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Scarce Large Blue (*Maculinea teleius*) hemolymph studies in different populations of Lithuania

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Scarce Large Blue hemolymph studies were conducted in different model populations of Lithuania. The analysis of the hemoplymph of caterpillars, pupas and adults revealed hemolymph changes during the life cycle of the Scarce Large Blue. Proleucocytes were only found in the hemolymph of caterpillars, with no such type hemocytes in the hemolymph of adults. The amount of macronucleocytes also deceased in the hemolymph of Scarce Large Blues as insects grew. Based on hemograms of adult Scarce Large Blues collected in different areas we can conclude that their physiological state is good and the percentage ratio of different types of hemocytes changes insignificantly in all study areas.

Key words: hemolymph**,** *Maculinea teleius,* hemograms, hemocytes

INTRODUCTION

Biotic and abiotic environmental factors have a decisive impact upon population numbers of insects. It is under their influence solely that insect fecundity, mortality and duration of their ontogenesis change. In determining the functional regularities of insect populations, research into their physiological state, which reveals the dynamics of internal processes within populations, is highly significant. Hemolymph is one of the key indices of the insect's physiological state. Hemolymph, as a very labile tissue taking part in different live processes, most promptly responds to any stimuli. Even the slightest metabolic irregularity is initially reflected in insect hemolymph. Therefore, hemolymph as a key index of the physiological state is of paramount importance in studying the dynamics of insect population numbers (Sagdi, 1991; Bartninkaitė, Tekoriutė, 1994; Bartninkaitė, 1998). The percentage ratio of different type form elements of hemolymph allows a precise diagnosing of the insect's physiological state which provides information about the abundance and viability of the population (Jonaitis et al., 2000).

The definition of an insect's physiological state is not only a basis for a precise forecasting of the insect population numbers, but also could be an important index of environmental pollution (Solodovnikova et al., 1990; Chkhoidze, 1991). Insect hemolymph studies can also be used in insect taxonomy because each type of species has an inherent species-specific percentage ratio and type-specific form and size (Sagdi, 1991; Bartninkaitė, 1995).

The physiological state of an insect depends not only upon the habitat, but upon the time of insect development as well (Bartninkaitė, 1998, 2002), which can be evidenced by hemolymph studies.

All this shows how versatile the importance of insect hemolymph is and why hemolymph studies are both of practical and theoretical significance.

The purpose of the present work was to study the hemolymph of moths of the *Maculinea teleius* species during their ontogenesis and determine their physiological state in different areas of the country to provide a basis for determining the viability of the population and forecasting the status of this protected moth species in Lithuania.

MATERIALS AND METHODS

Scarce Large Blue hemolymph smears were prepared from caterpillars and adults collected in four places of the country in 2005 by the accepted cytological methods. The selected sites were meadows of Joniškis district near the Mūša (56°07'27.6", 23°29'16.1"), Priekulė of Klaipėda district (55°31'40.2", 21°22'47.7"), Antvardė of Jurbarkas district (55°08'51", 22°43'07") and Saviečiai of Kėdainiai district (55°09'02.9", 23°59'00.7"). Hemolymph smears were fixed with methyl alcohol, dyed using the Giemza–Romanovski method and examined under an MBI-1 microscope. The classification of hemocytes, their percentage ratio as well as the amount of mitotic divisions of proleucocytes and macronucleocytes were estimated using the conventional cytological methods, and the data obtained were treated using the methods of variation statistics (Pristavko, 1971; Lakin, 1980) and STATISTICA 6 software package.

Hemolymph smears from different size caterpillars and adults were prepared for estimation of the dynamics of the hemolymph structure during the ontogenesis of the Scarce Large Blue. The physiological state of the Scarce Large Blue in separate areas was estimated according to their hemograms (Bartninkaitė, 2002; Russo J. et al, 2001).

RESULTS AND DISCUSSION

We have detected five types of hemocytes in the hemolymph of the Scarce Large Blue during its ontogenesis. The percentage ratio of hemocytes was found to depend upon the insect's development stage. We found all five types of form elements – proleucocytes, macronucleocytes, micronucleocytes, enocytoides and phagocytes – in the hemolymph of caterpillars and only three types of hemocytes – macronucleocytes, micronucleocytes and phagocytes – in the hemolymph of adults.

Proleucocytes are the smallest elements in the hemolymph of the Scarce Large Blue. A proleucocyte is an almost round cell (2.6–7.8 µk) with a large round nucleus (1.1–6.5 µk) surrounded by a thin cytoplasm layer (1–1.5 µk). Proleucocytes are elementary cells from which other hemocytes develop. They can divide by mitosis.

Macronucleocytes in the hemolymph of Scarce Large Blue caterpillars are large (8.6–15.9 µk) round cells with a large nucleus (5.2–12.0 µk). The cytoplasm layer encircling the nucleus is considerably wider than that in proleucocytes. One to three vacuoles can often be seen in the cytoplasm of macronucleocytes. Macronucleocytes are young and little differential cells. Some of them become mature in the course of ontogenesis and later perform the function of phagocytosis, whereas others differentiate to other types of hemocytes. Macronucleocytes can also divide by mitosis. They were found during their mitotic division in the hemolymph of Scarce Large Blue caterpillars.

As both proleucocytes and macronucleocytes are young and little differentiated cells, we have examined how their amounts change in the hemolymph of caterpillars as they grow and how the frequency of mitoses changes. In the course of the development of caterpillars, the number of proleucocytes in their hemolymph decreases on average from 15.3% in size III caterpillars to 10.4% in size V caterpillars (Table 1). Such difference in the number of proleucocytes in the hemolymph of different size caterpillars is statistically reliable. As in the course of development of caterpillars the number of primary cells considerably decreases, the number of their mitoses comes down, too. The average number of mitoses accounts for 21.6% of the total number of proleucocytes in the hemolymph of size III caterpillars and for merely 13.6% in the homolymph of size V caterpillars. Such difference is statistically reliable. The results show that when the body of insects ages, the process of mitotic division of cells in their hemolymph becomes slower.

The number of macronucleocytes in the hemolymph also decreases as caterpillars grow. They average 40.7% in the hemolymph of caterpillars of size II and 38.4% of size V (Table 1). The number of mitoses in macronucleocytes in the hemolymph of caterpillars is much lower than that in proleucocytes. Depending upon the caterpillar size, the number of mitoses in proleucocytes averages from 21.6 to 13.6%; in macronucleocytes, merely from 5.9 to 5.2%. Though the decrease in the amount of macronucleocytes in the hemolymph of caterpillars as they grow is not statistically reliable, the decrease in the number of their mitoses is reliable.

Investigations of the hemolymph of different size caterpillars of the Scarce Large Blue have demonstrated that as the body of an insect is ageing, not only the amount of young and little differentiated hemocytes in its hemolymph decreases but also the mitotic activity of such hemocytes is slowing down. Such process in the organism of an insect undoubtedly influences its life duration.

Micronucleocytes are small (4.7–10.9 µk) nearly round cells with a rather small round nucleus (1.8–3.6 µk) in the hemolymph of Scarce Large Blue caterpillars. In the cytoplasm of micronucleocytes, several vacuoles and various dark dying insertions can be noticed, which undoubtedly are nutrients carried by micronucleocytes as mature trophic cells around the organism of the insect. Numerous authors indicate that micronucleocytes perform the function of nutrient carriers in the organism and accumulate nutrients used

Table 1. **Dynamics of proleucocytes and macronucleocytes and their mitoses in the hemolymph of different size Scarce Large Blue caterpillars**

Caterpillar instar	Proleucocytes			Macronucleocytes		
	Amount of hemocytes %	Number of mitoses	Number of mitoses, %	Amount of hemocytes %	Number of mitoses	Number of mitoses %
III	15.3 ± 0.88	3.3 ± 0.34	21.6 ± 1.02	40.7 ± 1.20	3.7 ± 0.67	8.94 ± 1.34
V	10.4 ± 1.03	1.4 ± 0.24	13.6 ± 2.28	38.4 ± 0.51	2.0 ± 0.32	5.20 ± 0.82
t	3.24	4.63		2.07	2.65	
p value	<0.05	<0.01		>0.05	<0.05	

during insect metamorphosis (Sagdi, 1991; Bartninkaitė, 1995).

Micronucleocytes differentiate from proleucocytes and macronucleocytes. They do not divide by mitosis as they are mature trophic cells.

Enocytoides are among the largest cells (13.5–20.3 µk) in the hemolymph of Scarce Large Blue caterpillars. They are nearly round in their shape with a small (3.1–7.8 µk) round nucleus in the middle. Enocytoides are fully mature cells with no mitoses observed. Enocytoides perform the functions of elimination and secretion in the organism (Sirotina, Chernaya, 1965).

Phagocytes differ greatly in their shape from all other hemocytes found in the hemolymph of the Scarce Large Blue. They are spindle-shaped cells 18.2–26.5 µk long and 7.5–10.7 µk wide. The central part of phagocytes contains a small oval nucleus (5.2–8.6 µk long and 2.6–5.2 µk wide). It is noteworthy that phagocytes have no such long sprouts in the hemolymph of Scarce Large Blue caterpillars compared with those found in the hemolymph of most caterpillars of the order Lepidoptera, which sometimes reach 60 µk and more in length (Miseliūnienė, Murauskaitė, 1981; Bartninkaitė, 1995). Most probably shorter phagocytes of Scarce Large Blue caterpillars result from their specific way of life. Phagocytosis is the main function of phagocytes. They play a very important role in the organism of an insect because they eliminate dead cells from the hemolymph and other tissues.

The hemolymph of the Scarce Large Blue has been found to contain a different number of hemocyte types depending upon the stage of insect development. The number of each hemocyte type in the organism of an insect is closely related with the functional specialization of each particular hemocyte.

Proleucocytes can be found only in the hemolymph of caterpillars (Fig. 1). Proleucocytes were not detected in the hemolymph of adults. Proleucocytes accounted for 14–17% in the hemolymph of smaller caterpillars, and for mere 8–14% in the hemolymph of larger ones, which could be explained by the transformation of proleucocytes into other types of hemocytes and a gradual slowing down of the formation of new proleucocytes up to a complete termination of their formation process in the course of development of the insect.

The amount of macronucleocytes in the hemolymph of the Scarce Large Blue is also decreasing as the insect grows – from 40.7–38.4% in the hemolymph of caterpillars to 2.5–3.7% in the hemolymph of adults (Figs 1 and 2). As macronucleocytes are little differentiated cells, such a rapid decrease in their numbers during insect ontogenesis shows that macronucleocyte differentiation into other type hemocytes and their maturing become more intensive in the organism of an insect as it grows. Mature macronucleocytes perform the function of phagocytosis in the organism of an insect therefore, their abundance in the caterpillar stage shows that macronucleocytes carry the greatest burden of protective function in this particular stage wherefore their

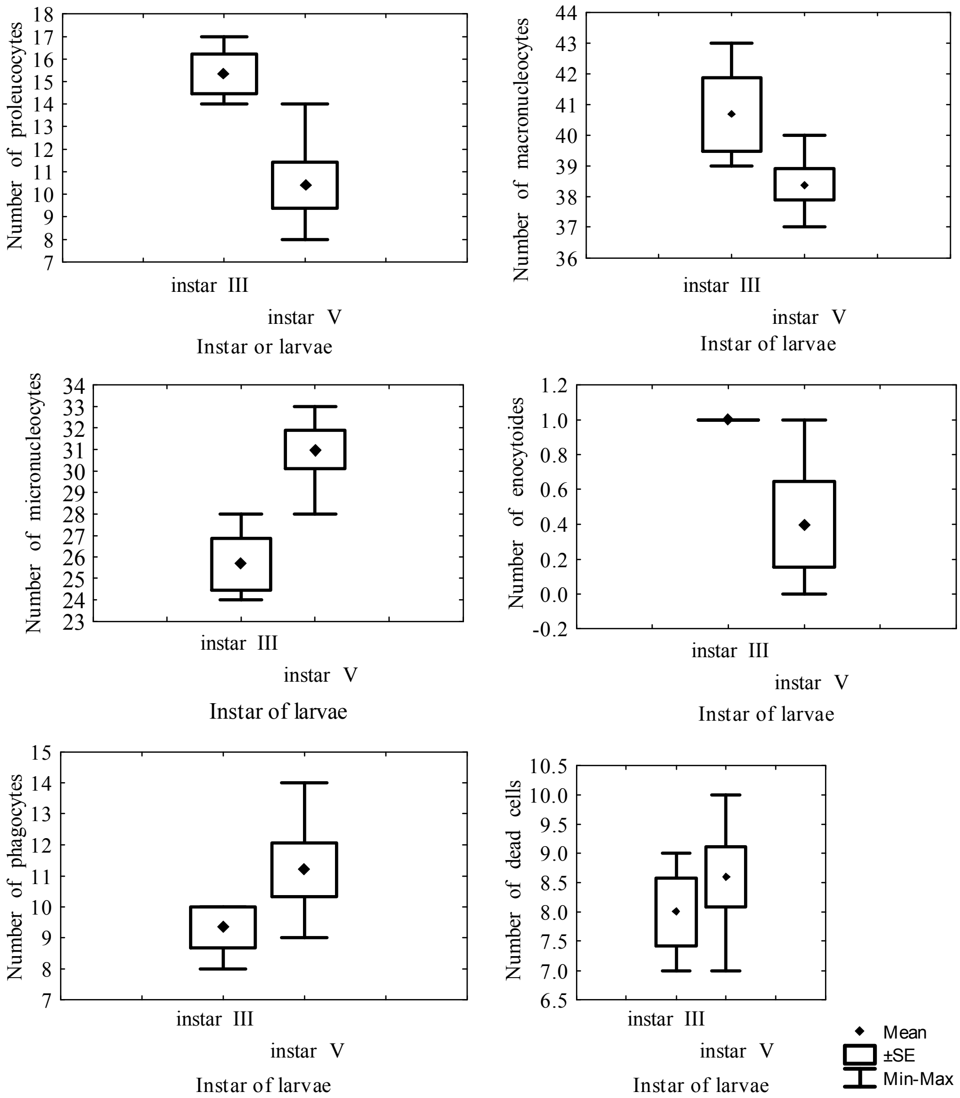

Fig. 1. Scarce Large Blue hemograms during the development of larvae

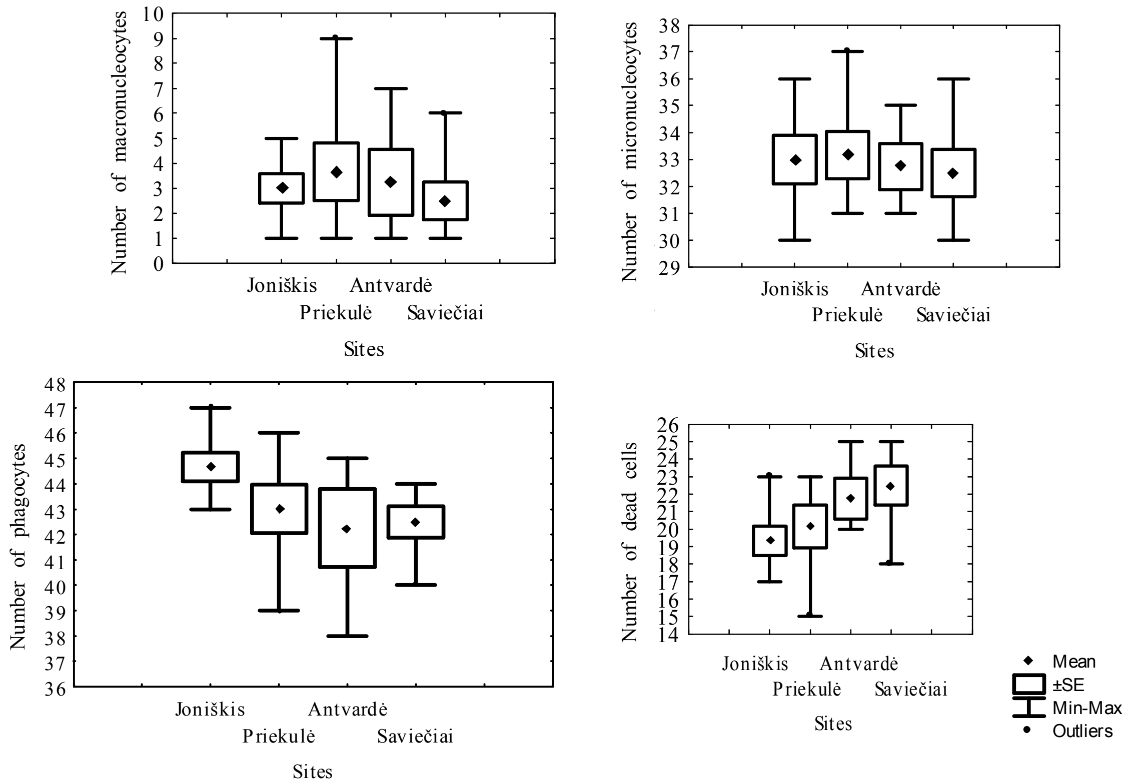

Fig. 2. Scarge Large Blue hemograms in various sites

mortality increases and consequently their numbers considerably decrease.

In the hemolymph of Scarce Large Blue caterpillars, the number of micronucleocytes reaches 24–32 and in adults 30–37 (Figs. 1 and 2).

We have found very small numbers of enocytoides in the hemolymph of caterpillars (Fig. 1) and have not found them in the hemolymph of adult Scarce Large Blues. Most authors reported analogous results as to the hemolymph of different representatives of the order Lepidoptera.

The amount of phagocytes during ontogenesis of the Scarce Large Blue increases from 9.3–11.2% in the hemolymph of caterpillars up to 42.2–44.7% in the hemolymph of adults (Figs. 1 and 2). It could be explained by a higher intensity of caterpillar feeding (as a consequence of which the amounts of different substances of alien origin getting into hemolymph become greater) and by a greater mortality of cells both in the hemolymph and neighboring tissues (particularly in pupa and adult stages). Therefore, great numbers of phagocytes appear in the hemolymph whose function is to eliminate dead cells and harmful substances from the organism, so the number of phagocytes as cells performing the protective function in the organism of an insect is indicative of the physiological state of the insect.

The number of dead cells in the hemolymph is also an important index in determining the physiological state of an insect. The smaller the number of dead cells in the hemolymph, the better is the physiological state of the insect, because it shows that hemocytes performing the protective function in the organism of the insect function actively and are able to eliminate different dead cells from the hemolymph. The number of dead cells in the hemolymph of the Scarce Large Blue increases from 8.0–8.6% in the caterpillar stage (Fig. 1) to 19.3–22.5% in the adult stage (Fig. 2).

A comparison of Scarce Large Blue hemograms with the hemograms of the other Lepidoptera insects shows that the physiological state of the Scarce Large Blue is rather good both in caterpillars and adults (Bauer et al., 1998, Jonaitis et al., 2000; Yamashita, Miyuki et al., 2001; Bartninkaitė, 2002, 2003). High numbers of hemocytes (macronucleocytes and phagocytes) specialized for the protective function in the hemolymph of Scarce Large Blue caterpillars are implied by a small number of dead cells – 8.0–8.6% on average (Figs. 1 and 2), which evidences that Scarce Large Blue caterpillars are of good physiological state and will succeed in their development. Besides, the considerably greater number of macronucleocytes compared with phagocytes allows assuming that macronucleocytes perform the main protective function in the caterpillar stage, and as the insect body is ageing, i.e. in the adult stage, the main protective function goes over to phagocytes, because their numbers in the hemolymph are tenfold the number of makronucleocytes at that time.

From the hemograms of Scarce Large Blue adults collected in different places (Fig. 2) we can conclude that their physiological state is good and similar in all study areas and the percentage ratio of different type hemocytes changes insignificantly. A statistically significant difference was detected only between the number of Enocytoides ($t = 2.6$, p value = 0.026) and dead cells ($t = 2.26$, p value 0.046) of imagos from Joniškis and Saviečiai. A greater amount of dead cells was found in the hemolymph of Scarce Large Blues collected in Antvartė and Saviečiai, which could be due to a later collection in the mentioned areas (7 days later than in Joniškis). High numbers of hemocytes fulfilling the protective and feeding functions show that Scarce Large Blue adults' fecundity and viability will ensure the normal functioning of the population provided the feeding basis is suitable and the meteorological state is favorable.

CONCLUSIONS

1. Five types of form elements – proleucocytes, macronucleocytes, micronucleocytes, enocytoides and phagocytes – have been identified in the hemolymph of caterpillars, and only three types – macronucleocytes, micronucleocytes and phagocytes – in the hemolymph of adult Scarce Large Blues.

2. Different percentage ratio of different type hemocytes in the hemolymph of the Scarce Large Blue depends upon the stage of development. In the course of development, the numbers of proleucocytes in the hemolymph decreases from 15.3 to 10.4% on average, and they entirely disappear in the hemolymph of adult insects. The amount of macronucleocytes decreases from 40.7–38.4% in the hemolymph of different size caterpillars to 2.5–3.7% in the hemolymph of adults. The number of micronucleocytes increases from 24–32 in the hemolymph of caterpillars to 30–37 in the hemolymph of adults, and the amount of phagocytes increases from 7–10 to 38–47, respectively.

3. Proleucocytes and macronucleocytes have been found to divide by mitosis in the hemolymph of caterpillars; as caterpillars grow, mitosis is slowing down from 21.6 to 13.6% for proleucocytes and from 8.9 to 5.2% for macronucleocytes.

4. Based on Scarce Large Blue hemograms we can conclude that the physiological state of both caterpillars and adults in different areas of the country is good and we expect a good fecundity and viability of the Scarce Large Blue in this population.

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KRAUJALAKINIO MELSVIO (*MACULINEA TELEIUS*) HEMOLIMFOS TYRIMAS SKIRTINGOSE LIETUVOS POPULIACIJOSE

S a n t r a u k a

Atlikti kraujalakinio melsvio hemolimfos tyrimai skirtingose Lietuvos modelinėse populiacijose. Atlikta lervų, lėliukių ir suaugėlių hemolimfos analizė, kuri atskleidė hemolimfos pokyčius vystantis kraujalakiniam melsviui. Melsvio hemolimfoje proleukocitai buvo aptikti tik vikšrų stadijoje, o suaugėlių hemolimfoje jų nebuvo. Makronukleocitų kiekis melsvio hemolimfoje jam vystantis taip pat mažėja. Pagal melsvio suaugėlių, surinktų įvairiose vietose, hemogramas, visose tirtose vietose jo fiziologinė būklė yra gera, o skirtingų tipų hemocitų procentinis santykis kinta nežymiai.

Raktažodžiai: hemolimfa, *Maculinea teleius*, hemogramos, hemocitai