

Differentiation of *Harmonia axyridis* Pall. according to Polymorphic Morphological Traits and Variability of the Mitochondrial COI Gene

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Abstract—The geographic variability of *Harmonia axyridis* Pall. has been studied with regard to three morphological features. A study of the cytochrome C oxidase subunit I (COI) polymorphism was begun. Two geographically separated zones inside the Russian part of the geographic range of the species are recognized. Interpopulational differences, as judged using polymorphic traits, are very small within each zone. In contrast, the differences between the populations of the western and eastern zones reach the subspecies level of. A zone of clinal variability of morphological traits is noted in Transbaikalia. It obviously resulted from secondary contact between the western and eastern groups of populations, which were separated during the last glaciation and then rejoined.

Keywords: *Harmonia axyridis*, geographic variability, polymorphism.

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Our earlier studies of the elytron patterns of the Asian lady beetle (*Harmonia axyridis* Pallas (1773), Coleoptera, Coccinellidae) suggested the presence of two subspecies [1, 2]. We tentatively drew the boundary between their ranges along the Baikal fault. The differentiation of the species and its evolutionary history can be better understood on the base of later studies of the geographic variability of elytron patterns in Transbaikalian populations conducted by our group and those of other scientists [3], as well as combined consideration of the variability in the presence of the elytral crest [4] and pronotum patterns [5].

No prolonged directional variation of any trait was found in the analysis of long-term variability in several populations of various regions of the geographic range [6], in comparison with the results reported by Dobrzansky [7]. For this reason, we pooled samples obtained in various years in the same populations in the course of our geographic variability analysis.

Table 1 presents the rates of major elytron phenotypes, including data not reported previously, and the rates of individuals with no elytral crests (recessive homozygotes). These data indicate that western populations, from Novosibirsk to Bolshoi Yaloman Village, clearly differ from all the others. They are practically monomorphic in elytron pattern, and the prevalence of individuals lacking the elytron crest shows no cline.

Samples of eastern populations from Kaptsegaitui Village (southeastern Chita Region) to Troitsa Bay (southern Primorsky Krai) are also similar. The axyri-

dis phenotype is very rare there; the rate of succinea is 85–94%; and the melanistic phenotypes *conspicua* and *spectabilis*, taken together, constitute 6–15%. Cluster analysis indicates that the variability among samples of this group has no distinct cline either.

The percentage of beetles lacking the elytral crest in these samples is 0.7–2.7%. Cluster analysis of populations of this group shows no cline [4]. The variation in the prevalence of pronotum phenotypes among eastern populations is also insignificant and shows no cline [5]. Thus, two relatively homogeneous geographically separated groups of populations can be recognized in the species according to morphological traits: western and eastern. The level of difference between these groups according to two morphological traits with known genetics meets the 75% criterion accepted in conventional systematics for the subspecies rank. Clear clinal variability in rates of elytron pattern phenotypes is observed between these population groups in the region from Irkutsk to Nizhnii Tsasuchei (Table 1). The rate of the axyridis phenotype decreases from 94 to 23% and the succinea phenotype increases from 6 to 74%. An abrupt change is observed near Lake Baikal, between the populations of Listvyanka Village and Baikalsk. The *conspicua* and *spectabilis* phenotypes appear east of Lake Baikal. However, the samples of near-Baikal populations do not differ from the Baikalsk sample in the presence of the elytral crest. As seen from the rate of individuals lacking the elytral crest in Nizhnii Tsasuchei, the zone of clinal variabil-

Table 1. Geographic variability of elytron patterns and rates of elytral crest absence

Locality	Sample size	Rates of elytron phenotypes, %				Percentage of individuals lacking the elytral crest
		conspicua	spectabilis	axyridis	succinea	
Novosibirsk	817			99.88 ± 0.12	0.12 ± 0.12	74.91 ± 1.52
Kemerovo	345			99.71 ± 0.29	0.29 ± 0.29	80.87 ± 2.12
Gorno-Altaiisk	362			100.00		69.74 ± 5.27
Bolshoi Yaloman Village	36			100.00		
Irkutsk	218			94.04 ± 1.60	5.96 ± 1.60	83.49 ± 2.51
Baikal Village	354			89.27 ± 1.64	10.73 ± 1.64	81.36 ± 2.07
Listvyanka Village	322			85.40 ± 1.97	14.60 ± 1.97	82.33 ± 2.42
Baikalsk	217	0.46 ± 0.46		49.77 ± 3.39	49.77 ± 3.39	84.93 ± 2.42
Maleta Village*	85	2.35 ± 1.64	2.35 ± 1.64	51.76 ± 5.42	43.53 ± 5.38	
Nizhnii Narym Village*	232			37.50 ± 3.18	62.50 ± 3.18	
Chita*	392	2.04 ± 0.71	2.55 ± 0.80	31.89 ± 2.35	63.52 ± 2.43	
Nizhnii Tsasuchei Village	61		3.28 ± 2.28	22.95 ± 5.38	73.77 ± 5.63	49.18 ± 6.4
Kaptsegaitui* Village	216	6.48 ± 1.68	5.09 ± 1.50		88.43 ± 2.18	
Shirokii Village	4285	6.98 ± 0.39	5.86 ± 0.36	0.21 ± 0.07	86.95 ± 0.51	
Arkharo Village	1737	4.49 ± 0.50	4.78 ± 0.51		90.73 ± 0.70	1.09 ± 0.25
Pashkovo Village	337	4.15 ± 1.09	6.82 ± 1.37		89.02 ± 1.70	1.48 ± 0.66
Teplozersk Village	250	8.00 ± 1.72	7.60 ± 1.68		84.40 ± 2.29	0.8 ± 0.56
Bastak Nature Reserve	83	7.23 ± 2.84	7.23 ± 2.84		85.54 ± 3.86	2.13 ± 1.22
Sikhote-Alin Nature Reserve	2908	4.37 ± 0.38	4.50 ± 0.38		91.13 ± 0.53	0.93 ± 0.23
Dubovyi Klyuch Village	866	5.54 ± 0.78	3.12 ± 0.59		91.34 ± 0.96	0.69 ± 0.28
Kedrovaya Pad Nature Reserve	772	3.37 ± 0.65	3.11 ± 0.62		93.52 ± 0.89	
Emor Bay	202	2.97 ± 1.19	3.47 ± 1.29		93.56 ± 1.73	
Vladivostok	11560	5.67 ± 0.22	6.35 ± 0.23	0.01 ± 0.01	88.20 ± 0.30	1.46 ± 0.12
Russkii Island	433	5.31 ± 1.08	6.47 ± 1.18		88.22 ± 1.55	
Popov Island	3011	4.42 ± 0.37	4.98 ± 0.40	0.03 ± 0.03	90.57 ± 0.53	
Troitsa Bay	441	7.03 ± 1.22	8.84 ± 1.35		84.13 ± 1.74	2.71 ± 0.77

* Reported by O. V. Korsun (2004).

Table 2. Variability of the mtDNA COI gene region among populations

Population	Sample size	Haplotype rates, %									
		G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
Gorno-Altaiisk	27	33.33	48.15	3.70	3.70	3.70	3.70	3.70			
Vladivostok	22	81.82							4.55	9.09	4.55
Denver	25	92.00							4.00	4.00	

ity in the rate of this trait is farther east. The prevalence of pronotum patterns in beetles with succinea elytra also shows the clinal variability in Transbaikalian populations [5]. These populations also show elevated genetic heterogeneity [2, 4–6]. The combination of similarly directed clinal variabilities of various traits in one geographic zone and elevated genetic heterogeneity in the same zone indicates that this zone is not a

center of adaptive divergence of the species, as was thought by Dobrzansky [7], but is a site of secondary hybridization.

To better understand the intraspecific differentiation of *H. axyridis* and draw boundaries of the hybridization zone, we commenced a study of the polymorphism of the mitochondrial *COI* gene. We analyzed the variability of a 648-bp long region of this gene in three

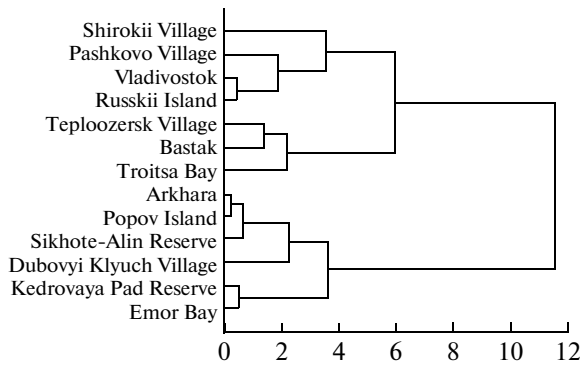


Fig. 1. Cluster analysis of samples of the eastern population group according to elytron phenotype rates.

H. axyridis populations: Gorno-Altaiisk, Vladivostok (Russia), and Denver (United States). It is believed that this species invaded the United States from China. We found ten haplotypes and submitted them to GenBank.

The dendrogram of nucleotide sequence similarity constructed by the neighbor-joining method according to the Kimura two-parameter model shows two clusters (Fig. 2). One cluster includes sequences that occur only in the Gorno-Altaiisk population, and the other, sequences of all the three populations. One haplotype is common for all samples, six occur only in Gorno-Altaiisk, and one occurs only in Vladivostok.

The prevalence of the haplotypes in the samples shows that the Gorno-Altaiisk population differs significantly from the Vladivostok and Denver ones, which are closely similar to each other (Table 2). This fact proves the profound difference between the western and eastern population groups.

We conclude that the pattern of *H. axyridis* geographic variability constructed according to the traits analyzed indicates that the contiguous geographic range of the species was split in two areas, which were entirely isolated from each other for a long time. Then the range restored its contiguity. Most likely, these events occurred during the last Pleistocene glaciation. The species survived in small isolated refugia in the present Russian Far East area and in Altai. With the onset of the Holocene warming, the isolated areas of the geographic range merged, and a secondary integration zone formed in Siberia.

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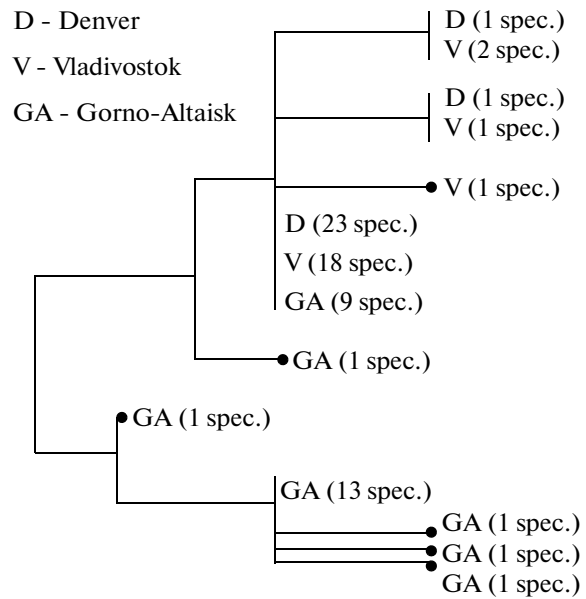


Fig. 2. Dendrogram of nucleotide sequences.

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