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Eco-Geographical Components of Natural Population Variability of Ectoparasites *Habrobracon hebetor* (Say, 1836) (Hymenoptera, Braconidae)*

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Abstract: The article presents original data on the specific development of geographically distant populations of *Habrobracon hebetor* (Say, 1836). We compared two populations from different ecological niches in Ukraine, Kiev, and Uzbekistan, Tashkent. In particular, their physiological processes, morphological features, and behavioral characteristics were studied. It has been established that there is a significant difference between populations by the following criteria: performance of the reproductive system, duration of life of females, sex ratio, linear sizes of imago and cocoon, the color of imago, motor activity, and the nature of oviposition. According to our observations, local populations are ecologically plastic with high adaptive characteristics. The outlandish population was marked by a violation of the physiological state. At the same time, as our studies have shown, it is quite expedient and promising to carry out the selection of geographically distant populations of ectoparasites to improve the technological and physiological characteristics of hybrids.

Keywords: Entomophagous mass breeding, ecosystem, adaptation, intraspecific changes, endogenous and exogenous factors, selection

1. Introduction

The plant protection strategy in the third millennium is built on an ecological basis. The European category of quality of life includes various indicators, among which the quality of the products we consume is crucial for maintaining the health of the nation. At the same time, modern agricultural technologies in Ukraine and some other countries provide for fairly intensive use of chemical pesticides to protect agrocenoses from the negative activity of weeds, phytophages, and pathogens. Despite their high efficiency, the negative consequences of the introduction of intensive technologies are also obvious. They are characterized by aggressiveness and high cost (Kysil, 2000).

Today, it is believed that the basis of organic agriculture is the use of biological means of crop protection. In particular, the regulation of the number of pests is carried out using the entomophagous organism, Acaridae, bacteria, fungi, etc. Among these bioagents, an effective link is occupied directly by entomophages (Kuzmenko, 2013).

Many years of practice have shown that the main means of biological control of phytophages in Ukraine and Europe, and this is about 80%, is carried out using a group of species of the *Trichogramma* spp. These insects parasitize more than 200 species of Lepidoptera phytophage. The second place after the *Trichogramma* spp. among the entomophagous occupy is parasitic wasps of larva's of Lepidoptera phytophages *H. hebetor*. In nature, parasitic wasps attack more than 60 species of phytophages. In most cases, *H. hebetor* is used in integrated technologies for the protection of vegetables and melons, as well as during the storage

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of products (Drozda, 2001; Drozda et al., 2017; Ismailov et al., 2017).

Numerous studies have been conducted on the use of *H. hebetor* Say in technologies of protection of long-term storage products (Kysil, 2000; Drozda and Zagayko, 2016). Parasitoids do not negatively affect the environment, humans, or beneficial organisms. Many scientists used laboratory parasite populations in experiments on wheat grain, corn, and wheat flour, and also on dried fruits. At the same time, a significant decrease in the density level of *Plodia interpunctella* (Hübner, 1813) and *Ephestia kuehniella* (Zeller, 1879) was observed (Cline et al., 1984; Mbata and Shapirol-Ilan, 2010; Drozda et al., 2017). In laboratory studies, *H. hebetor* demonstrated 97 % effectiveness against moths (Brower and Press, 1990).

In addition to long-term storage products, parasite wasps were widely used in agrocenoses. Analysis of the literature shown that the effectiveness of *H. hebetor* in agrocenoses has been studied quite deeply by several scientists from Russia, Kazakhstan, USA, Germany, Uzbekistan, Iran, and others (Rafiei et al., 2008; Saxena et al., 2012; Mahdavi et al., 2013; Kovalenkov et al., 2016; Ismailov et al., 2017). At the same time, the experiments were carried out in corn, chickpea, and cotton fields, apple, and plum orchards, and also partially in forest and forest parks. Studies show that laboratory cultures *H. hebetor* Say are effective against *Ostrinia nubilalis* (Hübner, 1796) up to 22%, *Lacanobia oleracea* (Linnaeus, 1758) - up to 35%, *Helicoverpa armigera* (Hübner, 1808)- up to 45%, *Autographa gamma* (Linnaeus, 1758)- up to 30%, and *Polychrosis botrona* (Denis & Schiffermüller, 1775) up to 35% (Mbata and Shapiro-Ilan, 2010). At the same time, the effectiveness of the *H. hebetor* in integrated technologies for the protection of agrocenoses using microbiological preparations and other types of entomophages (*Trichogramma* spp., *Chrysopa* spp.) reaches approximately 70-90% (Drozda, 2001).

It is known that the development of scientifically sound technologies for mass cultivation and rational use of entomophages can be carried out based on a detailed study of their species biodiversity, morphological specifics, and some eco-geographical features that are associated with their adaptation to specific living conditions. In particular, *H. hebetor* are characterized by a wide distribution area, a relatively short life cycle, and high efficiency in regulating the number of Lepidoptera phytophages. However, a number of issues concerning the adaptive strategy of local populations of some eco-geographical features of the reproductive strategy of females and their searchability in the conditions of Ukraine have not been fully studied.

The aim of our research was to reveal the adaptation strategies of eco-geographically distant populations of ectoparasites depending on environmental conditions, the formation of their morphological and physiological characteristics.

2. Materials and Methods

2.1. Breeding of ectoparasite *H. hebetor* and host insect *E. kuehniella*

For a long period (2016-2019 years) at the Department of Integrated Protection and Plant Quarantine, of the National University of Life and Environmental Sciences of Ukraine, the laboratory culture of ectoparasites *H. hebetor* is maintained, which are grown using popular technologies in the author's modification (Kovalekov et al., 1995; Mbata and Shapiro-Ilan, 2010; Drozda and Statkevych, 2018).

Field and laboratory studies were carried out according to generally accepted methods in the field of entomology, parasitology, and plant protection (Cline et al., 1984; Ahmed et al., 1985; Dospekhov, 1985; Brower and Press, 1990; Antolin et al., 2003; Kil et al., 2018). In particular, a culture of ectoparasites was formed by collecting parasitic larvas of scoops and leafworms from the untouched ecosystems of Ukraine and Uzbekistan (Vyktorov, 1976). The natural ectoparasite population consisted of 118 paralyzed phytophage larvas, of which 468 adults were resurrected. The parasitoid was reared on the last instar larva of *E. kuehniella* in glass containers of rearing jars at 25±3 ºC, with a 16-hour photoperiod and relative humidity 70±5% (Vyktorov, 1976; Dospekhov, 1985; Kovalenkov et al., 1995; Mbata and Shapiro-Ilan, 2010).

Population *E. kuehniella* was selected at flour mills in flour products. Caterpillar cultivation *E. kuehniella* provided for the use of wheat bran with the addition of vitamins A_1, B_1, B_2, B_{12}. Glycerol and agar were added to maintain moisture in the culture medium. Larvas were bred in plastic containers. The breeding mode was carried out at a temperature of 28±1.5 °C, the relative humidity of 70±5%, and with a 16-hour photoperiod (Kovalenkov et al., 2016; Statkevych, 2019).

2.2. Study of the ecological and geographical variability of natural populations of *H. hebetor*

Ecological and geographic variability of the natural populations of *H. hebetor* investigated by comparing the adaptive properties of two

geographically distant ectoparasite colonies. In particular, entomophage populations from such natural niches, Ukraine- Kiev, and Uzbekistan-Tashkent, were compared. The morphological, physiological, and ethological criteria of *H. hebetor* were evaluated.

2.3. Statistical analysis

Analysis of variance (ANOVA) was performed to determine changes in fertility, linear sizes of adults and cocoons, the life expectancy of females, and preimaginal phases of development. Statistical analysis was performed using StatGraphics Plus software (Anonymous, 1980). Least Significant Difference (LSD) tests were performed to see the magnitude of structural changes in both geographically distant populations of *H. hebetor* (Medhi, 1992; Steel et al., 1997).

The average fecundity of females of ectoparasites was determined by the Equation 1.

F= A/N whereat (1)

F - average fecundity of females; A is the total number of eggs laid by all females; N - number of females. Average of how many females - 30.

3. Results and Discussion

Analysis of literature data indicates the importance of studies of geographically remote populations of ectoparasites. The long-term practice of using entomophages in the world has shown that the best effect of biological control is achieved when using local species adapted in the process of long evolution to local conditions. Such similar studies have been successfully carried out on *Trichogramma spp.* Scientists have found that the basis for the effective use of *Trichogramma spp.* in biological plant protection is the correct selection of species and intraspecific forms (Fursov and Storozheva, 1990; Sorokina, 1993; Melnychuk et al., 2008). The variety of landscape zones, the abundance of cultivated crops, the specificity of the climate of Ukraine necessitate the identification of the local type of ectoparasite and the study of their ecology.

It is known that the territory of Uzbekistan is diverse and most of it is not suitable for life, therefore this share belongs to deserts, steppes, and mountains. The climate here is somewhat specific and is characterized as sharply continental, hot, and dry. The Ukrainian climate is different from Uzbekistan. In particular, it is predominantly moderately continental, while the summer is warm and long, in the east and south it is hot and dry, in the west it is warm and humid. Mild winters are observed in the south and west of the country, the coldest winters are observed in the northeast. Both states have different environmental conditions. Specific to all the ecological and geographical features of countries, we analyzed two different populations of *H. hebetor* (Khromov and Petrosyants, 2006).

The materials of Tables 1 and 2 illustrate several differences (physiological, ethological, and morphological) between the colonies of ectoparasites of Ukraine and Uzbekistan. Physiological parameters (Table 1) were noted of reproductive efficiency, while the Ukrainian population laid an average of 85.81 eggs per female, and the Uzbek population laid 65.13 eggs per female in the Ukrainian climate. Given the temperature regime (25±3 °C), the duration of the ontogenesis of the premimaginal stages of the local

Table 1. Analysis of physiological parameters of the viability of eco-geographical distant populations of ectoparasites *H. hebetor*

Population geographic niche	Physiological parameters of ectoparasites that are evaluated							
	Duration of ontogenesis of preimaginal stages, days			Female life span, days	Fertility, eggs per female	The number of adults that have revived from one host caterpillar is	Sex correlation ♀:♂	Deformed adults, %
	Egg	Larva	Pupa					
Ukraine (Climate: mostly temperate continental)	1.31	3.99	3.85	13.36	85.81	6.73	1:1	1.03
Uzbekistan (climate: sharply continental, hot and arid)	2.98	5.71	5.87	10.72	65.13	4.36	0.42:1.75	3.71
Mixed (crossbreeding of Ukrainian and Uzbek populations)	1.65	4.61	4.91	14.46	96.55	8.81	1:1	0.66
LSD	0.54	1.51	0.75	1.01	2.03	1.76	-	2.26

Table 2. Analysis of morphological and ethological features of eco-gerophically distant populations of ectoparasite *H. hebetor*

Population geographic niche	Morphological features				Ethological features	
	The length of the female (mm)	Length of males (mm)	The length of the cocoon (mm)	Imago color	Motility and searchability of females	The nature of the oviposition
Ukraine (Climate: mostly temperate continental)	3.42	3.01	3.51	Dark brown	Pronounced high locomotor ability and directionally respond to larva	Compact groups and single oviposition between larva segments (protected) Fig. 1, expressed selectivity of a suitable substrate
Uzbekistan (climate: sharply continental, hot, and arid)	2.44	2.25	3.04	Light brown with an orange tinge	Moderate locomotor activity parasitizes larva's after prolonged monitoring	Compact groups of 3-5 eggs
Mixed (crossbreeding of Ukrainian and Uzbek populations)	4.01	3.45	3.71	Dark brown	Females have excellent flying activity, actively move and react directionally to larva's	Pronounced selectivity of a suitable substrate, lay in groups of 3-4 eggs
LSD	1.13	0.91	0.73	-	-	-

colonies of *H. hebetor* lasted from 8 to 9 days. In return Uzbek population was characterized by a somewhat prolonged development of individual preimaginal phases, including the process itself lasting 12-13 days. This significant difference between populations is explained by the fact that a foreign population develops at higher temperatures (30 °C and higher) that are characteristic of it, and if this factor is violated, changes in development immediately occur. We found that some parts of the Uzbek colony of the parasite had not adapted to local conditions, resulting in the death of insects. In this case, the average number of regenerated adult individuals was 4.36 individuals from one caterpillar of the *E. kuehniella*, and it was 6.73 specimens in local populations. Disturbance of physiological processes in the population of parasitic wasps of Uzbekistan has led to an imbalance in the sex ratio. The study showed that during the revival of ectoparasites, a significant predominance of males over females was observed. This indicator was 0.42: 1.75, compared with the Ukrainian population 1:1, which is considered as a serious deviation.

Studies have also been conducted in a mixed population of ectoparasites. The process of outbreeding of geographically distant populations improved the adaptive properties of *H. hebetor* to some extent. We found that the population was characterized by excellent physiological parameters. At the same time, the fecundity was 96.55 eggs per female, the female lifespan averaged 14.46 days, and the ontogenesis of the preimaginal stages lasted 7-8 days.

A comprehensive analysis of morphological and ethological features revealed a number of significant differences between populations. In particular, it was found that the local population has no deviations in the size of imago and cocoon. At the same time, females on average had a length of 3.42 mm, males 3.18 mm, and cocoons 4.02 mm, respectively (Table 2). The linear sizes of the Uzbek population had a significant difference from local populations, which make up the length of females 2.44 mm, males 2.25 mm, cocoons 3.04 mm. Again, we draw attention to the fact that the linear dimensions of the mixed colony during outbreeding increased.

It is known that the color and size of insects depend on a number of factors. But the main ones are such as abiotic and biotic factors, as well as from the diet of insects (Sorokina, 1993). Morphological analysis of *H. hebetor* populations showed that they are characterized by peculiar changes in the geographical variability of the colonies.

First of all, the observations showed that the characteristic difference between the populations is the color of ectoparasites imago, in particular, in Figure 1 the Uzbek entomophage was noted to be

light brown with an orange tinge in Figure 1, the local appearance of ectoparasites was characterized by a dark brown color (Figure 1).

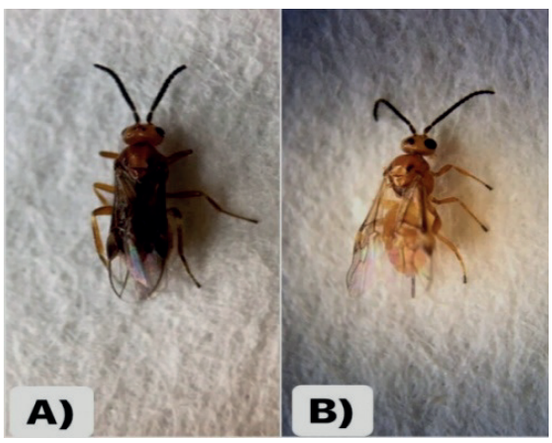

Figure 1. Imago of ectoparasites *H. hebetor*: A) imago from the Ukrainian ecological niche; B) imago with Uzbek ecological niche

Significant differences between populations are manifested by ethological characteristics. In particular, they manifested themselves in physical activity, the search ability of the entomophage, as well as in the process of paralysis of prey and fertility of female parasitic wasps (Table 2). Our visual observations recorded a pronounced and high locomotor ability of the local *H. hebetor* populations, at the same time, the females actively and directedly responded to the host insect larva's. First, a female parasitic wasp monitored physiologically healthy larvas with pulsating movements of the antennae. Then the females paralyzed the prey, fed on the hemolymph of the larva's, and proceeded to oviposition.

Our research on the ethological characteristics of a foreign population of parasitic wasps showed that their motor activity was moderate and dull, responds to larvas after prolonged monitoring. In some cases, the ectoparasite did not attack the larvae, and they remained viable.

A series of laboratory studies found that the active selectivity of local populations of *H. hebetor* larva's in its size and physiological state is a sign of such a phenomenon as care for offspring. In particular, the egg-laying (Figure 2) in this variant was characterized by compact groups and single between the segments of the larvas, in this way the female fixes the egg and protects it from the influence of negative external factors. Regarding the females of the Uzbek population, they lay many eggs on each paralyzed caterpillar and are compact. It was also observed that a large number of ectoparasite larvae develop simultaneously on one caterpillar, sometimes significantly more, which results in the larvae lacking a nutrient substrate for further development. It was established that such a population does not show sufficient care for its offspring, limiting itself to caterpillar paralysis and laying an erratic number of eggs.

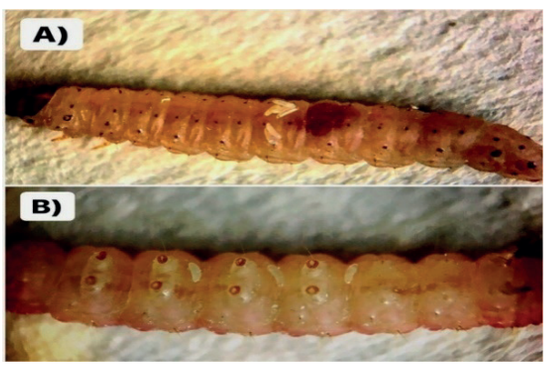

Figure 2. The character of the oviposition of female *H. hebetor*: A) the population of Uzbekistan; B) the population of Ukraine

4. Conclusions

As a result of studying various remote populations of ectoparasites *H. hebetor*, it can be concluded that in the process of adapting to specific conditions, they are ecologically and geographically variable, that is, they underwent a number of certain changes: physiological, morphological, and ethological characters**.** It has been established that the local population is ecologically plastic by high adaptive characteristics. Therefore, given the geographical variability, the use of ectoparasites in crop protection technologies should be accompanied exclusively by the native population *H. hebetor*.

As for the mixed population, pronounced adaptive properties of ectoparasites are observed. Parasitic wasps were characterized by high quality, the ability to search, and paralyze larva's in a wide range of temperatures and humidity in natural conditions. This implies the real need for techniques that prevent the loss of biologically valuable traits in the mass cultivation of *H. hebetor*. As the accumulated scientific and practical experience shows, the ectoparasite breeding technology provides for the outbreeding of geographically distant populations of parasitic wasps.

Our studies have shown that in addition to standard techniques, in particular, a saturation of laboratory cultures with natural populations of ectoparasites, it is quite advisable to maintain an appropriate level of heterogeneity of laboratory cultures by mating them with a Central Asian culture.

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