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COMPARATIVE AND ONTOGENIC PHYSIOLOGY

To the 100th Anniversary of A.S. Danilevsky

Genetic Heterogeneity and Adaptive Strategies of the Ladybird *Harmonia axyridis* Pall. (Coleoptera, Coccinellidae)

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Abstract—Analysis of microstatial variability of the phenetic composition of populations of the ladybird *Harmonia axyridis* Pall. is performed in Baikal region of the Primorskii krai. It is revealed that the phenetic composition of the Baikal *H. axyridis* population is significantly more labile than that in Primorie. In individual micropopulations the phenoimage is clarified due to a local increase in frequency of the morph *succinea*. Individual and family testing of *H. axyridis* individuals from the Primorie and Baikal populations is performed by the quantitative parameters affecting the adaptability—the larval and pupal survival at starvation, critical weight of pupation, the imago weight and fertility. At starvation of larvae of the IV instars for 3—4 days, only the morph *succinea* individuals from the Primorie population reached the imago stage. The oviposition value in these imagoes corresponded to the mean for the population (31.2 ± 1.06 eggs). The obtained result confirms the earlier expressed suggestion that the morph *succinea* is distinguished by a high ecologic plasticity. This morph is able to survive under conditions of the food deficit and is characterized by the intermediate, but stable reproduction.

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Key words: *Harmonia axyridis*, polymorphism, color pattern, Baikal region, Primorskii krai.

INTRODUCTION

The ladybird *Harmonia axyridis* (Pallas 1773) is distributed widely in the forest zone of Siberia and Far East. In the North, this area reaches the zone of permafrost, in the South it includes the subtropical regions of Nepal and China [1, 2]. *H. axyridis* inhabits the broad-leaved and coniferous broad-leaved forests and is the massive species in the agrocenoses, on the ruderal and weedy vegetation. *H. axyridis* is a predator preferring aphids and, besides, consuming psyllae, red spider mites, eggs of lepidopterous and coleopterous insects [3].

H. axyridis is the object of numerous population-genetic and ecophysiological studies started in the beginning of the XX century by Dobzhansky [4] and continuing until the present time. For the last 15 years the researchers have been particularly interested in acclimatization and the area widening of *H. axyridis* in America and Europe, to which the ladybird was introduced for the fight with pests [5, 6].

Characteristic of the *H. axyridis* imago is polymorphism in the elytra color pattern. The differences between the morphs are determined by numerous alleles of one locus [7, 8]. The lightly stained color morph *succinea* dominates in Far East, America and Europe [9, 10]. Siberia and South Japan are the zones of distribution of darkly stained morphs [11, 12, 13]. In Dobzhansky works [14, 15] it is emphasized that the melanist forms are concentrated in regions with high humidity and low temperature, whereas the light forms, on the contrary, are more thermophilic and prefer low humidity.

The high ecologic plasticity and invasive potential of *H. axyridis* are ascribed to the picture polymorphism that allows this species to use the mostly completely heterogeneity of the habitat and to be fast adapted to its changes. The maintenance of the genetically determined intrapopulation polymorphism for the elytra color pattern is considered to be the ground for the species adaptive strategy [9].

The main functions of the elytra picture in *H. axyridis* are, like in the majority of ladybirds, are as follows:

1. *Thermoregulation.* The darkly stained beetles are warmed in the sun faster. In spring the sun increases the sexual and exploratory activity of melanists, while in summer, on the contrary, can produce their overheating and decrease the activity, which affects in both cases the individual's reproductive success.

2. *Protection from predators.* To enhance the protective effect of the repellent color, the sympatric ladybird species often form the "rings" of mimicry [10]. We have failed to find in the literature the data about the mimetic relations of *H. axyridis.* But since the harmonia is the massive species with highly-toxic hemolymph, it can be suggested that it serves in mimicry rings as a model imitated by the rarer and less protected species. The effect of the color of sympatric species of coccinellidae on the elytra color pattern, in our opinion, is highly improbable.

The data accumulated for the last 100 years on the intra- and interpopulational variability of the phenetic composition of the *H. axyridis* populations cannot be explained only by the thermoregulating and protective function of the elytra color pattern. One of the unsolved questions is as to why the Far East *H. axyridis* populations preserve a high stability of the phenetic composition in the significant areal regions that include different by the climatic conditions areas. The morph *succinea* dominate in territories of the Amur region, Primorskii krai, the north-eastern and central China regardless of the insolation level and other climatic conditions [9, 16]. Possibly, this is due to that the intrinsic to the morph *succinea* is the terminal melanism (the area of black spots in elytra increased if larvae and nymphs developed at low temperatures). This is what principally distinguishes *succinea* from all other morphs. The modification variability of the elytra color pattern allows individuals of the morph *succinea* to adapt to different weather and climatic conditions. However, if the problem of thermoregulation is solved, why in the *H. axyridis* populations there are present the rare morphs whose pattern does not depend on temperature?

In this work we consider some ecologo-genetic mechanisms of maintenance of the intra- and interpopulational polymorphism of *H. axyridis* by the elytra color pattern as well as its adaptive significance for this species.

MATERIALS AND METHODS

The *H. axyridis* imagoes, nymphs, and larvae were collected at the territory of the Irkutsk oblast (Irkutsk, port Baikal, Listvyanka, Slyudyanka, and Baikalsk) at the second decade of July 2009–2010 as well as in Primorskii krai (Ussuriisk, Kamen-Rybolov, and Vladivostok) in July 2009.

The imagoes were developed at laboratory from the collected nymphs and larvae, the larvae being fed by the aphid species that were food for the predators in the habitat.

The sex was determined by the structure of the terminal segment, in which the *H. axyridis* males have a notch. Imagoes were sorted out by the elytra color pattern by separating morphs *succinea*, *conspicua, spectabilis, axyridis* and *aulica* [7].

For the individual and familial analysis of the main parameters of the reproductive potential (fertility, survival, and weight), the imago were kept by pairs in Petri dishes (9 cm in diameter, 0.8 cm in height), larvae—in plastic containers (15 cm in diameter, 5 cm in height). The usual aphid grown on wheat plants was the food.

The experiments on resistance of *H. axyridis* to starvation were performed with larvae of the 4 instars, as it at this age that the ladybirds consume

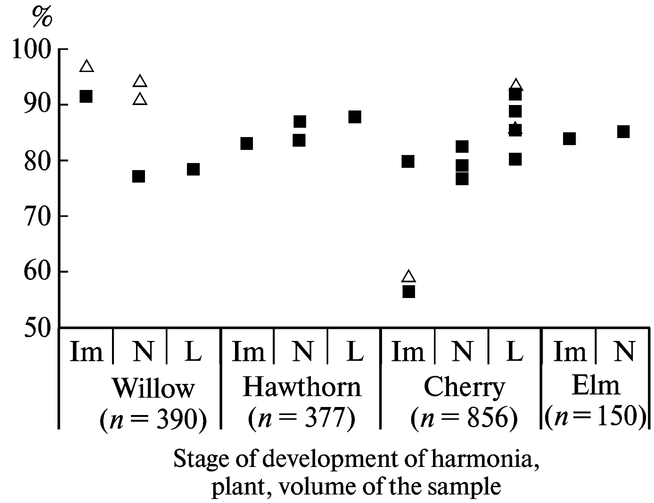

Fig. 1. The microstatial variability of the phenetic composition of populations of the *Harmonia axyridis* (Irkutsk oblast). *Abscissa*: Im—imago, N—nymph, L—larva, *ordinate*: frequency of morph *axyridis* (%). Collections: white triangles—in 2009, black squares—in 2010. Significant differences for percentage of morph *axyridis* are revealed between samples from the bird cherry ($p < 0.001$, $\chi^2 = 29.45$) as well as from willow ($p < 0.001$, $\chi^2 = 16.26$).

the greatest amount of food (about 70% of the total voraciousness at the larva stage). The larvae were chosen for the experiment 1–6 days after molting at the IV instars and were weighed. Then the experimental animals maintained individually without food until pupation or death. The weighing was performed daily by using a Shinko HTR-80CE scale.

The statistical treatment of data was performed by using the package of the statistical software SPSS v.13.0.

RESULTS AND DISCUSSION

The *H. axyridis* morphs are suggested to differ by biotopic confinement and by gigrothermal and nutritional preferences [9, 17]. To evaluate this hypothesis, we performed analysis of the microstatial variability of the phenetic structure of the Far East and Siberian populations of *H. axyridis* in different biotopes.

The beetles were collected in Primorskii krai during summer of 2009 in agrocenoses of soybean and maize, in river bottom-lands on willow as well as on the weedy vegetation (corn sowthistle, wormwood). In spite of various kinds of plants and victims, the part of the dominating morph *succinea* was in all samples similar and amounted to 85–90%, the frequency of dark-stained individuals (*conspicua* and *spectabilis*) not exceeding 15%. The frequencies of the morphs revealed in Primorskii krai coincide with the literature data accumulated in this region for the last 20 years [9, 18]. The Far East *H. axyridis* populations are characterized by the many-year seasonal and microstatial stability of phenetic image.

Possibly, the steadily low frequency of the melanized *conspicua* and *spectabilis* morphs in Primorskii krai is maintained owing to increased adaptation of heterozygotes in alleles determining these morphs. The selection maintains heterozygotes and eliminates homozygotes. This, on one hand, restricts frequency of melanized morphs, but, on the other hand, allows the melanists to be preserved in population in the case of sharp changes in the weather conditions at the imago fly period (for example, at cooling off, when the dark color of elytra will increase chances for survival). The adaptive strategy based on the increased adaptation of heterozygotes has been described in populations of twin spot ladybird *Adalia bipunctata* L. [19].

For analysis of the microstatial variability and of the phenetic structure of Siberia populations, the *H. axyridis* populations of imagoes, nymphs, and larvae were collected in 2009–2010 in the Irkutsk oblast (Fig. 1). The percentage of the morph *axyridis* amounted, on average, to 85% on the elm and 86% on the hawthorn. Oscillations of this parameter in samples from the bird cherry and sallow are significant ($p < 0,001$, χ^2), which did not allow their pooling for evaluation of the weight–average frequency of morph *axyridis* on these plants. The part of the light-color morph *succinea* did not exceed, as a rule, 20%, but in occasional samples from sallow it reached 40–45%.

Since diapason of oscillations of ratio of the *H. axyridis* morphs in samples from one biotope exceeds differences in this parameter among different stations, we failed to reveal the biotopic confinement of morphs *axyridis* and *succinea* in the Baikal region.

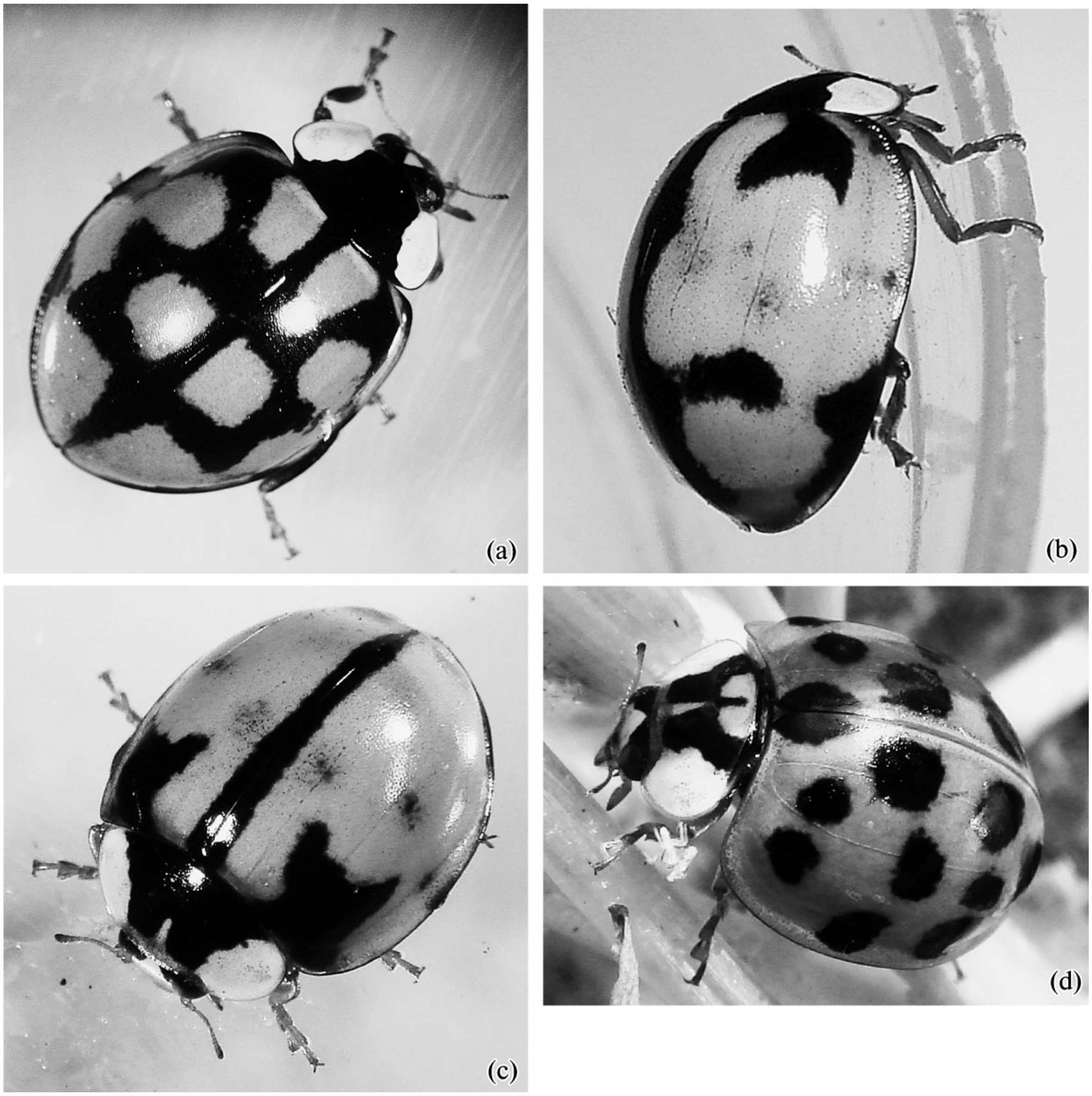

Fig. 2. Variability of the elytra color pattern in the Baikal area populations of *Harmonia axyridis* (Irkutsk oblast, 2009—2010). (a)–(c) Variants of morph *axyridis*; (d) morph *succinea*.

The phenetic structure of Baikal region populations of *H. axyridis* is significantly more labile than in Primorskii krai. The lighting of phenetic image occurs in occasional micropopulations due to a local increase of portion of the morph *succinea*. Besides, a significant variability of the morph *axyridis* is revealed in Baikal region populations There are present individuals, in which clearances between black spots are fused in different combinations; on the apical elytra parts a large light-colored area is formed, while the dark color pattern is reduced to two small sickle-shaped spots located on elytrae near the border with pronotum (Fig. 2).

Theoretically, the "clarification" of the Baikal region populations can occur not only due to an increase of the portion of the morph *succinea*, but also due to spreading of light-color variants of the morph *axyridis*. This is the reserve mechanism of change in the phenetic image of population. The situation is similar in Far East populations. The main mechanism of maintenance of the high frequency of the light-color individuals is the stable

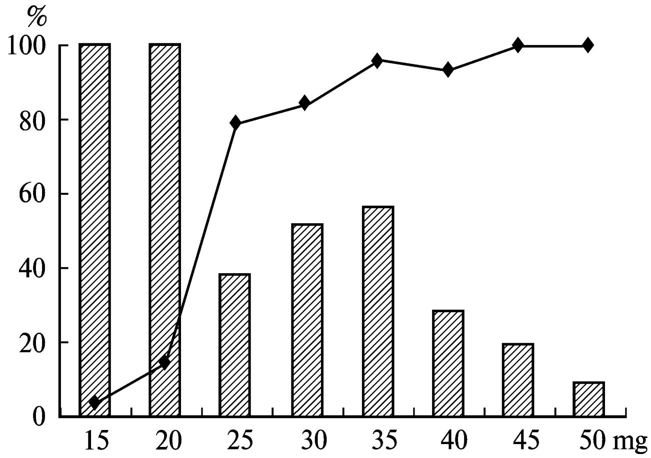

Fig. 3. Percentage of individuals *Harmonia axyridis* reaching the imago stage depending on the initial body weight of larva before starvation. Broken line—percentage of flying out imagoes (%), columns—percentage of males (%).

dominance of the morph *succinea*. However, less than 1% of rare light-colored morphs *aulica* and *intermedia* is present in this population. The part of the rare morphs is increased several times in the rarest cases. For example, in 2007, we described similar anomaly in the Chegu Island (Republic Korea), where the total part of morphs *aulica* and *intermedia* amounted to 22% [20]. Thus, the reserve mechanism of change in the phenetic image of population sometimes operates due to the rare morphs.

Some authors suggested that there are differences among morphs in the reproductive potential and adaptive abilities. This hypothesis is confirmed particularly by the experimental data, obtained at laboratory [21, 22].

Under laboratory conditions there are revealed the increased voraciousness and lifespan in the light-colored morph *aulica* as compared with the melanized morph *nigra* at feeding with peach aphid [21]. The individuals of morph *succinea* exceeding at feeding with a substitute of the natural food (the eggs of meal moth) the melanists by duration of life and of oppositional period, which, in the authors' opinion, indicates a high ecological plasticity of the morph *succinea* [22]. The differences revealed in laboratory experiments among the morphs are manifested at the artificial light whose intensity is lower than that of the solar light. Hence, differences between the morphs cannot be explained by different amount of the absorbed solar energy. Possibly, they are determinated genetically.

To check hypothesis about the existence of ecophysiological differences between the morphs, we performed individual and familial testing of *H. axyridis* individuals from the Far East and Siberia populations by the quantitative parameters affecting adaptation of the individual—survival of larvae and nymphs in starvation, critical weight of pupation, the imago weight and fertility.

In the experiment with different regime of nutrition of the *H. axyridis* larvae, the lethality depended on the initial weight of larvae: the higher the weight before starvation, the lower the lethality. The revealed dependence has the threshold character and can be described by the S-like curve (Fig. 3). The threshold weight, at which about 50% of individuals survive, is within the diapason of 20–25 mg. The larvae of *H. axyridis* reach the threshold weight for 3–4 days after molting (at temperature of 24–25°C) regardless of morph.

At analysis of the sex ratio in different variants of the experiment, it is revealed the following: the lower the initial weight of larva before starvation, the higher the portion of males among the individuals reaching the imago stage. The male larvae weighing 15–20 mg have pupation, whereas females die (Fig. 3). The sex ratio 1:1 was detected in the imago from larvae with initial weight of 25–35 mg before starvation. Among the larvae with the initial weight of more 40 mg, the percentage of males decreased to 10–30%.

It is to be noted that at the longest starvation (3–4 days), only individuals of the morph *succinea* from the Primorie population reached the imago stage. The egg-laying in these imagoes corresponded to the mean value for the population (see table).

Earlier it was suggested in literature that the morph *succinea* differed from others by the higher plasticity [22]. Possibly, the adaptive strategy of the *H. axyridis* Far East populations, where the morph *succinea* dominates, is based on separation of individuals in the population to universals and specialists. These universals, such as *succinea*, can survive under the conditions of food deficit and are distinguished by intermediate, but steady

Daily average fertility of females and the size of oviposition of the *H. axyridis* Primorskii population at nutrition by the grain aphid

Variant	The number of pairs	The daily average fertility	The number of ovipositions	The mean number of eggs in clutch
Primorskii population	80	17.7 ± 1.22*	435	32.6 ± 1.10
Morph *succinea* with the body weight lower than 20 g	15	11.1 ± 1.07	53	31.2 ± 1.06

Note: Asterisk designates the error of the mean. The daily average fertility was evaluated for the first 10 days of the oviposition period.

reproduction in the wide specter of victims. The universals form the nucleus of the population. The specialists are distinguished by the presence of the more narrow gigrothermal and alimentary preferences. The specialists under the optimal for them conditions can be ahead of the universal by reproduction. The specialists serve for the more complete assimilation of the habitat heterogeneity [23].

The maintenance in population of the large diversity of elytra color patterns allows *H. axyridis* to be adapted the most completely to the weather conditions and to peculiarities of new biotopes at spreading. At any changes of weather, in the population there always will be found the individuals that will obtain the adaptive preference due to the color, i.e., more correctly, due to the degree of its melanization.

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