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Polymorphism of the Harlequin Ladybird *Harmonia axyridis* (Coleoptera, Coccinellidae) Baikal Population

N. A. Belyakova

All-Russia Institute of Plant Protection, Russian Academy of Agricultural Sciences, St. Petersburg, 196608 Russia
e-mail: belyakovana@yandex.ru

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Abstract—The phenotypic composition of the Baikal population of the harlequin ladybird *Harmonia axyridis* (Pallas, 1773) was analyzed based on the material collected in Irkutsk Province in 2009–2010. Significant microgeographic phenotypic variation was revealed on the Baikal coast and in the city of Irkutsk. The morph *axyridis*, widespread in Siberia, was dominant (80–90%) but the light-colored morph *succinea* was also present with high frequencies (43–59%) in some samples. Some individuals possessed an elytral ridge, their frequency in the Baikal population being low (4–6%). An increase in the number of the morph *succinea* in Irkutsk Province is not related to hybridization between the populations from the Far East and Siberia.

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The harlequin ladybird *Harmonia axyridis* (Pallas, 1773) is widely distributed in the Russian Far East and in South Siberia (Kuznetsov, 1993). The species is characterized by polymorphism of the elytral pattern (Dobzhansky, 1924), the difference between morphs being determined by multiple alleles of one autosomal locus (Tan, 1946).

During the last 15 years *H. axyridis* has become acclimated in Europe and America where this ladybird had been introduced for the purpose of pest control (Koch et al., 2006). In Europe, *H. axyridis* has spread as far as Norway to the north and as far as Latvia and the Transcarpathia to the east (Staverløkk et al., 2007; Barševskis, 2009; Marko and Pozsgai, 2009). The species is very likely to appear in the western regions of Russia within 2–3 years.

In the newly colonized areas, *H. axyridis* occupies the dominant position in the coccinellid complexes (Soares et al., 2008). Due to rapid expansion and considerable influence on the local insect fauna, *H. axyridis* has attracted great attention of researchers and is considered as a model species for studying invasions (Roy and Wajnberg, 2008). The ladybirds used for introduction were taken from the Far Eastern populations; it is therefore possible that only these populations and not the entire species *H. axyridis* are capable of active invasion.

The Far Eastern populations of *H. axyridis* are characterized by the prevalence of the light-colored morph *succinea* and thus clearly differ from the Siberian populations which mostly consist of the melanistic morph *axyridis*.

According to a number of authors, the differences in the phenotypic composition of the Far Eastern and Siberian populations of *H. axyridis* are at the level of different subspecies (Vorontsov and Blekhman, 2001; Zakharov and Blekhman, 2001).

The Transbaikalia is the zone of hybridization between the Siberian and Far Eastern populations (possibly subspecies) of *H. axyridis* (Blekhman, 2009). Within the contact zone, the two subspecies may merge or one of them may replace the other. Considering the fact that the Far Eastern *H. axyridis* acts as an active invasive species in Europe, its westward expansion in the Transbaikalia and enlargement of its zone of hybridization with the Siberian populations may also be expected. In view of this, of special interest are the populations from Irkutsk Province, the region directly adjacent to the Transbaikalian hybridization zone. The published data on the phenetic composition of *H. axyridis* populations from Irkutsk Province were obtained early in the XX century, and also in 1982–1983 and 1998 (Dobzhansky, 1924; Blekhman, 2009). The aim of this work was to analyze the recent phenetic composition of *H. axyridis* in the Baikal region.

MATERIALS AND METHODS

Adults, pupae, and larvae of *H. axyridis* were collected in Irkutsk Province (the city of Irkutsk, Baikal

Fig. 1. Elytral patterns in Siberian populations of *Harmonia axyridis*: morphs SUC (*a*), AXY (*b*), and rare variants of AXY (*c*–*e*) [(*a*–*d*) after Dobzhansky, 1924; (*e*) after Filippov, 1961].

Port, Listvyanka, Slyudyanka, and Baikalsk) during the second decade of July in 2009 and 2010.

The collected pupae and larvae were brought to adults in the laboratory; the larvae were fed with aphids of the same species which they consumed in the nature. All the samples together contained 1647 adults.

For phenetic population analysis, adults of *H. axyridis* were sorted by their elytral pattern, distinguishing the morphs *succinea* (designated below as SUC) and *axyridis* (AXY) (Fig. 1).

The frequency of individuals possessing the elytral ridge (a transverse cuticular ridge located in the apical portion of the elytra) was also estimated. The presence of the elytral ridge is a dominant autosomal character with monogenic inheritance independent from that of the elytral pattern (Hosino, 1939; Komai and Chino, 1969).

RESULTS AND DISCUSSION

The Phenetic Composition of H. axyridis Populations in the Baikal Region

Our analysis of the phenetic composition of *H. axyridis* populations has shown the morph AXY to be prevalent over the entire Baikal region. The fraction of the morph SUC did not exceed 20% in most samples from the Baikal coast, regardless of the collection site and the plant species on which individuals of *H. axyridis* were collected (Fig. 2). According to the published data, in 1982–1983 the frequency of SUC on the Baikal coast (Listvyanka, Baikal Port) was similar: 11–16% (Blekhman, 2009).

Higher frequencies of the morph SUC (40–59%) were observed in three samples which consisted of individuals collected as adults (Fig. 2: dashed line). The frequency of the morph SUC among the adults reared in the laboratory from the pupae and larvae collected on the same plants was 10–20% (Fig. 2). At first glance this might indicate a higher mortality of pupae and larvae of the morph AXY, as compared to SUC, under the natural conditions. However, this conclusion was not confirmed by data on the phenetic composition of samples from the city of Irkutsk, in which *H. axyridis* individuals collected at different stages showed no differences in the morph frequency (Fig. 2). In samples from Irkutsk, the samples of adult beetles did not differ in their phenetic composition from those of pupae or larvae which reached the adult stage under laboratory conditions, with the same diet as in the nature.

It is noteworthy that samples of adults from Baikalsk (2010) with high frequencies of the morph SUC were taken at the beginning of the flight period of the second generation of *H. axyridis*. We did not observe mating of these adults and did not find any laid egg. Many of the captured adults still had soft integuments. One might assume that adults with the phenotype SUC were the first to emerge, resulting in their fraction being overestimated in the early samples. However, samples of adults from the Angara River, which had also been taken at the beginning of the flight period of the second generation, revealed no increase in the fraction of the morph SUC (Fig. 2).

The equal ratio of the morphs AXY and SUC, recorded in our material in some samples from Irkutsk Province, was previously observed in collections from Baikalsk (Vorontsov and Blekhman, 1986). In Chita Province the morph SUC also comprised about 50%

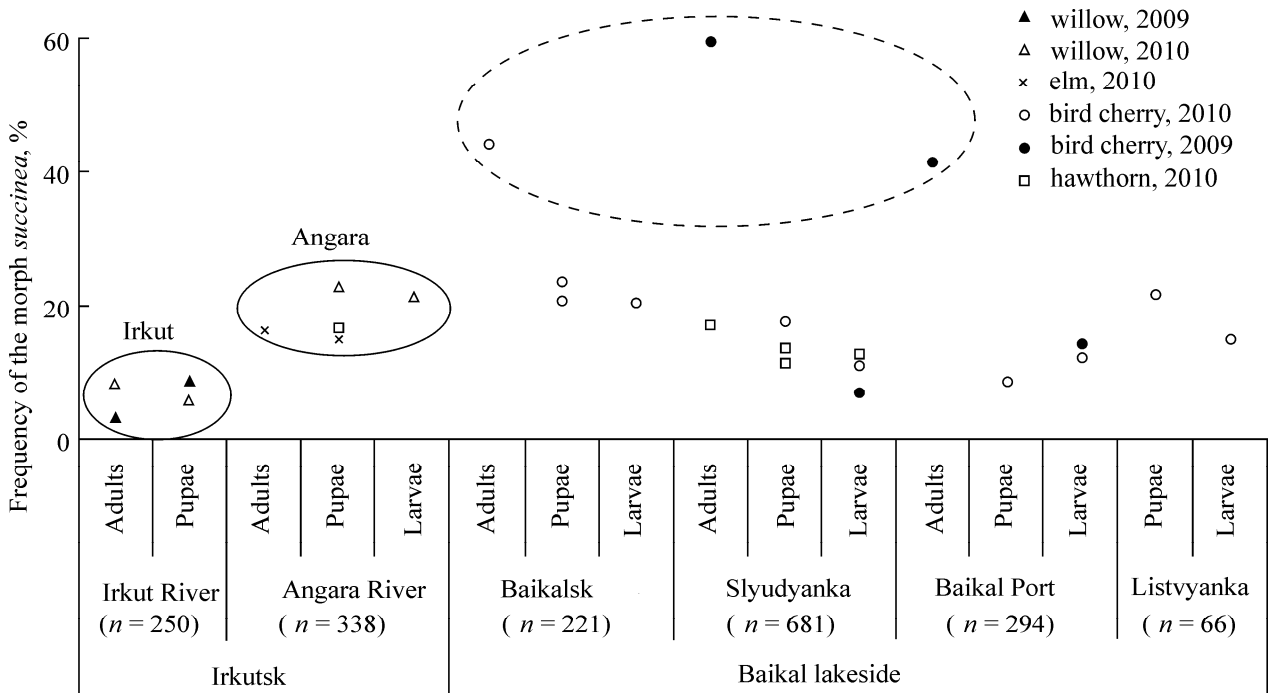

Fig. 2. The frequency of the morph SUC in *Harmonia axyridis* populations from Irkutsk Province (2009–2010). The clusters of samples "Irkut" and "Angara" were found to be significantly different in the frequency of the morph SUC ($p < 0.001$, $\chi^2 = 16.46$). The cluster showing a higher frequency of the morph SUC is encircled in the dashed oval.

(Butko, 2005; Blekhman, 2009). However, the similar phenetic composition of *H. axyridis* populations of the East Transbaikalia and Irkutsk Province has been achieved by different mechanisms.

The East Transbaikalia is justly considered the zone of hybridization between the Siberian and Far Eastern populations of the ladybirds (Blekhman, 2009). The frequency of the morph SUC increases from west to east in parallel with an increase of the fraction of individuals with the elytral ridge. The ridge and the SUC pattern are characteristic of the Far Eastern populations of *H. axyridis*.

In Irkutsk Province, the frequency of the morph SUC increases locally on the Baikal coast; however, the frequency of the elytral ridge in these samples remains low, which is typical of the Siberian populations of *H. axyridis* (table).

If genetic material exchanged actively between the Far Eastern and Siberian *H. axyridis* in the Baikal region, the hybrid individuals would possess the elytral ridge since this character, typical of the Far Eastern populations, is dominant (Komai et al., 1950). However, the ridge is absent in most individuals from Irkutsk Province; therefore, the observed changes in

The phenetic composition of *Harmonia axyridis* populations in the Baikal region

Collection site	Number of samples	Total number of ind. in samples**	Frequency of occurrence, %			χ^2
			morph SUC	morph AXY	elytral ridge	
Irkutsk (Irkut River)	4	250	6.8	93.2	4.5	2.113
Irkutsk (Angara River)	5	338	18.3	81.7	5.5	2.587
Baikalsk*	3	185	21.6	78.4	3.6	0.254
Slyudyanka*	6	605	13.2	86.8	3.9	3.605
Baikal Port*	3	203	10.7	89.3	3.7	1.938
Listvyanka*	2	66	18.2	81.8	3.6	0.603

Notes: * Collection of larvae and pupae.

** The samples were united because they showed no significant differences in the frequency of the morph SUC ($p > 0.05$, χ^2 test).

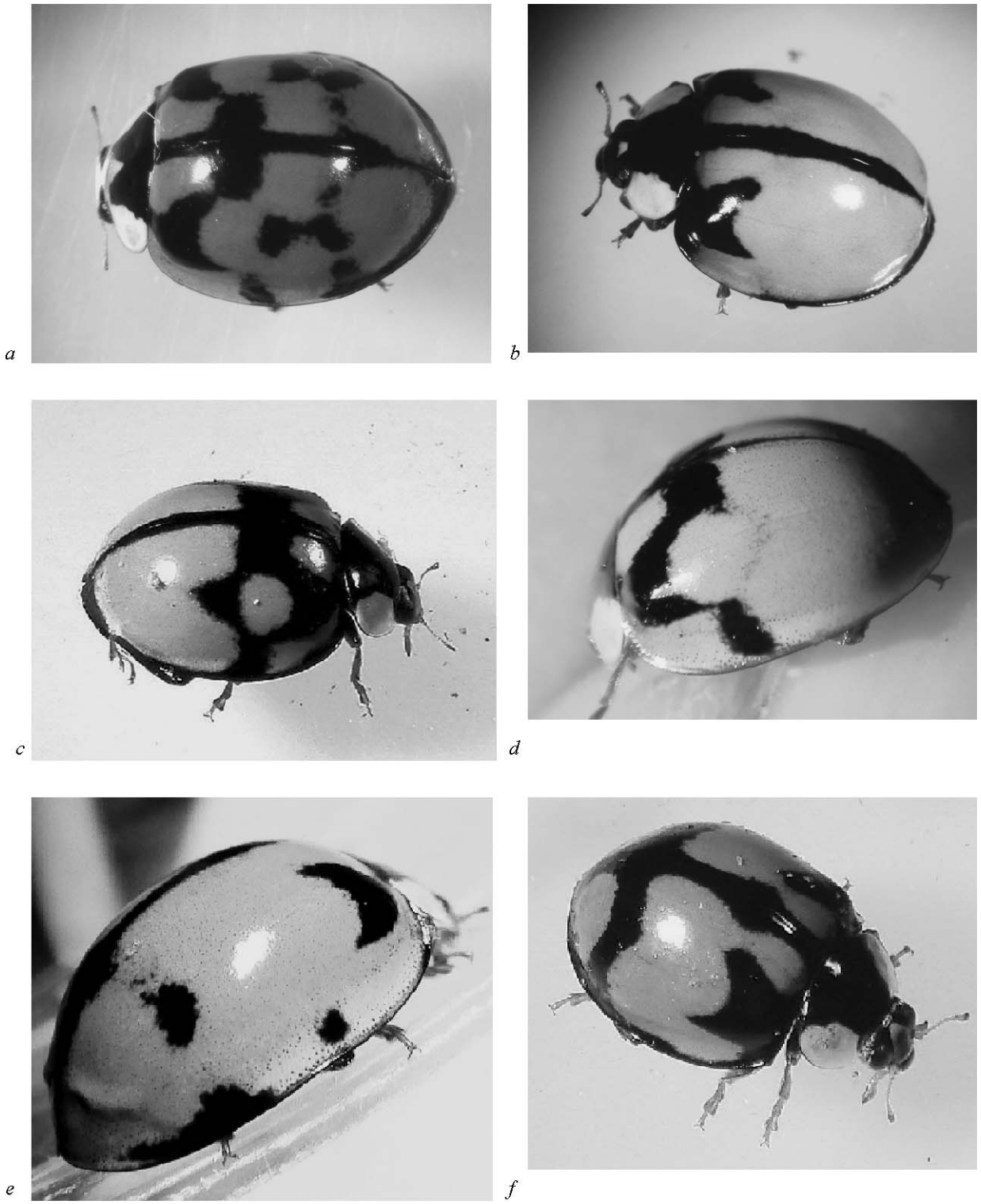

Fig. 3. Rare variants of the elytral pattern of *Harmonia axyridis* from the Baikal populations (2009–2010).

the phenetic aspect of the Baikal populations are not related to interpopulational hybridization between the Siberian and Far Eastern *H. axyridis*. Individuals homozygous for allele SUC must have been favored by selection, which has increased the frequency of this morph in some samples.

Microgeographic Variation of H. axyridis Morph Frequency in the Territory of Irkutsk

Collections of *H. axyridis* were carried out in two localities within the city of Irkutsk and its environs: on the left bank of the Angara (Irkutsk downtown) and on

the bank of the Irkut (Smolenshchina Vill.). The distance between the sampling localities was no more than 10 km but the difference in the frequency of the dominant morph AXY was statistically significant ($p < 0.001$, $\chi^2 = 16.46$) (Fig. 2: "Irkut" and "Angara" clusters).

According to the published data (Blekhman, 2009), the frequency of the morph AXY in Irkutsk (the Akademgorodok area) in the autumn of 1998 was 94%, which corresponds to our data for the Irkut collection locality.

Microgeographic variation of *H. axyridis* morphs frequency in the territory of Irkutsk seems to be determined by the wintering conditions within the urban area, which provides many shelters suitable for diapausing adults. The local urban micropopulations probably do not mix during wintering, so that their differences in the morphs frequency are retained. In natural biotopes, *H. axyridis* forms large wintering aggregations that include hundreds of thousands of beetles from vast territories; this facilitates mixing of individuals from different micropopulations and makes the morphs frequency more uniform.

Microgeographic differences in morphs frequency were also observed in *Adalia bipunctata* L. in large cities, such as St. Petersburg and Stockholm. The actual results did not always agree with the frequencies predicted based on the possible influence of pollution or the maritime climate (Zakharov, 2003). Microgeographic fluctuations of morphs frequency in ladybirds, including *H. axyridis*, may not always be adaptive. Instead, they may result from population densities in different localities leading to genetic drift.

Variation of the Elytral Pattern in the Morph axyridis in the Baikal Region

The elytral pattern of the morph AXY consists of 7 black spots (Figs. 1*e*, 3*a*) which usually merge leaving 6 gaps on each elytron (Fig. 1*b*). The semilunar basal spot represents a stable element in the AXY pattern, whereas the remaining spots vary in their size, shape, and degree of merging. This variation determines considerable diversity of the elytral pattern in the morph AXY which was described for the populations from Japan (Hosino, 1939; Komai, 1956) and Siberia (Dobzhansky, 1924; Filippov, 1961; Butko, 2005).

Our material of 2009–2010 from the Baikal region included *H. axyridis* individuals with the following rare variants of the elytral pattern:

(1) a semilunar spot at the boundary with the pronotum (Fig. 3*b*);

(2) four spots in the basal part of the elytron (Fig. 3*c*);

(3) three spots in the basal part of the elytron (Fig. 3*d*);

(4) three spots or a band in the apical part of the elytron combined with a basal semilunar spot (Fig. 3*e*, 3*f*). A similar pattern with a band was described as morph *forficula* in the populations from Japan (Hosino, 1939) and the Korean Peninsula (Seo et al., 2007).

The Japanese researchers have suggested that the elytral pattern of *H. axyridis* is determined by several genes in a complex locus, and that the rare variants may appear due to recombination within this locus (Komai and Chino, 1969). A similar genetic mechanism determining the elytral pattern is known in *A. bipunctata* (Zakharov, 1995).

To summarize the results of analysis of the phenetic composition of *H. axyridis* populations from the Baikal region, it should be noted that light-colored individuals comprise 15–20% of the population and are mostly represented by the morph SUC.

The rare light-colored morphs together comprise 0.4%. For comparison, in Primorskii Territory the rare morphs (*aulica*, *intermedia*, etc.) comprise 0.3–0.6% (Kholin, 1988). Thus, the Baikal population of *H. axyridis* is similar to that from Primorskii Territory in the level of phenotypic diversity. However, the set of rare morphs found in the Baikal region in 2009–2010 was entirely different from that present in the Far East. The only exception is the morph *forficula* which was recorded, besides the Baikal region, also in Japan and the Korean Peninsula (Hosino, 1939; Seo et al., 2007).

The Baikal population of *H. axyridis* is characterized by considerable microgeographic variation of its phenetic composition. The relatively high frequencies of light-colored variants observed locally in the Baikal region are not related to the process of intrapopulational hybridization between the Far Eastern and Siberian *H. axyridis*. Therefore, no westward expansion of the Far Eastern populations of *H. axyridis* is taking place.

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