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SHORT COMMUNICATIONS

Population Variation of Elytral Ridge Occurrence in Ladybirds *Harmonia axyridis* Pallas

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Abstract—Intra- and interpopulation variation of occurrence of elytral ridge (neutral morphological character with the known genetic determination) in some populations of *Harmonia axyridis* from the Russian part of the species area was studied for the first time. Comparative analysis of the frequencies of the recessive allele *r* of a corresponding gene and the frequencies of recessive homozygotes in 32 samples from 16 localities has shown the lack of temporal and microgeographic variations of this trait. The character of its geographic variation confirms the hypothesis advanced earlier on the basis of analysis of elytral pattern variation about the existence of two subspecies of *H. axyridis* and suggests the existence of a zone of secondary hybridization between them in the central part of the area.

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The ladybird *Harmonia axyridis* Pallas (1773) has long been a classical object of population genetic investigations. However, only the variation of elytral patterns has so far been studied in detail in this species. The elytral pattern is a character whose genetic determination was established in the middle of the 20th century [1–4]. The data on the geograpic variation of this character suggest the existence of at least two subspecies of *H. axyridis*, the dividing line between them being located in the zone of the Baikal fracture [5]. However, to prove such suggestions, to localize the boundaries between possible subspecies, and to solve many other microevolutionary problems, it is necessary to study the population variation of a complex of characters. Another morphological trait is known in *H. axyridis*, the inheritance of which was studied experimentally [2, 6]. It is an elytral ridge (a transverse chitinous torus on the lower elytral ends) whose development is controlled by a single autosomal diallelic gene. The allele *r* determining the absence of a ridge is recessive, and individuals carrying the dominant allele *R* have an elytral ridge. The size of the ridge may vary. This character is inherited independently of elytral coloration. The geographic and temporal elytral ridge variations were comprehensively studied only in populations from Japan and very little in populations from Korea and China [7]. In connection with this, the intra- and interpopulation variations of this character in some populations of the Russian part of the species area has been studied for the first time in the present work.

The material for the work were the following samples of *Harmonia axyridis* (the collection of the Kol'tssovInstitute of Developmental Biology) from 16 localities of the Russian part of the area: (1) Kemerovo, autumn 2006, A.D. Polyakov; (2) Novosibirsk (Akademgorodok), autumn 1983, V.I. Telegin; Oct. 10–15, 1983, A.P. Kryukov; Sept. 26–30, 2006, I.A. Zakharov; Obskoye sea coast, Oct. 16, 1983, A.P. Kryukov; (3) Gorno-Altaisk, Sept. 2005, I.A. Sakharov; Aug.18, 2006, A.S. Bogdanov; (4) Irkutsk (Akademgorodok), autumn 1998, Timoshkin; (5) settlement of Listvyanka, Sept. 6–9, 1960, A. Popova; Sept. 13–27, 1983, A.V. Blekhman; (6) village of Baikal, Angara river source, autumn 1982, Obolkina; (7) Baikal'sk (12 km to northeast, Baikal lake shore), Sept. 2–10, 1983, A.V. Blekhman; (8) settlement of N. Tsasuchei (Ononskii raion, Chita oblast), March 19, 2006, V.P. Korablev; (9) settlement of Arkhara (south of the Amur oblast), Oct. 14, 1983, Yu.A. Darman; (10) settlement of Teploozersk (Khabarovsk krai, Obluchenskii raion, Jewish Autonomous Oblast, Bira river), Sept. 29, 30, 1989, S.V. Baptidanov; (11) settlement of Pashkovo (Khabarovsk krai, Obluchenskii raion, Jewish Autonomous Oblast), May 6–8, 2004, N.A. Formozov; (12) reserve Bastak (Khabarovsk krai, Jewish Autonomous Oblast), Oct. 2000, May 2001, M.F. Biserov; (13) Sikhote-Alin' reserve, cordon Ust'-Serebryanyi, Oct. 18, 1982, E.N. Smirnov; (14) settlement of Dubovyi Klyuch (Primorye, 30 km from the Ussuriyskii reserve), Oct. 26, 1981, V.N. Kuznetsov; (15) Vladivostok: 2nd river, Oct. 13, 20, 2003, A.V. Zimenko; Botanical garden, Oct. 17, 1995, V.P. Korablev; Akademgorodok, Sept. 28, 1995, Oct. 4–10, 2002, Oct. 4, 2003, autumn 2004, V.P. Korablev; st. Sedanka, Oct. 10–13, 2002, May 12, 2003, Oct. 6, 7, 2003, V.P. Korablev; Oct. 11, 2004, M.V. Tsvirka; st. Ugol'naya, Oct. 6–8, 2003, V.P. Korablev; (16) bay Troitsa (Primorye, Khasanskii raion, biostation TIBOKh), Oct. 6, 7, 2003, A.V. Zimenko.

Table 1. Intrapopulation variation of elytral ridge occurrence

Locality, sample			Sample size	Proportion of recessive homozygotes (*rr*), %	χ^2	*p*
Novosibirsk	Obskoye sea coast	16.10.1983	455	77.58 ± 0.02	4.41	0.22
	Akademgorodok	10, 15.10.1983	81	69.14 ± 0.05		
		autumn 1983	117	70.94 ± 0.04		
		26–30.09.2006	164	73.17 ± 0.03		
Gorno-Altaisk		Sept. 2005	57	70.18 ± 0.06	0.02	0.89
		18.08.2006	19	68.42 ± 0.11		
Listvyanka		Sept. 1960	74	68.92 ± 0.05	6.24	0.01
		Sept. 1983	249	82.33 ± 0.02		
Bastak		Oct. 2000	83	2.41 ± 0.02	0.08	0.78
		May 2001	58	1.72 ± 0.02		
Vladivostok	2nd river	Oct. 2003	204	0.98 ± 0.01	7.66	0.74
	Botanical garden	17.10.1995	3995	1.53 ± 0.00		
	Akademgorodok	28.09.1995	1146	0.96 ± 0.00		
		4–10.10.2002	1725	1.68 ± 0.00		
		4.10.2003	414	1.93 ± 0.01		
		21.09.2004	634	1.74 ± 0.01		
		29.10.2004	162	0.62 ± 0.01		
	Sedanka	10–13.10.2002	130	0.77 ± 0.01		
		12.05.2003	130	1.54 ± 0.01		
		Oct. 2003	475	0.84 ± 0.00		
		11.10.2004	150	1.33 ± 0.01		
	Ugol'naya	6–8.10.2003	113	2.65 ± 0.02		

Beetles were collected during the mass autumn migration to overwintering sites (or during the spring migration after overwintering). In Listvyanka (1983), in the vicinity of Baikal'sk (1983), and in Gorno-Altaisk (2006) ladybird beetles were collected from still reproducing colonies.

Beetles without an elytral ridge, i. e., recessive homozygotes (*rr*), were counted in the samples, and the frequency of the corresponding allele (q_r) was calculated by the standard procedure following the Hardy–Weinberg law. The significance of differences between the samples was determined by the χ^2 criterion [8]. All calculations and statistical analysis of the data were made using the standard Statistica 6.0 package.

To estimate the intrapopulation variation of the frequencies of alleles of the gene controlling the presence–absence of an elytral ridge, the frequencies of recessive homozygotes $Q_{(rr)}$ were determined in available repeated samples from some populations, and statistical significance of the detected intrapopulation differences was calculated. These data are presented in Table 1. Only the differences between the 1960 and 1983 samples from Listvyanka can be considered to be statistically significant ($P = 0.01$). The differences in the frequencies of the *r* allele in the spring and autumn samples from the Bastak reserve and from Vladivostok (Sedanka) are not statistically significant by the χ^2 criterion. No statistically significant differences were observed between samples from different places in Vladivostok.

To estimate the geographic elytral ridge variation, homogenous repeated samples were united, and from the settlement of Listvyanka the sample of 1983 was taken into consideration. The frequencies of recessive homozygotes $Q_{(rr)}$ and the corresponding q_r allele are presented in Table 2. The data show that the populations of the western part of the area (from Kemerovo to Baikal'sk) have very high frequencies of the analyzed allele, and the populations from the south of the Amur oblast, Khabarovsk krai, and Primorye (from Arkhara to the Troitsa bay) are very low. Within these groups, the differences between the populations are statistically significant, but not pronounced, and have no clear geographic direction. The sample from N. Tsasuchei (east-

Table 2. Geographic variation of elytral ridge occurrence

Locality	Sample size	Proportion of recessive homozygotes (*rr*), %	*r* allele frequency (q_r)
Kemerovo	345	80.87 ± 2.12	0.90 ± 0.01
Novosibirsk	817	74.91 ± 1.52	0.87 ± 0.01
Gorno-Altaisk	76	69.74 ± 5.27	0.84 ± 0.03
Irkutsk	218	83.49 ± 2.51	0.91 ± 0.01
Baikal village	354	81.36 ± 2.07	0.90 ± 0.01
Listvyanka	249	82.33 ± 1.55	0.91 ± 0.01
Baikal'sk	219	84.93 ± 2.49	0.92 ± 0.01
N. Tsasuchei	61	49.18 ± 6.40	0.70 ± 0.05
Arkhara	1739	1.09 ± 0.25	0.10 ± 0.01
Bastak reserve	141	2.13 ± 1.22	0.15 ± 0.04
Pashkovo	337	1.48 ± 0.66	0.12 ± 0.03
Teploozersk	250	0.80 ± 0.56	0.09 ± 0.03
Sikhote-Alin' reserve	1819	0.93 ± 0.23	0.10 ± 0.01
Dubovyi Klyuch	868	0.69 ± 0.28	0.08 ± 0.02
Vladivostok	9278	1.46 ± 0.12	0.12 ± 0.01
Troitsa bay	442	2.71 ± 0.78	0.16 ± 0.02
Total	17213		

ern Transbaikalia, Chita oblast) is intermediate by the *r* allele frequency between the samples from populations of the western and eastern parts of the area.

The data on the intrapopulation dynamics of the *r* allele (Table 1) point out to the lack of microgeographic and seasonal elytral ridge variations in the populations under study. Temporal variation was discovered only in the population from Listvyanka and not discovered in Novosibirsk, Gorno-Altaisk, and Vladivostok. Similar data were previously obtained for populations of Japan [7], where significant changes of q_r in the period from 1925 to 1944 were found only for two populations from the southern islands Shikoku (Matuyama) and Kyushu (Fukuoka) and not found for the remaining ones. The above-mentioned changes resulted in the equilization of the *r* allele frequencies observed in these populations and in the approximation of their values to those in the neighboring populations. q_r changes in Listvyanka also led to the diminution of differences by this parameter between this and neighboring populations (Tables 1, 2). It can be assumed that such temporal variation is determined by an increase of the interpopulation flow of genes due to a less isolation of neighboring populations and not by their response to ecological changes.

Comparison of our data on the macrogeographic elytral ridge variation (Table 2) with the known literature data [2, 7] permits two large geographic zones markedly differing in the occurrence of the recessive allele *r* to be distinguished within the species area. One of them, in which the populations are characterized by high q_r values, occupies a territory from the western boundary of the area to the eastern shore of the Baikal lake. The second zone is even more extensive. It begins at least in the south of the Amur oblast and occupies the whole eastern and southeastern parts of the species area (except for the Japanese islands), including China and Korea. The populations in this zone are characterized by low values of q_r. Interpopulational differences in the frequencies of this allele within these zones are insignificant, they have no geographic regularity and seem to be determined by stochastic processes.

At the same time, it is possible to distinguish two zones within the species area, in which a clinal variation of the *r* allele frequency is observed. One of them includes the Japanese islands, where the populations exhibit a smooth increase in the *r* allele frequency in the direction from northeast to southwest from 7% on Hokkaido (which corresponds to the analogous parameter in the group of eastern populations) to 94% on Kyushu [7]. The elytral ridge variation in the populations of the central part of the area (eastern Transbaikalia and the northern part of the Amur oblast) is poorly studied. However, the q_r value in the sample from N. Tsasuchei (Chita oblast) is intermediate between those characteristic of the western and eastern populations (Table 2), suggesting the existence in this part of the area of the second zone of clinal variation of the characater under study. Further studies are required to determine its boundaries.

It should be noted in conclusion that the functional role of the elytral ridge is so far unknown. Its temporal variation is missing in most populations. There are large and ecologically different parts of the area with insignificant interpopulation variation of this trait. The available facts demonstrate that the elytral ridge is a neutral morphological character with the known genetic determination. The pattern of its geographic variation reflects the history of formation of the area of the species. Apparently, in the previous evolution of *H. axyridis* the species area was divided in two parts (western and eastern) isolated from each other for a long time, in which the frequencies of the *r* allele (as well as alleles of the gene controlling the elytral pattern) were essentially different [3, 5]. Following the restoration of the area integrity, a hybridization zone with intermediate allelic frequencies was formed between the western and eastern parts. Further studies of the elytral ridge population variation will make it possible to localize the boundaries of this zone and to assertain the character of the microevolutionary processes that took place there in the past and are taking place now.

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