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Seasonal Phenotypic Plasticity of the Polyvoltine White Butterfly *Pieris napi* L. (Lepidoptera: Pieridae) in the Southern Urals

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Abstract—Seasonal variations in the body weight and the forewing size of imagoes have been studied based on the example of different seasonal generations of the white butterfly *Pieris napi* L. in the Southern Urals. It has been found that the body weight of imagoes during the flight of one generation decreases in the process of reproduction. In this case, reserve substances in the abdomen are spent most intensively during the life of an insect: as the flight activity of a seasonal generation begins, the mean relative abdominal weight of imagoes is always higher than at the end of its flight. The wings of imagoes in the spring generation are the smallest. Those in the summer generations are the largest. Thus, these generations are capable of long and energy-consuming flights. Therefore, seasonal generations of *P. napi* are distinguished based on morphophysiological features associated with their fertility and adaptation of imagoes to settle in new habitats.

Keywords: phenotypic plasticity, *Pieris napi*, seasonal variation, fertility, body size

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In recent years, the role of phenotypic plasticity in the process of evolution has been widely discussed in the literature (West-Eberhard, 2003; Pigliucci et al., 2006). Phenotypic plasticity is defined as phenotypic expression of a single genotype across a range of environments in the context of its reaction norm (Shmal'gauzen, 1983). Seasonal morphological variation in animals that have several generations per year is a convenient model to study the phenomenon of phenotypic plasticity. Thus, seasonal generations of many insect species may differ in color, body size, lifecycle, behavior, and fertility (Shapiro, 1976; Fric and Konvicka, 2002; Karlsson and Johansson, 2008).

In lepidopterans, as in many other groups of insects, the fertility of females directly depends on their body size. As a rule, larger female lepidopterans have a bigger abdomen, where they accumulate nutrient reserves to produce eggs (Karlsson and Wickman, 1990). The chemical composition and nutritional value of food consumed by females also have a significant influence on their fertility. One limiting factor is nitrogen reserves derived by larvae, because the eggs and spermatophore of lepidopterans are rich in protein (Marshall, 1982; Karlsson and Wickman, 1990; Boggs, 1992). Lepidopteran larvae feed on green parts of plants that contain large amounts of protein and carbohydrates, while imagoes of most species prefer nectar, which is free of amino acids. The feeding type of imagoes does not have any significant effect on further fertility. The only exception to this rule is heliconians (Lepidoptera: Heliconidae) (Stjernholm and Karlsson, 2000).

Male lepidopterans can also influence the fertility of females. In species with monogamous females, a male transfers a relatively small spermatophore bag during mating. If females are capable of mating several times over their lifespan (polyandry), this spermatophore bag is, as a rule, large (up to 15% of the male's total body weight) and rich in nutrient substances, including amino acids (Stjernholm and Karlsson, 2000; Bergstrom et al., 2002). Nutrient substances of the spermatophore also enter the eggs (Wilklund et al., 1993).

Owing to the phenomenon of phenotypic plasticity, imagoes from different seasonal generations of lepidopterans have unequal fertility. This is influenced by the distribution of reserves that are accumulated at the stage of larval development between the body segments of imagoes in the process of ontogenesis. The spring generation of the polyvoltine white butterfly *Pieris napi* L. (Lepidoptera: Pieridae) in the territory of Europe is distinguished from the summer generation by a larger abdomen, as well as smaller forewings and thorax (Fric et al., 2006; Karlsson and Johansson, 2008).

In the green-veined white *P. napi*, two or more generations, depending on climatic conditions, are recorded during the summer season (Korshunov, 2000; Gorbunov, 2001). The first (diapause) and second (non-diapause) generations are easily distinguishable by their wing patterns: spring specimens show greater wing melanism than those that emerge in the summer. In the Southern Urals, the time of flight of imagoes in these generations does not coincide (Shkurikhin et al., 2011). Nevertheless, the second and third non-diapause generations have only slight morphological differences: it is known from the literature that specimens in the autumn are smaller than those in the summer. Since the time of flight in such imagoes coincides, it is difficult to refer them to any particular summer–autumn generation.

Both male and female imagoes of lepidopterans spend nutrient reserves in their abdomen for reproduction (Stjernholm and Karlsson, 2000; Stjernholm et al., 2005). Polyandric species may undergo histolysis of flight muscles in their thorax by the end of their lifspan; thus, amino acids that are released during this process are also used to produce fertility products (Stjernholm and Karlsson, 2008). Therefore, a reproducing specimen continuously spends its weight accumulated at the larval stage of its development. The absolute and relative body weights of various segments in fresh imagoes are different from those in specimens that have already taken part in reproduction. For this reason, these parameters may be used as morphophysiological indicators. The morphophysiological method (Shvarts et al., 1968) was developed for vertebrate animals, but its principles are quite universal. We made an attempt to apply this method on insects by using seasonal generations of the dominant polyvoltine lepidopteran species *P. napi* as a model. It was assumed that the mean relative abdominal weight of specimens during the mass flight of one generation would be higher than that in imagoes at the end of the flight of the given generation.

The aim of this study was to estimate the phenotypic plasticity of seasonal generations of *P. napi* as a model species and to use its morphophysiological features to determine the number of non-diapause generations and the time of mass flight in imagoes.

MATERIALS AND METHODS

The material was sampled during the field seasons of 2010 and 2011 near the village of Metlino (Chelyabinsk oblast, Kaslinskii district). To estimate the abundance dynamics of *P. napi* imagoes, route censuses were performed (Yamamoto, 1975). The route was about 4 km in length and 20 m in height. Half of it runs through the village. Another part is located at the outskirts, along the margin of a birch forest and a country road along the field. In 2010, 313 males and 230 females were caught. In 2011, 390 males and 330 females were caught.

After the route census (on the same day, under the conditions of a field station), three parameters were measured in the captured imagoes of *P. napi*: body weight with wings, thorax weight, and abdomen weight. For this purpose, an electronic balance (Kern 440-21N, accuracy 1 mg) was used. Every weighing was performed in two replicates. The obtained data were averaged. Electronic images of wings were taken using a Canon Eos 450D digital camera. The wing area was calculated in the tpsUtil 1.40 program (Rohlf, 2008). In advance, marks were set on the wing images, where veins cross the wing edge. Only left forewings were used in this work.

The data were statistically processed using the PAST 2.17 (Hammer et al., 2001) and Statistica 8.0 (StatSoft., Inc.) programs.

RESULTS AND DISCUSSION

The dynamics of changes in the absolute and relative thorax and abdomen weights of *P. napi* imagoes during the flight of a generation was estimated using regression analysis. The spring generation of 2011 was used as a model. The flight times of the studied generation were determined on the basis of route censuses. Observations began on the first day when imagoes of this species were caught. The statistically significant regression dependence of the abdominal weight in imagoes on the day of capture was recorded in both males and females: the abdominal weight decreases during the flight of the first generation (Fig. 1a). The regression equation takes the following form: $y = -0.144x + 19.615$ ($R^2 = 0.36$, $p < 0.01$) in males, $y = -0.574x + 37.031$ ($R^2 = 0.78$, $p < 0.01$) in females. It is seen from the figure that females lose their abdominal weight much faster than males. In the middle of May, immediately after the mass flight of imagoes of the first generation, the abdominal weight of females was almost twice that of males. By the end of the flight, these values were almost equal. Therefore, females spend much more of the reserve substances that accumulate in the abdomen to produce and lay eggs.

It was demonstrated under experimental conditions (Stjernholm and Karlsson, 2008) that *P. napi* imagoes begin to use fertility products of amino acids to produce eggs by the end of their lifespan. These products come from their flight muscles. In this context, we estimated the dynamics of the thorax weight in *P. napi* males and females from the spring generation of 2011 (Fig. 1b). The regression dependence was statistically significant only in females: $y = -0.148x + 17.506$ ($R^2 = 0.64$, $p < 0.01$). The thorax weight of males during the flight of the first generation varies insignificantly. In contrast to females, males spend more time flying, because they search for virgin females. The flight activity of females is significantly lower. They spend most of their time in the herb layer and fly only short distances. The histolysis of flight muscles in their thorax is more active than that of males, because the ability for long and rapid flights is more important for males.

Figure 1 shows the dynamics of changes in the relative abdominal weight of imagoes of *P. napi* males and females during the flight of the spring generation. It was revealed that they both underwent a statistically significant decrease in the mean relative abdominal weight of the population by the end of flight of the first generation. The regression equation takes the follow-

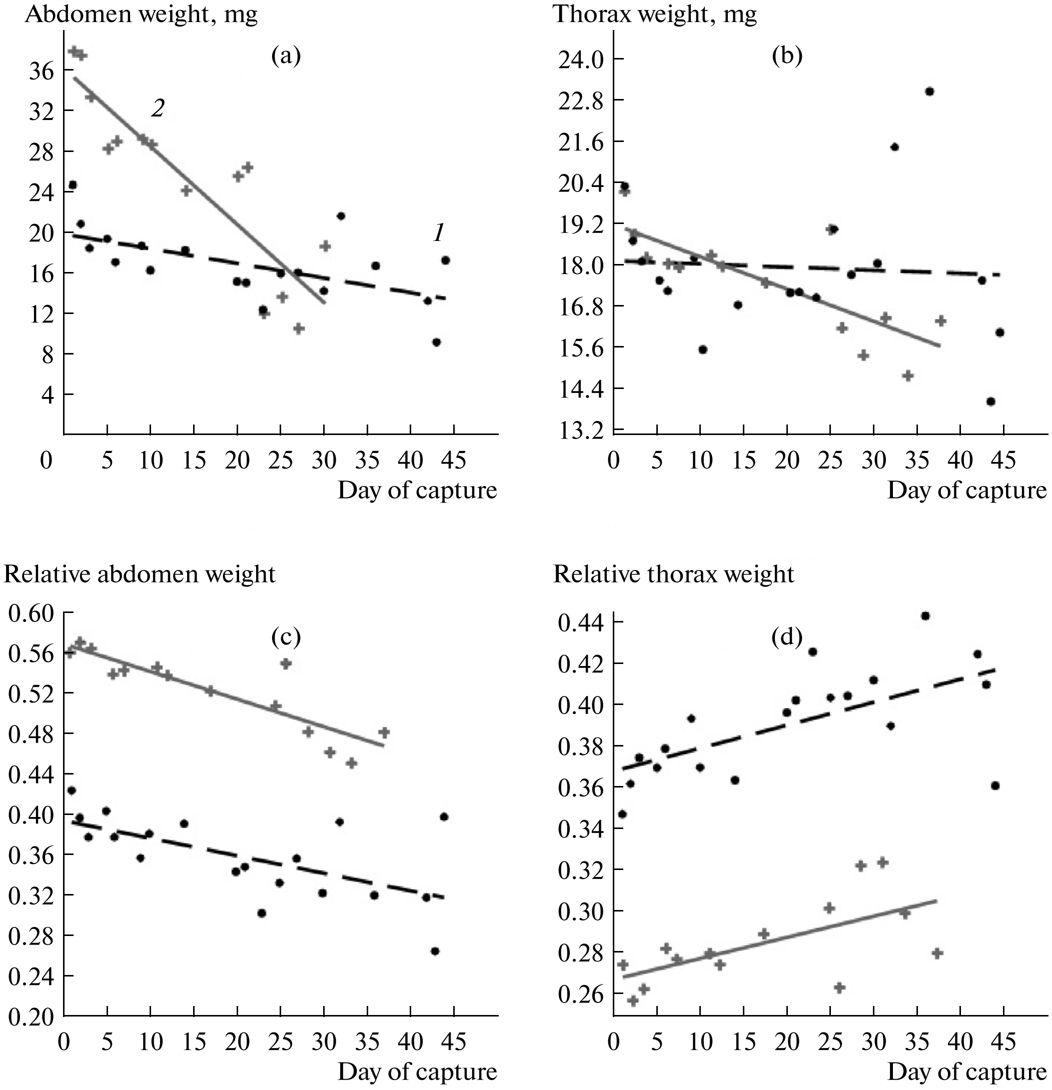

Fig. 1. The dynamics of the absolute and relative abdominal weight (a, c), as well as of the absolute and relative thorax weight (b, d) in male (*1*) and female (*2*) imagoes of *P. napi* during the flight of the summer generation in 2011.

ing form: $y = -0.003x + 0.568$ ($R^2 = 0.77, p < 0.01$) in females, $y = -0.002x + 0.393$ ($R^2 = 0.38$, $p < 0.01$). The relative abdominal weight in imagoes of both sexes during the flight of a generation decreases at the same rate, while, based on the absolute body weight, females spend reserve substances in their abdomen more intensively than males. During their lifespan, the body proportions of females differ from those of males: at any time of the flight of the studied generation, the relative abdomen size of females is always higher than that of males.

Changes in the relative thorax weight of *P. napi* imagoes of both sexes during the flight of the first generation were estimated using regression analysis (Fig. 1d). The regression dependence is statistically significant in the case of both females and males: $y = 0.001x + 0.267$ ($R^2 = 0.39$, $p < 0.01$) and $y = 0.001x + 0.371$ ($R^2 = 0.39$, $p < 0.01$), respectively. It is seen from the figure that the relative thorax weight during the flight of the spring generation becomes slightly higher in imagoes of both sexes. The histolysis of flight muscles in imagoes lowers the speed and maneuverability of flight, as well as its length. Therefore, they should be less able to escape attacks of predators in the air, as well as not being as active when searching for mating partners and nectar. Nevertheless, reserve substances in the abdomen are more intensively spent for reproduction by imagoes than that via the histolysis of flight muscles that takes place. Thus, the relative thorax weight in breeding specimens does not decrease and even becomes slightly higher. The initial elevating capacity of thorax muscles in imagoes becomes excessive by the end of their lifespan; thus, the excess protein is redirected to produce fertility products.

Based on the results of regression analysis, the wing area of *P. napi* males and females does not change over

The results of a two-factor multivariate analysis of variance of seasonal variations in the weight of the body and its segments (the thorax and abdomen) of *P. napi* imagoes in 2011

Factor	λ Wilks	*F*	Degrees of freedom of the factor	Significant level (*p*)
“Sex”	0.305	520	3	$p < 0.001$
“Period”	0.442	43.5	15	$p < 0.001$
“sex” × “period”	0.956	2.1	15	$p = 0.009$

the entire flight of its spring generation. After the flight of imagoes, the wing of an insect does not grow or stretch. During the entire flight period, the mean size of imagoes that emerged from pupae remained almost the same. Therefore, the revealed decrease in the weight of the body segments is explained by the process of reproduction rather than by the flight of smaller specimens by the end of the flight season of the given generation.

The seasonal dynamics of absolute and relative parameters of the body weight in imagoes, as well as the size of wings, were analyzed based on the summer seasons of 2010–2011. According to the abundance dynamics of imagoes, the entire flight period of this species was divided into six periods. The length of each period was two decades, which corresponds to the maximal lifespan of imagoes under laboratory conditions (Stjernholm and Karlsson, 2008). During the first period (the two last decades of May), the mass flight of imagoes of the spring generation occurs. During the second period, the flight of this generation ends. In 2010, *P. napi* imagoes of the spring generation finished their flight by the second decade of June. In 2010, they flew during all of June. Accordingly, the third period (mass flight of imagoes of the summer generation) began at the end of June in 2010 and at the beginning of July in 2011. During the fourth period, the second generation ends its flight. During the fifth period, the autumn generation begins its flight, while imagoes of the second generation end their flight. During the sixth period, imagoes of the autumn generation fly.

Seasonal variations in the weight of the body, thorax, and abdomen in imagoes of both sexes were estimated in 2011 using multivariate analysis of variance (MANOVA). During the summer season, the mean weight of body segments in imagoes does not vary in a statistically significant manner in either sex (see table). The significance of the differences between particular periods was estimated using the post-hoc Tukey test.

During the flight of the first and second generations, the body weight of imagoes becomes lower in a statistically significant manner, mostly due to the abdominal weight (Fig. 2). The largest specimens were caught during the mass flight of imagoes of the summer generation (period 3). As a result of the overlap of

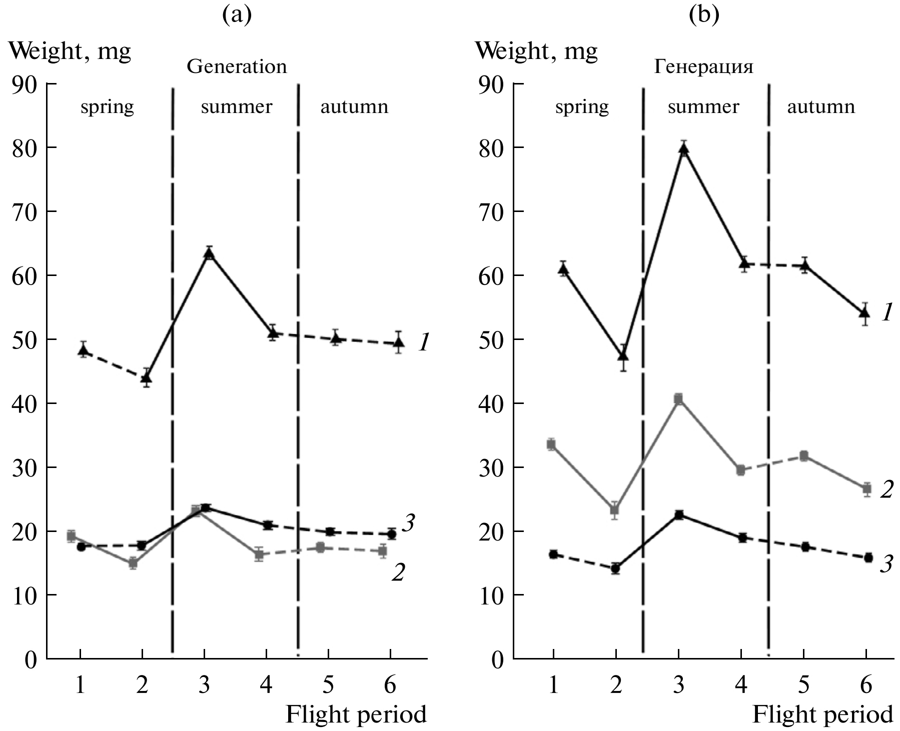

Fig. 2. Seasonal dynamics of the body weight (*1*), abdomen (*2*), and thorax (*3*) in males (a) and females (b) of *P. napi* in 2011. The figure shows the mean values and standard errors. The solid line designates statistically significant variations in the weight from one period to another, the broken line designates insignificant variations.

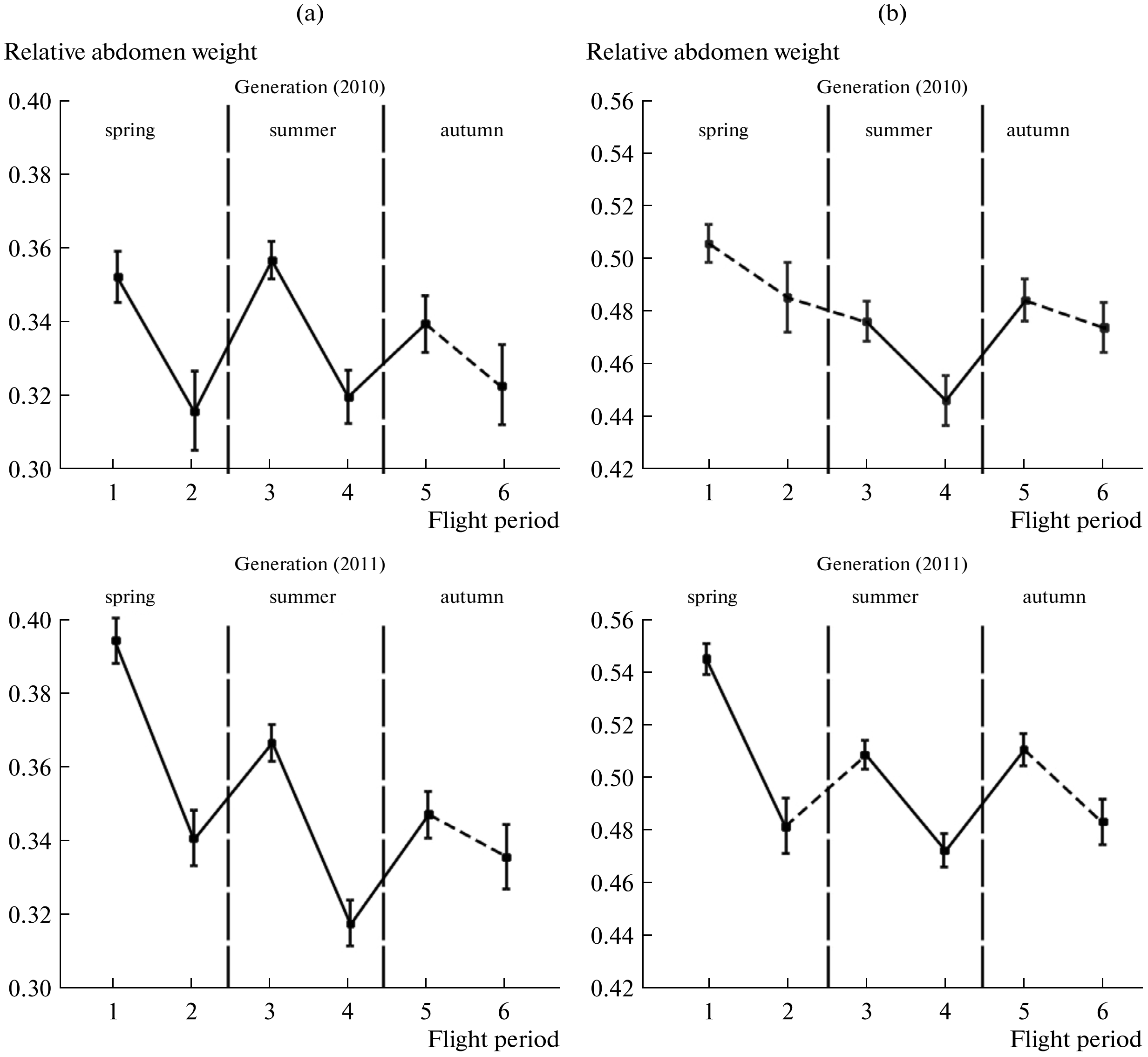

Fig. 3. Seasonal variations in the relative abdominal weight of males (a) and females (b) of *P. napi* in 2010–2011. The figure shows mean values and standard errors. A solid line designates statistically significant variations in the relative abdominal weight from one period to another, a broken line designates insignificant variations.

two flight periods in the summer and autumn generations, it is difficult to determine the date when the flight of one generation ends and that of another generation begins based on the analyzed weight values. If two generations overlap in time, the number of old and light specimens decreases, while that of fresh and heavy imagoes increases. Owing to this, when a change of overlapping generations occurs, the mean weight of the body and its segments is almost the same.

Substances that accumulated in the abdomen during the lifespan of an imago are sent most intensively. Thus, the maximal relative abdominal weight will be in flying specimens that did not take part in the process of reproduction. During the entire summer season, the relative abdominal weight should be maximal at the moment when the flight of imagoes from any of the generations starts. Then, it gradually decreases until the flight of another generation begins. Seasonal variations in the relative abdominal weight of males and females of *P. napi*, which were caught in 2010 and 2011, were estimated using the one-way analysis of variance. In all cases, the analyzed parameter underwent statistically significant variations during the summer. The significance of the differences between individual periods was tested using the post-hoc Tukey test.

Seasonal variations in the relative abdominal weight of *P. napi* males and females in 2010 and 2011 are given in Fig. 3. In all cases, the measured parameter decreased in a statistically significant manner during the flight of summer generation decreased from period 3 to 4. This decrease in the relative abdominal weight is also observed during the flight period of the spring generation, viz., from period 1 to period 2. When the spring generation is replaced by the summer one (from period 2 to period 3), the relative abdominal weight gets significantly higher. The relative abdominal weight is also used to determine the beginning of flight in the autumn generation: the measured parameter gets significantly higher from period 4 to period 5 in all analyzed cases. The analysis of absolute body weight, as well as other phenotypic features, does not show the beginning of flight in the autumn generation.

During the entire summer season, the largest specimens were caught as imagoes of the summer non-diapause generation underwent their mass flight (in the first half of July). This generation develops without a diapause in the first half of summer under optimal temperature conditions. Thus, it is able to accumulate a large biomass in a short period of time. At the end of summer and the beginning of autumn, larvae of the autumn non-diapause generation and those overwintering in the state of diapause that form the spring generation of the next year develop at once. In the late summer, the total of the effective temperatures under the conditions of the Southern Urals is lower than in the first half of the summer. Thus, *P. napi* specimens do not have enough time to achieve a high biomass.

Imagoes of the autumn generation of 2010 and of the spring generation of 2011 are progenitors of the summer generation of 2010. Depending on the day length, the temperature of the environment, and the accumulated weight, each individual specimen may either continue its larval growth and overwinter as a pupa (the spring generation) or develops directly without a diapause (the autumn generation). Along with this, each larva follows one of two possible ways of development, which determines their different phenotypes. Non-diapause imagoes have large wings (194.5 mm^2 in males, 184.5 mm^2 in females) and a relatively low abdomen weight, while diapause specimens, in contrast, are characterized by small wings (152 mm^2 in males and 150 mm^2 in females) and a relatively large abdomen.

The morphological features in the described case are subject to seasonal phenotypic plasticity. They characterize fertility, as well as flight adaptations. Imagoes of the spring generation during the mass flight have the largest relative abdominal weight for the entire analyzed period. Thus, the weight of flight muscles in their thorax is lower. At the same time, imagoes of the spring generation are characterized by the high ratio between their body weight and wing area, which is called the loading on a wing (Dudley and Srygley, 1994; Srygley and Kingsolver, 2000), which equals 0.32 in males and 0.41 in females. In imagoes of the autumn generation, this ratio was 0.25 in males and 0.28 in females. The statistical significance of differences in the loading on a wing between the two generations was estimated using the Student's t-test: the differences turned out to be significant in the cases of both males ($t = 8.09$; $df = 73$; $p < 0.01$) and females ($t = 12.97$; $df = 67$; $p < 0.01$).

According to some authors, the spring generation of *P. napi* is distinguished by high fertility and low distribution (Fric et al., 2006). The high loading on the wing and the low relative weight of wing muscles favor short energy-consuming flights. Based on our observations, the population density of *P. napi* near the village of Metlino increases in the second half of the summer. Along with the population growth, the distribution activity of imagoes also becomes higher (Fric et al., 2006). The summer and autumn imagoes have a lower relative abdominal weight, a higher weight of their thorax muscles, and larger wings. The low loading of the wing and well-developed thorax muscles favor longer and less energy -consuming flights, where imagoes are distributed along a territory.

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REFERENCES

Bergstrom, J., Wiklund, C., and Kaitala, A., Natural variation in female mating frequency in a polyandrous butterfly: Effects of size and age, *Anim. Behav.*, 2002, vol. 64, pp. 49–54.

Boggs, C.L., Resource allocation: Exploring connections between foraging and life history, *Funct. Ecol.*, 1992, vol. 6, pp. 508–518.

Dudley, R. and Srygley, R.B., Flight physiology of neotropical butterflies: Allometry of airspeeds during natural free flight, *J. Exp. Biol.*, 1994, vol. 191, pp. 125–139.

Fric, Z. and Konvicka, M., Generations of the polyphenic butterfly *Araschnia levana* differ in body design, *Evol. Ecol. Res.*, 2002, vol. 4, pp. 1017–1033.

Fric, Z., Klimova, M., and Konvicka, M., Mechanical design indicates differences in mobility among butterfly generations, *Evol. Ecol. Res.,* 2006, vol. 8, pp. 1511–1522.

Gorbunov, P.Y., *The Butterflies of Russia: Classification, Genitalia, Keys for Identification (Lepidoptera: Hesperioidea and Papilionoidea)*, Yekaterinburg: Institute of Plant and Animal Ecology, Russian Academy of Sciences, 2001.

Hammer, O., Harper, D.A.T., and Ryan, P.D., PAST: Paleontological Statistics software package for education and data analysis, *Palaeontol. Electr.*, 2001, vol. 4, no. 1, p. 9.

Karlsson, B. and Johansson, A., Seasonal polyphenism and developmental trade-offs between flight ability and egg laying in a pierid butterfly, *Proc. R. Soc. B: Biol. Sci.*, 2008, vol. 275, pp. 2131–2136.

Karlsson, B. and Wickman, P.-O., Increase in reproductive effort as explained by body size and resource allocation in the speckled wood butterfly, *Pararge aegeria* (L.), *Funct. Ecol.*, 1990, vol. 4, pp. 609–617.

Korshunov, Yu.P., *Bulavousye cheshuekrylye Severnoi Azii* (Rhopaloceran Butterflies of Northern Asia), Moscow: KMK, 2002.

Marshall, L.D., Male nutrient investment in the Lepidoptera: What nutrients should males invest?, *Am. Nat.*, 1982, vol. 120, pp. 273–279.

Pigliucci, M., Murren, C.J., and Schlichting, C.D., Phenotypic plasticity and evolution by genetic assimilation, *J. Anim. Ecol.,* 2006, vol. 209, pp. 2362–2367.

Rohlf, F.J., *TpsDig Version 2.10. Ecology & Evolution (Program)*, New York: Suny at Stony Brook., 2006.

Rohlf, F.J., *TpsUtil Version 1.40. Ecology & Evolution (Program),* New York: Suny at Stony Brook., 2008.

Schmalhausen, I.I., *Puti i zakonomernosti evolyutsionnogo protsessa. Izbr. trudy* (Pathways and Trends of the Evolutionary Process: Selected Works), Moscow: Nauka, 1983.

Shapiro, A.M., Seasonal polyphenism, *Evol. Biol.*, 1976, vol. 9, pp. 259–333.

Shkurikhin, A.O., Oslina, T.S., and Zakharova, E.Yu., Protandry in mono- and polyvoltine species of pierid and satyrid butterflies (Lepidoptera, Pieridae, Satyridae) under conditions of Southern Ural forest–steppe, *Vestn. Tomsk. Gos. Univ.: Biol.*, 2011, vol. 4, no. 16, pp. 80–91.

Shvarts, S.S., Smirnov, V.S., and Dobrinskii, L.N., *Metod morfofiziologicheskikh indikatorov v ekologii nazemnykh pozvonochnykh* (The Method of Morphophysiological Indicators in the Ecology of Terrestrial Vertebrates), *Tr. Inst. Ekol. Rast. Zhiv. Ural. Fil. Akad. Nauk SSSR*, vol. 58, Sverdlovsk, 1968.

Srigley, R.B. and Kingsolver, J.G., Effects of weight loading on flight performance and survival of palatable neotropical *Anartia fatima* butterflies, *Biol. J. Linn. Soc.*, 2000, vol. 70, pp. 707–725.

StatSoft Inc., STATISTICA for Windows. Version 8.0. 2007 (Data analysis software system). http://www.statsoft.ru/

Stjernholm, F. and Karlsson, B., Nuptial gifts and the use of body resources for reproduction in the green-veined white butterfly *Pieris napi, Proc. R. Soc. B: Biol. Sci.*, 2000, vol. 267, pp. 807–811.

Stjernholm, F. and Karlsson, B., Flight muscle breakdown in the green-veined white butterfly, *Pieris napi* (Lepidoptera: Pieridae), *Eur. J. Entomol.,* 2008, vol. 105, pp. 87–91.

Stjernholm, F., Karlsson, B., and Boggs, C.L., Age-related changes in thoracic mass: Possible reallocation of resources to reproduction in butterflies, *Biol. J. Linn. Soc.*, 2005, vol. 86, pp. 363–380.

West-Eberhard, M.J., *Developmental Plasticity and Evolution*, Oxford: Oxford Univ. Press, 2003.

Wiklund, C., Kaitala, A., Lindfors, V., and Abenius, J., Polyandry and its effect on female reproduction in the green-veined white butterfly (*Pieris napi* L.), *Behav. Ecol. Sociobiol.*, 1993, vol. 33, pp. 25–33.

Yamamoto, M., Notes on the methods of belt transect census of butterflies, *J. Fac. Sci. Hokkaido Univ. VI: Zool.*, 1975, vol. 20, no. 1, pp. 93–116.

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