	0.60	1.7	0.93	7 (46.7)
ST	22	9	0.41	2.4	0.80	8 (88.9)
PT	19	11	0.58	1.7	0.94	7 (63.6)
On average	27.4	13.9	0.51	2.2	0.87	7.7 (55.7)

Note: *N*, sample size; *G*, number of different genotypes; *G/N*, genotypic diversity; *N/G*, number of plants per clone; *D*, Simpson's diversity index for clonal plants [27, 28].

ulations, among these 13 genotypes were detected in two closely located populations. Four genotypes were found in three to five populations ("widely distributed" genotypes, according to [28, 29]). The average number of multilocus genotypes found in each population was 13.9 (Table 5). The lowest number of genotypes was 6 (BK), while the highest number was 37 (OR). The index of clonal diversity (G/N), reflecting the proportion of individual genotypes in certain population sample, was rather high and varied from 0.32 in population BK to 0.64 in population RM. The mean G/N value obtained in the populations of *A. contorta* (0.51) was higher than the values reported for 23 out of 26 clonal plant species [28], and the mean value determined for 45 clonal plant species (G/N = 0.27 [39]). At the species level, the value of G/N index constituted 0.377. Upon maximum genotypic diversity, the value of G/N was equal to one, i.e., in this case each plant represents an independent genet [27], and it can be treated as an indicator of sexual propagation, as it was reported for *Humboldtia brunonis* [40]. Correspondingly, the level of sexual propagation for *A. contorta* constituted 37.7%, and the level of clonal reproduction, 62.3% (1–G/N).

The values of Simpson's diversity index (*D*) in all but one (BK) populations examined were considerably high (Table 5) relative to mean value for clonal plants ($D = 0.62$ [28]; $D = 0.75$ [39]). Similarly high genotypic diversity indices were reported for the populations of other vine species, *Pueraria lobata* [22], *Monitopetalum chinense*, and clonal plant *Humboldtia brunonis* [40]. All localities examined in the present study were multiclonal. High level of genotypic diversity along with the presence of different clones can be explained in terms of either initial colonization of this habitat by the group of plants with different genotypes, or by the present propagation by seeds within the localities. This suggestion is supported by the presence of a certain number of unique genotypes in each population. The proportion of such varies from 21.4% in population BR to 88% in population ST. High proportion of unique genotypes per the small number of clones in populations ShT, ST, and PT points to the highest level of sexual reproduction in these localities. Noteworthy, in the population BR with the highest polymorphism level (Table 4), the lowest number of unique genotypes along with the rather high level of clonal reproduction was observed. These findings allow suggestion on the fixation of adapted heterozygous genotypes due to vegetative reproduction and (or) apomixis. Identical genotypes within one population can be the clones of a single plant, and the number of plants per clone (N/G) varies from 1.6 in populations RM and OR to 3.2 in population BK. The mean clone size constituted 2.2 plants. At the same time, the presence of identical genotypes in two closely located but isolated by distance and barriers populations allows suggestion on propagation of plants from apomictic seeds as a result of their dispersal by wind and water. Similarly, in the course of the analysis of sexual and vegetative reproduction in *Hieracium pilosella* it was decided to characterize the plants with identical genotypes and located at a distance more than 2 m from one another as grown from apomictic seeds (the distance was defined taking into account the ability of plants to clonal growth) [33]. Furthermore, in this study it was established that in more severe (arid) conditions the proportion of sexual propagation is increased.

Variability of the population genetic diversity level revealed is thought to be partly associated with different anthropogenic load intensity across the territory

Table 6. Analysis of population structure in *Aristolochia contorta*

Locus	F_{IS}	F_{IT}	F_{ST}
Aat-1	0.011	0.216	0.207
Fe-2	–0.292	–0.075	0.168
Fe-3	–0.099	0.030	0.117
Lap	–0.719	–0.611	0.063
6-Pgd	–0.162	0.179	0.293
Over all loci	–0.282	–0.067	0.168

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

examined. The highest influence of anthropogenic factor was observed in population BK, which, in turn, led to the reduction of the population number or in other words, to the increase of the gene drift influence. At the same time, population differences in the level of genetic diversity can be associated with the differences in the contributions of sexual and clonal propagation, or reflect the presence of sexual and apomictic reproduction, as it was demonstrated for *Bryonia alba* [23]. Habitation in more severe conditions at the range boundary can affect the ratio between the types of reproduction of *A. contorta*. Remarkable plasticity of the reproduction strategy makes it possible for the species to fixate on suitable territories within narrow ecological limits, as well as to survive in non-optimal conditions, and to use different mechanisms of reproduction for maintenance of genetic diversity.

Estimation of the population partitioning based on Wright's inbreeding coefficient indicates that interpopulation variation of *A. contorta* constitute 16.8% of total genetic variation observed (Table 6). Rather high level of population differentiation was close to that in *Pueraria lobata* ($G_{ST} = 0.199$) [22], to the mean indices for cross-pollinating species ($G_{ST} = 0.197$), and to the species capable of sexual and asexual reproduction ($G_{ST} = 0.213$) [31]. The gene flow index ($N_e m$), determined based on the F_{ST} index, constituted 1.24. The level of interpopulation differentiation discovered is associated with the present-day range fragmentation, as a result of anthropogenic influence, as well as with specific features of reproduction. Such factors as significant pollen defectiveness, negatively affecting the process of fertilization, as well as low productivity, and poor survival of seedlings due to competitive interactions, restrict the gene exchange and result in genetic population divergence. It is noteworthy that the divergence deepening is impeded by the formation of seeds as a result of sexual process, and the seed dispersal by anemo- and hydrochory. Negative F_{IS} value ($F_{IS} = -0.282$) points to complete absence of inbreeding and the excess of heterozygotes, highly improbable in case of free crossing. These findings can be expressed in terms of either self-incompatibility in the species of interest, or by selection in favor of heterozygotes. In any case, the most adapted heterozygous genotypes are reproduced through non-sexual propagation.

Thus, genetic population structure formed in the northern part of the range of *A. contorta*, as well as the genetic diversity level and distribution pattern, reflect the interaction of several processes. The basement is formed by the long-term history of the species, which includes northward dispersal, range fragmentation, and population isolation. Considerable contribution is made by the gene drift, associated with these processes. The influence of selection in stressful conditions of the range periphery cannot be also excluded. However, in our opinion, the main contribution is made by the species reproduction system along with its flexible reproduction strategy. The species ability to cross-pollination and the high proportion of the individuals of sexual origin provide maintenance of the genetic diversity level. At the same time, in the conditions impeding sexual reproduction, reproduction occurs at the expense of clonal propagation. It can be suggested that apomixis is also switched on as a backup processes in case of the absence of pollinators and the partners upon the development and fixing on the new territories. Clonal reproduction, as well as apomixis, provides the conservation and reproduction of adapted (mostly heterozygous) genotypes.

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