A Novel Form of Phenotypic Plasticity of the Thermal Reaction Norms for Development in the Bug *Graphosoma lineatum* (L.) (Heteroptera, Pentatomidae)

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Abstract—The temperature dependence of the developmental rate of ectothermic organisms may change under the influence of abiotic and biotic environmental factors that modify the thermal reaction norms for development. These reaction norms are characterized by the lower temperature threshold, the regression coefficient (i.e., the degree of thermal sensitivity of development), and the sum of degree-days. Such manifestation of phenotypic plasticity of the thermal reaction norms for development has been described in many species of insects. We studied the effect of photoperiodic conditions on the development of the bug *Graphosoma lineatum* (L.) from St. Petersburg (59.9° N) and Bryansk (53.2° N) populations. Two experiments were performed with the bugs from either population that were collected in the field at different times. Two photoperiodic regimes were used (12 vs. 22 hours of light per day for the St. Petersburg population and 12 vs. 18 hours of light per day for the Bryansk population) and five constant temperatures (20, 22, 24, 26, and 28°C) in each photoperiodic regime. Photoperiodic conditions did not affect egg development in all of the experiments. Under the short-day conditions, there was a disproportionate (i.e., more pronounced at low temperatures) acceleration of nymphal development in bugs from the Bryansk population (in both experiments) and in bugs from the St. Petersburg population (in the experiment with late-season collection of parental adults). Thus, under short-day conditions, thermal sensitivity of development and the sum of degree-days did not change, whereas the temperature threshold became lower. The nymphs from the St. Petersburg population reared under long-day conditions showed different thermal reaction norms for development, depending on the "early" or "late" date of collection of adults in the field. The latter response was presumably explained by different physiological age of the parental individuals. Thus, the thermal reaction norms for nymphal development in the bug *G. lineatum* exhibit phenotypic plasticity during the summer season, depending on the oviposition date and currently experienced photoperiodic conditions. There were also interpopulation differences in the thermal reaction norms for egg and nymphal development. Bugs from both populations showed a greater body mass after developing at higher temperatures.

Environmental temperature is among the key ecological factors and plays a defining role in the control of seasonal cycles of insects and other ectothermic organisms. It directly affects all parameters of the life cycle, including growth and development rates, body mass, fecundity, survival, etc. These organismal properties may exhibit phenotypic plasticity, facilitating adaptation of insects to their environment (James and Partridge, 1995; Liefting et al., 2009; Bouton et al., 2011). Phenotypic plasticity induced by biotic (diet, competition, predator pressure, etc.) and abiotic (temperature, day length, humidity, etc.) factors has been revealed in numerous poikilothermic organisms. This plasticity is characterized by means of reaction norms (Groeters, 1992; Stearns, 1992; Danks, 1994; Nylin, 1994; Nylin and Gotthard, 1998; Roff, 2002; Angilletta and Dunham, 2003; Kingsolver et al., 2004). When shaped by natural selection, the diversity of norms of reaction to temperature mirrors adaptive specialization of local populations (Angiletta, 2009).

The complete thermal reaction norm for development is an asymmetrical bell-shaped curve with a linear portion in the range of temperatures that are most fa-

vorable for the organism's activities. It is the linear part of the thermal reaction norm for development that is most important ecologically and evolutionarily because it corresponds to the natural thermal conditions under which development most often takes place (Campbell et al., 1974; Ikemoto and Takai, 2000). Within this temperature range, insect developmental rate linearly increases with the temperature rise. The dependence of developmental rate on temperature is frequently described using a linear regression equation of the form $R = a + bT$, where R is developmental rate (inverse of the duration of development), a is the y-intercept of the regression line, b is the linear regression coefficient, and T is developmental temperature.

The coefficient of linear regression b, or the coefficient of thermolability of development (Kozhanchikov, 1961; Mednikov, 1966, 1987) equals the tangent of the angle between the regression line and the x-axis. This is a measure of thermolability, or temperature-sensitivity, of development. This coefficient shows how strongly developmental rate depends on temperature. The more developmental rate increases or decreases with a change in temperature (i.e., the more sensitive development is to temperature), the greater the coefficient.

The point where the regression line crosses the x-axis is a lower temperature threshold for development, i.e., the temperature at which developmental rate approaches zero. The inverse of the regression coefficient is known in the Russian-language sources as the sum of effective temperatures and in the international literature as the sum of degree-days (Kipyatkov and Lopatina, 2010). It is equivalent to the sum of above-threshold temperatures that has to accumulate for development to be completed (Mednikov, 1966; Ratte, 1985; Groeters, 1992; Atkinson, 1994). The lower temperature threshold, the coefficient of temperature-sensitivity, and the sum of degree-days are often referred to as the parameters of temperature-sensitivity of development. These parameters characterize the linear dependence of developmental rate on temperature and describe the thermal reaction norms for development.

Insect thermal reaction norms for development exhibit phenotypic plasticity so that the sensitivity of developmental rate to temperature changes may be modified by various abiotic and biotic factors. For example, the linear regression coefficient and the lower temperature threshold for development may become greater or smaller resulting from a different diet (Lopatina et al., 2014), under the influence of social factors (Ryzhkova and Lopatina, 2015a) or photoperiodic regimens (Lopatina et al., 2007), etc. Our studies on the linden bug *Pyrrhocoris apterus* L. were the first to show that photoperiodic conditions do not merely accelerate or decelerate development of nymphs but modify the thermal reaction norms for their development. As a result, photoperiodic regimens have an equivocal influence and may speed up development at some temperatures while at other temperatures their effect may be opposite (Lopatina et al., 2007). Later on, a wide diversity of patterns of photoperiodic modification of thermal reaction norms for development was revealed and it was proved that phenotypic plasticity of thermal requirements for insect development was widespread and ecologically meaningful (Kucherov and Kipyatkov, 2011; Lopatina et al., 2011c, Kutcherov et al., 2011, 2015; Ryzhkova and Lopatina, 2015a, 2015b; Gusev and Lopatina, 2018; Kutcherov et al., 2018; Lopatina, 2018). New patterns of temperature-photoperiod interaction are likely to be discovered in studies on various insect species.

STUDY SPECIES

The stink bug *Graphosoma lineatum* is widely distributed in the Palaearctic and frequently encountered in Europe. The species prefers open and well insolated biotopes such as meadows, forest edges, floodplains, kitchen gardens, and wastelands. Its primary host plants are umbellifers (Apiaceae).

The duration of nymphal development in this bug has been shown to be under photoperiodic control. Under short-day conditions, development is accelerated (Musolin and Saulich, 1995). The bug overwinters only at the adult stage. Winter diapause is induced by decreasing day length. Voltinism of *G. lineatum* varies with latitude. In warmer years, a second generation is possible in the forest-steppe zone (Musolin and Saulich, 2001).

Phenological observations on the development of this bug under natural conditions in Sergievka park (Stary Petergof, St. Petersburg) have shown that adult *G. lineatum* emerge from their winter quarters in the second half of May. After a period of feeding on umbelliferous plants, males and females begin reproduction early in June. Adults are rather long-lived and constantly mate. Oviposition lasts into the middle of July. New generation nymphs feed, grow, and reach the adult stage

by the second half of August. Straggler nymphs of the fifth (final) instar may be seen on host plants as late as in the first days of September. Thus, the earliest nymphs in the season develop under long-day conditions, whereas those hatching from the last eggs complete development under short-day ones. In such a case, manifestation of photoperiodic plasticity of thermal reaction norms for development is expected to be found.

MATERIALS AND METHODS

Bugs from St. Petersburg (59.9° N) and Bryansk (53.2° N) were used in the experiments. Adults of the St. Petersburg population were collected in Stary Petergof, in Sergievka park, from 25.VI to 30.VI.2014 (late-season sample) and from 28.V to 15.VI.2016 (early-season sample). Bugs of the Bryansk population were collected in the vicinity of the city of Bryansk on 13.VII.2015 (late-season sample) and from 5.VI to 10.VI.2016 (early-season sample). The bugs were most often found on cow parsley *Anthriscus sylvestris*, goutweed *Aegopodium podagraria*, and hogweed *Heracleum sibiricum*.

Field-collected males and females were kept by 10–15 pairs in plastic containers (179 × 132 mm, 1000 ml) with a sheet of paper on the bottom. The bugs were kept at a temperature of 25°C under a long-day photoperiod (22 h of light per day for the St. Petersburg population and 18 h for the Bryansk population) and fed daily with goutweed *Ae. podagraria* and cow parsley *A. sylvestris*. To provide additional moisture, Eppendorf tubes filled with water and stoppered with cotton wool plugs were put into containers.

Males and females constantly mated during their maintenance in containers. Eggs laid by females were collected once every day over a period of 2.5 weeks until there were at least 100 eggs in each experimental regimen. Thus, eggs from females of the St. Petersburg population were collected from 1.VII to 11.VII.2014 (late-season eggs) and from 6.VI to 24.VI.2016 (early-season eggs). Eggs from females of the Bryansk population were collected from 16.VII to 8.VIII.2015 (late-season eggs) and from 14.VI to 24.VI.2016 (early-season eggs). Clutch size varied from 5 to 35 eggs and averaged at 16 ± 0.3.

Besides the laboratory experiments, we measured the surface temperature of the bugs' integument and that of the substrate on which the bugs were found in Sergievka park on sunny and cloudy days. This was done in order to assess the ability of the bugs to warm up by basking. The measurements were performed using an Optris LaserSight pyrometer in late August – early September.

Developmental durations (*D*) for each egg batch and each individual nymph were transformed into rates (1/*D*). After that, regression analysis was carried out using the entire dataset of individual developmental rates obtained at all of the experimental temperatures and photoperiods. The linear regression coefficient and the lower temperature threshold T_0 were calculated from the equation $R = a + bT$. The sum of degree-days was calculated as the reciprocal of the regression coefficient (1/*b*). Standard errors were also calculated for all the parameters (Campbell et al., 1974; Sokal and Rohlf, 1995). Two regression lines were compared by slope and threshold with the *t*-test, given standard errors of these parameters.

Sexual size dimorphism, or SSD, was quantified as (body mass of the larger sex / body mass of the smaller sex – 1) × 100 (Lovich and Gibbons, 1992). The effects of photoperiodic and thermal conditions on SSD were analyzed with ANOVA followed by the Tukey's HSD *post-hoc* pairwise comparisons test. To test the relationship between the fraction of surviving males and temperature within each photoperiodic regimen we used Kendall's rank correlation. In such a way, sex ratio in the experimental regimens was assessed.

The effects of the factors (temperature, photoperiod, sex, and the period of collection of adults and eggs) on development time and SSD were analyzed with ANOVA followed by Tukey's HSD *post-hoc* pairwise comparisons.

Prior to carrying out the ANOVA, the homogeneity of variances was verified using the Levene's test and the correlation between means and variances was checked. In the cases of heteroscedasticity as well as violation of normality assumption (Sokal and Rohlf, 1995), the dependent variables were natural log- or square root-transformed. When such transformations did not improve the properties of the dataset, the non-parametric Kruskal-Wallis test with subsequent multiple comparisons was used. The effects of temperature and photoperiod on survivorship were also analyzed with the Kruskal-Wallis test followed by multiple comparisons.

Significance of the differences in the surface temperature between bug integument and surrounding substrate was analyzed using one-way ANOVA. The temperature contrast between bug integument and surrounding substrate under different illumination was compared with the *t*-test.

All the calculations and their initial statistical processing were carried out in a custom-designed template DevRate 4.4 (© V. E. Kipyatkov, 1998–2010) realized in QuattroPro 9.0 (© Corel Corporation 1998–2000). All the statistical tests were performed in Statistica 7.1 (© StatSoft, Inc. 1984–2006).

RESULTS

Life-History Parameters of the St. Petersburg Population of the Bug Graphosoma lineatum (L.)

Survivorship. The percentage of St. Petersburg eggs and nymphs of *G. lineatum* that survived to the next stage under different conditions of temperature and photoperiod is shown in Fig. 1. Egg viability did not depend on either temperature ($H_{4, 133} = 4.4$, $p > 0.3$) or photoperiod ($H_{1, 133} = 1.2$, $p > 0.2$). These factors had a significant influence on nymphal survival rate: temperature, $H_{4, 133} = 29.2$, $p < 0.0001$ and photoperiod, $H_{1, 133} = 8.4$, $p < 0.004$. Mortality was higher under the short-day regimen at low temperatures (Fig. 1).

Duration of preimaginal stages. The higher was the rearing temperature, the faster the eggs developed (2014: $F_{4, 104} = 768.2$, $p \ll 0.0001$; 2016: $F_{4, 122} = 579.2$, $p \ll 0.0001$) (Table 1). Egg development time was unaffected by photoperiodic conditions (2014: $F_{1, 104} = 0.6$, $p > 0.4$, and 2016: $F_{1, 122} = 0.2$, $p > 0.6$) (Table 1). A comparison of experimental results for early- and late-season samples did not reveal any significant difference either ($F_{1, 236} = 0.7$, $p > 0.3$).

Differences in nymphal development time between males and females turned out to be nonsignificant (2014: $F_{1, 1116} = 0.5$, $p > 0.4$, and 2016: $F_{1, 611} = 2.9$, $p > 0.09$; Tukey's HSD: $p > 0.9$) (Table 2). Therefore, in all subsequent analyses and thermal reaction norm estimation, we used pooled data on eggs (years combined) and nymphs of both sexes.

In both experiments, nymphal development time significantly depended on the temperature (early-season: $F_{4, 621} = 3709$, $p \ll 0.0001$; late-season: $F_{4, 1128} = 6130$, $p \ll 0.0001$) and the photoperiod (early-season:

$F_{1, 621} = 497$, $p \ll 0.0001$; late-season: $F_{1, 1128} = 517$, $p \ll 0.0001$) (Table 1). Interaction of these factors was also significant (early-season: $F_{4, 621} = 3$, $p < 0.03$; late-season: $F_{4, 1128} = 31$, $p \ll 0.0001$). The higher was the temperature, the faster did the nymphs develop. Under short-day conditions, development proceeded faster at all the temperatures than under long day ones (Tukey's HSD test, $p < 0.0001$).

Nymphal development time differed significantly between experiments ($F_{1, 1749} = 39$, $p \ll 0.0001$) (Table 1). In the late-season experiment, nymphs developed faster under the long-day photoperiod at 26 and 28°C but slower at 20 and 22 °C (Tukey's HSD test, $p < 0.0001$). In the short-day regimens, there were no significant differences in nymphal development time between the two experiments.

Adult body mass. A comparison of the results from two experiments did not reveal any regularities and significant differences were found in only three cases out of twenty. Therefore, we combined the data from both experiments within each photoperiodic regimen.

There were significant effects of temperature ($F_{4, 1747} = 275$, $p \ll 0.0001$), photoperiod ($F_{1, 1747} =$
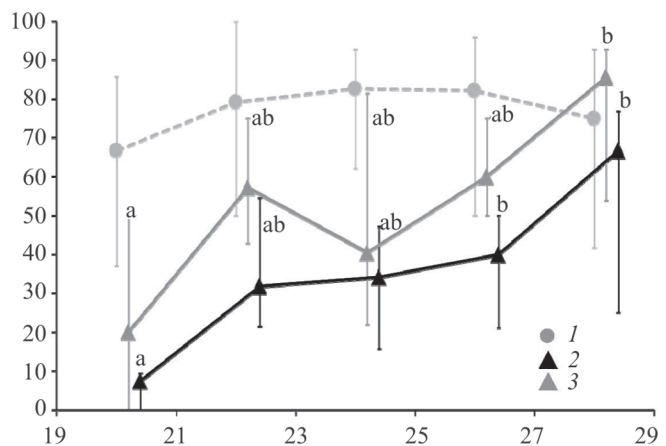
Fig. 1. Percentage of surviving eggs and nymphs of the bug *Graphosoma lineatum* (L.) from the St. Petersburg population under various conditions of temperature and photoperiod. Abscissa: temperature (°C), ordinate: survival rate (%). *1*, eggs; *2*, long-day nymphs; *3*, short-day nymphs. The data shown are the median values. Vertical bars denote 25% and 75% percentiles. Significant differences between temperature treatments (multiple comparisons following the Kruskal-Wallis test, $p < 0.01$) are denoted with different superscripts. Differences between photoperiodic regimens are nonsignificant. Here, and in subsequent figures, symbols are slightly set apart along the abscissa axis for clarity.

Table 1. Durations (in days) of preimaginal developmental stages of the bug *Graphosoma lineatum* (L.) from the St. Petersburg population under various conditions of temperature and photoperiod

Temperature (°C)		Day length (h)	Eggs	N[1]	Nymphs	Total	N[2]
set	real						
Early-season experiment (2016)							
20	20.0	12	12.0 ± 0.43^{a}	12	$47.3 \pm 1.42^{a*}$	$59.1 \pm 1.30^{a*}$	10
	20.0	22	11.9 ± 0.80^{a}	15	$53.5 \pm 3.73^{a*}$	$64.5 \pm 4.14^{a*}$	41
22	22.0	12	8.8 ± 0.38^{b}	13	$32.9 \pm 1.94^{b*}$	$41.8 \pm 1.86^{b*}$	54
	21.9	22	8.8 ± 0.55^{b}	13	$37.6 \pm 1.78^{b*}$	$46.0 \pm 2.52^{b*}$	81
24	22.8	12	7.4 ± 0.85^{c}	14	$29.5 \pm 2.15^{c*}$	$38.2 \pm 3.87^{c*}$	43
	24.1	22	7.2 ± 0.44^{c}	13	$29.0 \pm 1.90^{c*}$	$36.2 \pm 1.90^{c*}$	66
26	26.0	12	5.9 ± 0.64^{d}	13	$19.7 \pm 1.13^{d*}$	$25.4 \pm 1.12^{d*}$	70
	26.0	22	6.2 ± 0.44^{d}	13	$23.0 \pm 1.62^{d*}$	$29.0 \pm 1.75^{d*}$	81
28	27.3	12	5.2 ± 0.38^{d}	13	$17.0 \pm 0.94^{e*}$	$22.0 \pm 0.97^{e*}$	77
	28.0	22	4.9 ± 0.28^{e}	13	$18.6 \pm 1.56^{e*}$	$23.5 \pm 1.64^{e*}$	108
Late-season experiment (2014)							
20	19.9	12	$13.2 \pm 0.75^{a*}$	11	$53.4 \pm 4.53^{a*}$	$66.1 \pm 4.55^{a*}$	8
	20.3	22	$12.1 \pm 0.79^{a*}$	12	$60.8 \pm 3.40^{a*}$	$72.8 \pm 3.13^{a*}$	54
22	21.7	12	8.9 ± 0.51^{b}	12	$35.0 \pm 2.41^{b*}$	$43.9 \pm 2.41^{b*}$	128
	21.8	22	9.5 ± 0.52^{b}	12	$41.1 \pm 3.61^{b*}$	$50.6 \pm 3.57^{b*}$	136
24	23.9	12	6.4 ± 0.51^{c}	12	$25.6 \pm 2.65^{c*}$	$32.1 \pm 2.83^{c*}$	131
	23.9	22	6.8 ± 0.40^{c}	11	$28.7 \pm 1.54^{c*}$	$35.7 \pm 1.44^{c*}$	105
26	26.2	12	5.9 ± 0.29^{c}	12	$19.3 \pm 1.20^{d*}$	$25.3 \pm 1.22^{d*}$	134
	25.8	22	5.5 ± 0.52^{d}	11	$22.0 \pm 0.95^{d*}$	$27.6 \pm 0.91^{d*}$	134
28	27.9	12	4.8 ± 0.40^{d}	11	$16.6 \pm 0.69^{e*}$	$21.5 \pm 0.75^{e*}$	145
	28.0	22	4.9 ± 0.32^{d}	10	$17.4 \pm 1.26^{e*}$	$22.3 \pm 1.12^{e*}$	163

Durations are expressed as means ± SD. Significant differences (Tukey's HSD test, $p < 0.01$) within photoperiodic regimens are denoted with different superscripts. Asterisks denote significant differences (Tukey's HSD test, $p < 0.01$) between contrasting photoperiodic regimens. N[1]) the number of clutches; N[2]) the number of individuals that successfully completed immature development.

224, $p \ll 0.0001$), and sex ($F_{1, 1747} = 303$, $p \ll 0.0001$) on adult body mass as well as a significant temperature by photoperiod interaction ($F_{4, 1747} = 16$, $p \ll 0.0001$). Under both photoperiods, the body mass increased with the temperature rise (Fig. 2). The bugs were smaller under short-day conditions (Tukey's HSD test, $p < 0.0001$). Only at 20 and 24°C was the difference between photoperiods nonsignificant. Females were larger than males in all the experimental regimens (Tukey's HSD test, $p < 0.0001$) (Fig. 2).

In all of the experimental regimens, sex ratio was not significantly different from 1:1. The temperature did not have a significant influence on SSD ($F_{4, 169} = 1.9$, $p > 0.1$) while the effect of photoperiod was marginally significant ($F_{1, 169} = 5.8$, $p > 0.01$) and so was the interaction of these two factors ($F_{4, 169} = 2.6$, $p > 0.02$).

Thermal reaction norms of preimaginal stages. The parameters of linear regression equations for the dependence of developmental rate on the temperature are summarized in Table 3. In contrast to long-day conditions, comparisons of short-day nymphal development time between two experimental years did not reveal any significant differences. Thus, thermal requirements for nymphal development under the short-day regimen were calculated using combined data and separately for either year under long-day conditions.

A comparison of egg and nymphal thermal reaction norms showed that the regression coefficient for eggs

Table 2. Durations (in days) of preimaginal development in female and male bugs *Graphosoma lineatum* (L.) from the St. Petersburg population under various conditions of temperature and photoperiod

Temperature (°C)		Day length (h)	Females	N	Males	N
set	real					
Early-season experiment (2016)						
20	20.0	12	47.3 ± 1.67	8	47.5 ± 0.71	2
	20.0	22	53.1 ± 4.24	24	53.8 ± 2.96	17
22	22.0	12	32.3 ± 1.93	25	33.3 ± 1.72	29
	21.9	22	37.6 ± 1.55	43	37.7 ± 2.03	38
24	22.8	12	29.3 ± 2.49	20	29.7 ± 1.85	23
	24.1	22	29.1 ± 2.02	34	29.0 ± 1.76	32
26	26.0	12	19.5 ± 1.29	31	19.9 ± 0.97	39
	26.0	22	22.7 ± 1.56	40	23.2 ± 1.65	41
28	27.3	12	16.9 ± 0.93	40	17.0 ± 0.96	37
	28.0	22	18.6 ± 1.72	47	18.6 ± 1.45	61
Late-season experiment (2014)						
20	19.9	12	58.0 ± 0.00	1	52.7 ± 4.46	7
	20.3	22	60.7 ± 4.12	24	60.9 ± 2.76	30
22	21.7	12	34.8 ± 2.11	57	35.3 ± 2.61	71
	21.8	22	41.3 ± 3.70	62	40.9 ± 3.55	74
24	23.9	12	25.0 ± 2.58	66	26.3 ± 2.60	65
	23.9	22	29.0 ± 1.78	55	28.4 ± 1.17	49
26	26.2	12	19.2 ± 1.29	54	19.3 ± 1.13	80
	25.8	22	21.9 ± 0.99	65	22.0 ± 0.91	68
28	27.9	12	16.6 ± 0.71	83	16.6 ± 0.66	62
	28.0	22	17.5 ± 1.30	78	17.3 ± 1.22	85

Durations are expressed as means ± SD. N is the number of individuals that successfully completed immature development.

was significantly higher and the temperature threshold for development significantly lower than the corresponding nymphal values ($p < 0.01$) (Table 3).

The thermal reaction norms for nymphal development under the long-day photoperiodic regimen differed between the experiments with different sampling dates. The regression coefficient and the temperature threshold for development were significantly higher for late-season than for early-season individuals ($p < 0.01$) (Table 3, Fig. 3).

Comparisons of thermal requirements for nymphal development between the two photoperiodic regimens yielded the following results. In the late-season experiment, linear regression coefficients (i.e., temperature sensitivity of nymphal development) and the sum of degree-days did not differ between the short-day and long-day regimen. However, the temperature threshold for nymphal development was significantly higher under long-day conditions ($p < 0.01$) (Table 3, Fig. 3). Early-season (2016) nymphs under long-day conditions had significantly lower values of the temperature threshold and regression coefficient than under short-day ones ($p < 0.01$) (Table 3, Fig. 3). The sum of degree-days in the case was greater, as it is the reciprocal of the linear regression coefficient.

Ability to Warm up by Basking in G. lineatum Bugs from St. Petersburg

The results of measurements of surface temperature of the bug body and that of the surrounding substrate in sunny and cloudy weather are shown in Table 4.
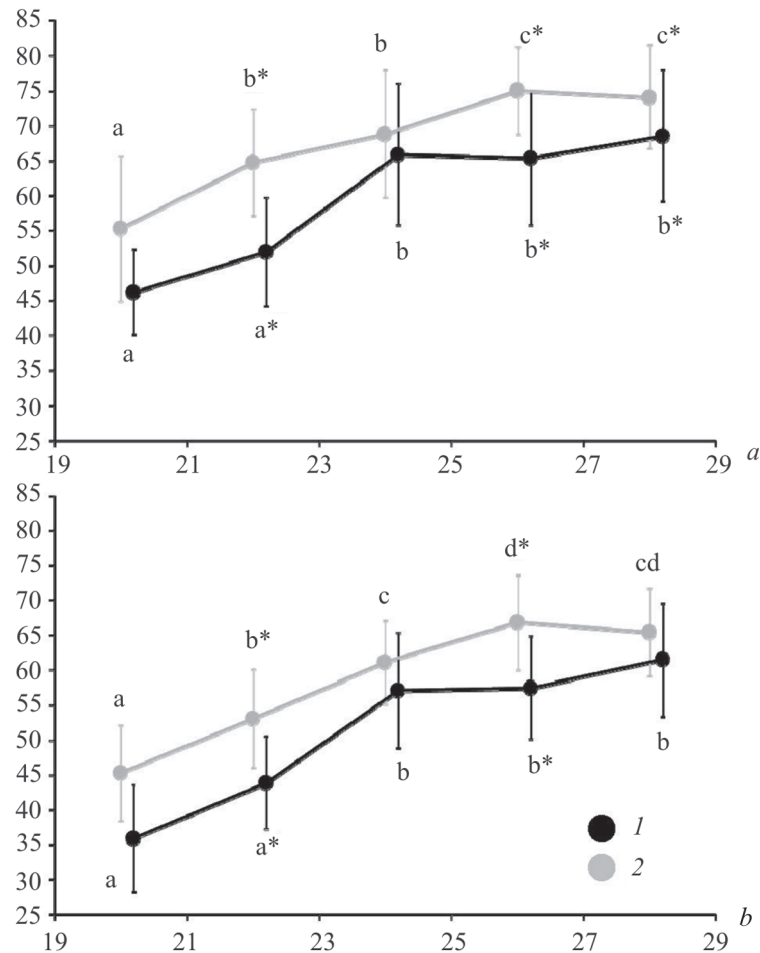
Fig. 2. Female and male body mass in the bug *Graphosoma lineatum* (L.) from the St. Petersburg population under various conditions of temperature and photoperiod (data from two experiments combined). Abscissa: temperature (°C), ordinate: body mass (mg). (*a*) Females, (*b*) males. Photoperiodic conditions: *1*, day length 12 h; *2*, day length 22 h. The data shown are the mean values. Vertical bars denote standard deviations. Significant differences between temperature treatments within photoperiodic regimens (Tukey's HSD test, *p* < 0.01) are denoted with different superscripts. Asterisks denote significant differences (Tukey's HSD test, *p* < 0.01) between contrasting photoperiodic regimens.

ANOVA showed that the temperature on the surface of the bug's integument was significantly higher than that of the substrate surface ($F_{1, 90} = 12.983, p < 0.001$). However, pairwise comparisons showed significant differences for the sunny weather only (Tukey's HSD test, $p < 0.01$). The temperature contrast between the bug surface and the substrate surface was significantly greater under maximum insolation ($p < 0.01$).

Life-History Parameters of the Bryansk Population of G. lineatum (L.)

Survivorship. The percentage of Bryansk eggs and nymphs of *G. lineatum* that survived to the next stage under various experimental conditions is shown in Fig. 4. Neither temperature ($H_{4, 202} = 4.7, p > 0.3$) nor

photoperiod ($H_{1, 202} = 0.7, p > 0.4$) affected egg viability. The nymphal survival rate was significantly influenced by temperature only ($H_{4, 198} = 54.2, p < 0.0001$). Cooler temperatures resulted in lower numbers of individuals that successfully completed development.

Duration of preimaginal stages. The higher was the rearing temperature, the faster the eggs developed (2015: $F_{4, 62} = 1522.5, p \ll 0.0001$, and 2016: $F_{4, 120} = 557.5$, $p \ll 0.0001$) (Table 5). Photoperiodic conditions did not have a significant effect on their development (2015: $F_{1, 62} = 0.1, p > 0.7$, and 2016: $F_{1, 120} = 0.6, p > 0.5$). The duration of egg development marginally differed between the two experiments ($F_{1, 192} = 5.8, p > 0.01$) (Table 5). Pairwise comparisons by Tukey's HSD test showed a significant difference at 20°C only ($p < 0.002$).

Table 3. Thermal requirements for preimaginal development in the bug *Graphosoma lineatum* (L.) from the St. Petersburg and Bryansk populations under various conditions of temperature and photoperiod either early or late in the season

Experiment	Day length (h)	Stage	N	R^2	Linear regression coefficient (1/°C×d)	Temperature threshold (°C)	SDD (°C×d)
St. Petersburg population							
Pooled data*	Pooled data**	Egg	246[1]	0.92	0.01489 ± 0.000277	14.4 ± 0.18	67 ± 1.3
Pooled data*	12	Nymph	802[2]	0.94	0.00536 ± 0.000048	16.5 ± 0.08	187 ± 1.7
Early	22	Nymph	379[2]	0.95	0.00442 ± 0.000055	15.9 ± 0.11	227 ± 2.8
Late	22	Nymph	592[2]	0.96	0.00533 ± 0.000042	17.2 ± 0.06	188 ± 1.5
Bryansk population							
Pooled data*	Pooled data**	Egg	202[1]	0.93	0.01593 ± 0.000316	15.1 ± 0.18	63 ± 1.3
Pooled data*	12	Nymph	381[2]	0.93	0.00452 ± 0.000065	15.0 ± 0.15	221 ± 3.2
Pooled data*	18	Nymph	342[2]	0.96	0.00464 ± 0.000051	16.1 ± 0.10	216 ± 2.4

Parameters of thermal reaction norms are expressed as means ± SE. R^2) coefficient of determination; N) sample size; SDD) sum of degree-days. *Early- and late-season data combined; **data from both photoperiodic regimens combined. [1]The number of clutches; [2]the number of individuals that successfully completed immature development.

Differences in development time between males and females turned out to be nonsignificant (2015: $F_{1, 170}$ = 5.1, $p > 0.02$; 2016: $F_{1, 511}$ = 3, $p > 0.1$; Tukey's HSD test: p > 0.9) (Table 6), similar to those in the bugs from the St. Petersburg population. Therefore, in subsequent analyses, we pooled the egg data from both experiments and the nymphal data for both sexes.

In either experiment, there were significant effects of temperature (late-season: $F_{4, 180}$ = 2931.9, $p \ll 0.0001$; early-season: $F_{4, 521}$ = 3488, $p \ll 0.0001$) and photoperiod (late-season: $F_{1, 180}$ = 227.5, $p \ll 0.0001$; early-season: $F_{1, 521}$ = 477, $p \ll 0.0001$), as well as their interaction (late-season: $F_{4, 180}$ = 19.4, $p \ll 0.0001$; early-season: $F_{4, 521}$ = 55, $p \ll 0.0001$), on nymphal development time (Table 5). Nymphal developmental rate increased with the temperature rise. Nymphs developed faster under the short-day photoperiod (Tukey's HSD test, $p < 0.001$) than under the long-day regimen at all the temperatures except 26°C in the late-season experiment and 28°C in the early-season experiment.

On the whole, in the late-season experiment (2015), nymphal development proceeded more rapidly than in the early-season one (2016) ($F_{1, 701}$ = 66, $p \ll 0.0001$) (Table 5), although significant differences were only found under the long-day regimen at 26 °C and under a the short-day one at 24 and 28°C (Tukey's HSD test, $p < 0.0001$). The mean durations of nymphal development across all of the temperature regimens did not differ between the two experiments under either photoperiod (one-way ANOVA, $p > 0.5$).

Table 4. Body surface temperature of the bugs *Graphosoma lineatum* (L.) and the temperature of the substrate on which they were found under different illumination conditions

Weather	Surface temperature (°C)		Δ t (°C)	N
	bugs	substrate		
Sunny	30.0 ± 0.94	26.5 ± 0.85	3.5 ± 0.75	25
Cloudy	21.3 ± 0.34	19.6 ± 0.41	1.7 ± 0.34	22

Temperatures are expressed as means ± SE. Δ t) the temperature contrast between the bug and the substrate; N) sample size (number of measurements).

Adult body mass. Based on the results from two experiments, temperature (2015: $F_{4, 170}$ = 24.3, $p \ll 0.0001$; 2016: $F_{4, 511}$ = 33.7, $p \ll 0.0001$), photoperiod (2015: $F_{1, 170}$ = 8.4, $p < 0.005$; 2016: $F_{1, 511}$ = 65.9, $p \ll 0.0001$), and sex (2015: $F_{1, 170}$ = 29.9, $p \ll 0.0001$; 2016: $F_{1, 511}$ = 126.9, $p \ll 0.0001$) had a significant effect on adult body mass (Figs. 5 and 6). The interaction of temperature and photoperiod in the early-season experiment (2016) was also significant ($F_{4, 511}$ = 8.5, $p \ll 0.0001$). In that experiment, male and female body mass increased with the temperature rise under the long-day photoperiod while, under a the

short-day one, it only increased over the range from 20 to 24°C and practically did not depend on the temperature at 24–28°C (Fig. 5). In the late-season experiment (2015), male and female body mass increased with the temperature rise under both photoperiodic regimens (Fig. 6).

On average, across all the temperature regimens used, male and female bugs were significantly larger under the long-day regimen than under the short-day photoperiod (one-way ANOVA, $p < 0.003$). However, in the early-season experiment, differences in body mass between the two photoperiods were only significant at 28°C for both sexes and at 22°C for females (Tukey's HSD test, $p < 0.004$). In the late-season experiment, differences in body mass between the two photoperiods were not significant either for males or for females (Fig. 6).

On average, across all the temperature regimens used, female bugs were significantly larger than males under both long-day and short-day conditions in the early-season experiment (one-way ANOVA, $p << 0.0001$). In that experiment, the difference in body mass between females and males was significant at 24, 26, and 28°C under both photoperiodic regimens (Tukey's HSD test, $p < 0.004$). In the late-season experiment, females were significantly larger than males when averaged across all the temperatures but only under long-day conditions (one-way ANOVA: $p << 0.0001$). However, the only pairwise comparison that yielded a significant difference between the sexes was that at 26°C. In the same experiment, the differences in body mass between males and females under short-day conditions were only marginally significant (one-way ANOVA: $p < 0.02$).

In general, late-season adults (in the 2015 experiment) were larger than those reared in the early-season experiment (2016) ($F_{1, 681} = 148.8$, $p << 0.0001$) (Figs. 5 and 6).

Sex ratio did not significantly deviate from 1:1 in all of the experimental regimens. There were no significant effects of either temperature ($F_{4, 78} = 0.3$, $p > 0.9$) or photoperiod ($F_{1, 78} = 0.01$, $p > 0.9$) on SSD. The interaction of these factors was not significant either ($F_{4, 78} = 0.8$, $p > 0.5$).

**Thermal reaction norms of preimaginal stages.** The parameters of linear regression equations for the dependence of developmental rate on the temperature

in the Bryansk population of *G. lineatum* are summarized in Table 3. Comparisons of nymphal development times between two experimental years did not reveal significant differences at most temperatures under both
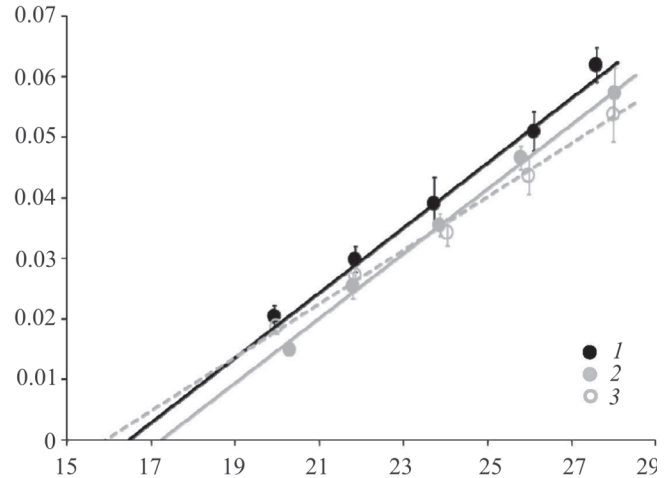
Fig. 3. Thermal reaction norms for nymphal development in the bug *Graphosoma lineatum* (L.) from the St. Petersburg population under different photoperiods. Abscissa: temperature (°C), ordinate: developmental rate (d^{-1}). Experimental conditions: *1*, day length 12 h (data from two experiments combined); *2*, day length 22 h, late-season experiment; *3*, day length 22 h, early-season experiment. The data shown are the mean values. Vertical bars denote standard deviations.
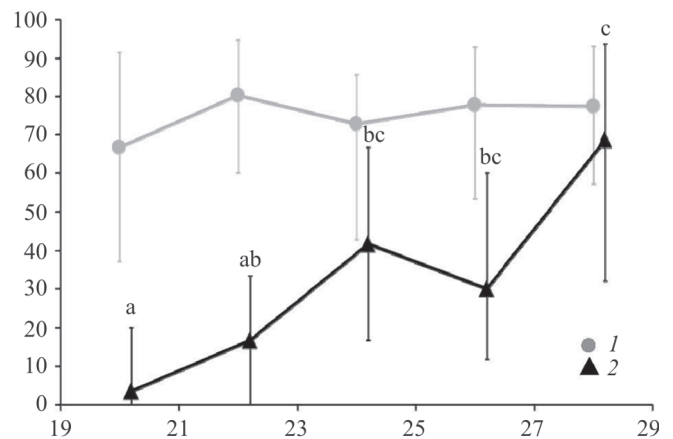
Fig. 4. Percentage of surviving eggs and nymphs of the bug *Graphosoma lineatum* (L.) from the Bryansk population at different rearing temperatures (data from two photoperiodic regimens and from two experiments combined). Abscissa: temperature (°C), ordinate: survival rate (%). *1*, eggs; *2*, nymphs. The data shown are the median values. Vertical bars denote 25% and 75% percentiles. Significant differences between temperature treatments (multiple comparisons following the Kruskal-Wallis test, $p < 0.01$) are denoted with different superscripts.

Table 5. Durations (in days) of preimaginal developmental stages of the bug *Graphosoma lineatum* (L.) from the Bryansk population under various conditions of temperature and photoperiod

Temperature (°C)		Day length (h)	Eggs	N[1]	Nymphs	Total	N[2]
set	real						
Early-season experiment (2016)							
20	19.7	12	12.3 ± 0.87[a]	12	48.1 ± 2.95[a]*	60.2 ± 2.83[a]*	16
	20.0	18	12.5 ± 0.66[a]	13	56.0 ± 4.85[a]*	68.4 ± 4.72[a]*	22
22	21.6	12	9.1 ± 0.62[b]	14	32.8 ± 1.99[b]*	41.7 ± 1.94[b]*	42
	21.9	18	9.3 ± 0.75[b]	13	38.7 ± 2.42[b]*	48.1 ± 2.34[b]*	39
24	22.8	12	7.3 ± 0.63[c]	13	30.3 ± 1.78[c]*	37.7 ± 1.86[c]*	63
	23.8	18	7.3 ± 0.47[c]	14	28.1 ± 1.38[c]*	35.4 ± 1.33[c]*	61
26	25.8	12	5.9 ± 0.83[d]	14	20.0 ± 1.12[d]*	25.8 ± 1.05[d]*	72
	26.0	18	5.7 ± 0.48[d]	13	23.3 ± 1.26[d]*	28.9 ± 1.55[d]*	47
28	28.2	12	4.9 ± 0.51[e]	12	17.4 ± 1.45[e]	22.1 ± 1.53[e]*	80
	27.7	18	5.1 ± 0.51[e]	12	18.5 ± 0.99[e]	23.4 ± 1.05[e]*	91
Late-season experiment (2015)							
20	19.9	12	13.3 ± 0.49[a]	7	46.4 ± 1.77[a]*	59.5 ± 1.69[a]*	8
	19.9	18	13.0 ± 0.00[a]	6	53.1 ± 1.07[a]*	66.1 ± 1.07[a]*	7
22	21.8	12	9.3 ± 0.49[b]	7	32.1 ± 1.45[b]*	41.7 ± 1.40[b]*	16
	21.9	18	9.4 ± 0.53[b]	7	36.7 ± 1.42[b]*	46.2 ± 1.36[b]*	20
24	24.0	12	7.0 ± 0.00[c]	7	24.1 ± 0.90[c]*	31.1 ± 0.90[c]*	24
	23.9	18	7.2 ± 0.41[c]	6	26.3 ± 0.90[c]*	33.4 ± 0.91[c]*	25
26	26.2	12	6.1 ± 0.35[d]	8	19.4 ± 0.91[d]	25.1 ± 0.90[d]*	25
	25.8	18	6.0 ± 0.00[d]	8	20.9 ± 0.47[d]	26.9 ± 0.47[d]*	14
28	28.1	12	5.0 ± 0.00[e]	8	16.6 ± 0.98[e]*	21.6 ± 0.98[e]*	35
	28.0	18	5.0 ± 0.00[e]	8	17.7 ± 0.70[e]*	22.7 ± 0.70[e]*	16

Durations are expressed as means ± SD. Significant differences (Tukey's HSD test, $p < 0.01$) within photoperiodic regimens are denoted with different superscripts. Asterisks denote significant differences (Tukey's HSD test, $p < 0.01$) between contrasting photoperiodic regimens. N[1]) the number of clutches; N[2]) the number of individuals that successfully completed immature development.

long-day and short-day photoperiodic conditions. Thus, thermal requirements for nymphal development under either day length were calculated using a combined dataset for two years.

Egg development was characterized by greater temperature sensitivity in comparison with nymphs ($p < 0.01$) (Table 3). The temperature threshold for egg development was significantly lower than that in long-day nymphs ($p < 0.01$), which, in turn, was significantly higher than the temperature threshold for nymphal development under the short-day regimen ($p < 0.01$). The coefficients of linear regression of nymphal de-

velopmental rate on temperature were not significantly different between the two photoperiods (Table 3, Fig. 7), indicating practically the same degree of temperature sensitivity.

Comparison of the Life-History Parameters in the Bug *G. lineatum* (L.) from the St. Petersburg and Bryansk Populations

Survivorship. There were no interpopulation differences in the percentage of individuals that successfully completed immature development ($H_{1, 335} = 0.01$, $p > 0.9$), and the effect of origin on nymphal survival
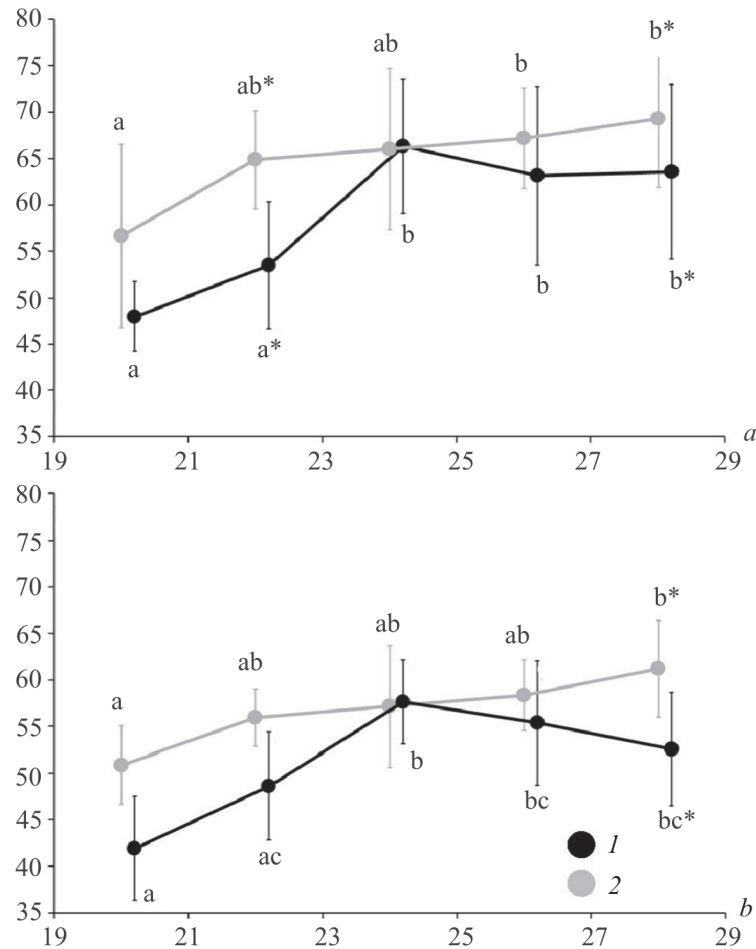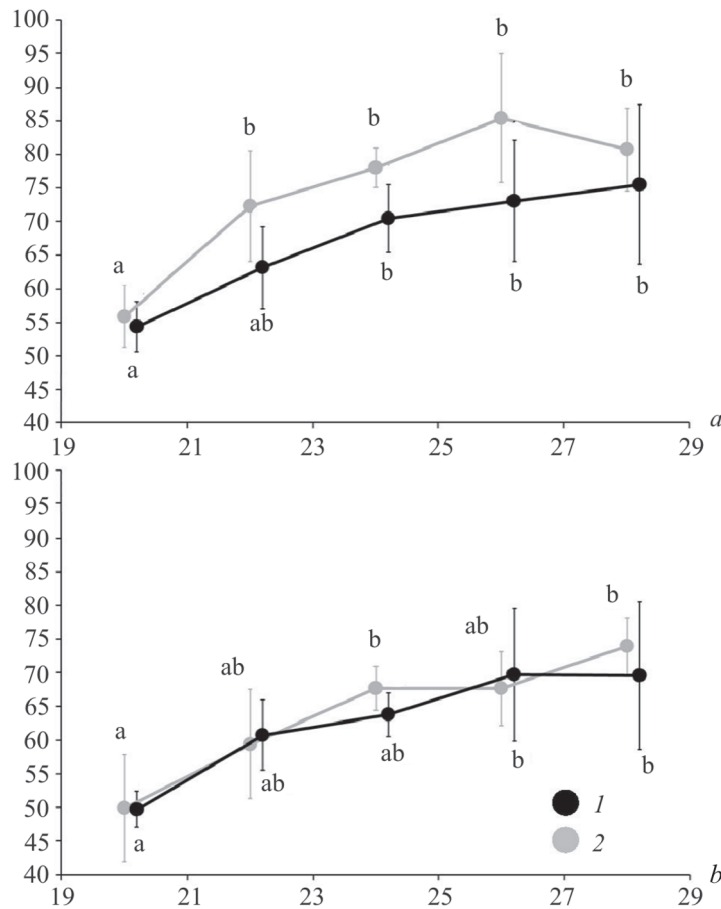
Fig. 5. Female and male body mass in the bug *Graphosoma lineatum* (L.) from the Bryansk population under various conditions of temperature and photoperiod (early-season experiment, 2016). Abscissa: temperature (°C), ordinate: body mass (mg). (*a*) Females, (*b*) males. Photoperiodic conditions: *1*, day length 12 h; *2*, day length 18 h. The data shown are the mean values. Vertical bars denote standard deviations. Significant differences between temperature treatments within photoperiodic regimens (Tukey's HSD test, $p < 0.01$) are denoted with different superscripts. Asterisks denote significant differences (Tukey's HSD test, $p < 0.01$) between contrasting photoperiodic regimens.

rates was marginally significant ($H_{1, 331} = 5.3$, $p > 0.02$). Nymphs of the St. Petersburg population fared somewhat better under experimental conditions.

Egg development. According to ANOVA, bugs from the two populations differed in egg development time ($F_{1, 438} = 10.2$, $p < 0.001$), although the difference was fairly small (Tables 1 and 5). Only the difference at 20°C turned out to be significant (Tukey's HSD test, $p < 0.0001$). Eggs of St. Petersburg bugs developed slightly faster at 20 and 22°C and eggs from the Bryansk population, at 26 and 28°C. The temperature by origin interaction was significant ($F_{4, 438} = 6.8$, $p < 0.0001$). The temperature thresholds for egg development and the corresponding regression coefficient values were significantly higher in the Bryansk population ($p < 0.01$) (Table 3, Fig. 8).

Nymphal development. ANOVA did not reveal any significant interpopulation differences in nymphal development time in either late-season or early-season experiments (Tables 1 and 5). Nymphal development time in the bugs from the two populations significantly differed at 24°C under the long-day regimen and at 22, 24, and 28°C under the short-day one (Tukey's HSD test, $p < 0.008$) but all these differences between the two populations were inconsistent. Nevertheless, the double temperature by origin interaction ($F_{4, 1142} = 6.1$, $p < 0.0001$) and the triple temperature by photoperiod by origin interaction ($F_{4, 1142} = 37.6$, $p < 0.0001$) turned out to be significant.

In the late-season experiments, nymphs of the St. Petersburg population developed slower than those from Bryansk in all of the experimental regimens ($F_{1, 1308} = $

Fig. 6. Female and male body mass in the bug *Graphosoma lineatum* (L.) from the Bryansk population under various conditions of temperature and photoperiod (late-season experiment, 2015). Abscissa: temperature (°C), ordinate: body mass (mg). (*a*) Females, (*b*) males. Photoperiodic conditions: *1*, day length 12 h; *2*, day length 18 h. The data shown are the mean values. Vertical bars denote standard deviations. Significant differences between temperature treatments within photoperiodic regimens (Tukey's HSD test, $p < 0.01$) are denoted with different superscripts. Differences between photoperiodic regimens are nonsignificant (Tukey's HSD test, $p > 0.08$).

128.6, $p \ll 0.0001$, Tables 1 and 5). Significant differences in nymphal development time under long-day conditions were found at the temperatures of 20, 22, and 24°C (Tukey's HSD test, $p < 0.0001$) and marginally significant ones, under the short-day conditions at 20 and 22°C (Tukey's HSD test, $p < 0.02$). There were significant interactions between the factors of origin and photoperiod ($F_{1, 1308} = 17.7$, $p < 0.0001$) and origin and temperature ($F_{1, 1308} = 23.8$, $p < 0.0001$).

Thermal reaction norms for nymphal development early in the season did not differ between the two populations under long-day conditions, whereas in late-season experiments, the linear regression coefficient and the temperature threshold were significantly higher in the bugs from the St. Petersburg population ($p < 0.01$).

In both early-season and late-season experiments, nymphal development in the bugs from the St. Petersburg population was characterized by significantly greater temperature sensitivity and temperature threshold ($p < 0.01$, Table 3).

Adult body mass. In the early-season experiments, male and female bugs of the St. Petersburg population were somewhat larger than their counterparts from Bryansk under both photoperiods ($F_{1, 1122} = 35.2$, $p \ll 0.0001$) (Fig. 9). In the late-season experiments, bugs of the Bryansk population turned out to be larger ($F_{1, 1286} = 155.7$, $p \ll 0.0001$) and there were significant interactions between the factors of origin and photoperiod ($F_{1, 1286} = 18.7$, $p < 0.0001$) and origin and temperature ($F_{1, 1286} = 8.4$, $p < 0.0001$).

Table 6. Durations (in days) of preimaginal development in female and male bugs *Graphosoma lineatum* (L.) from the Bryansk population under various conditions of temperature and photoperiod

Temperature (°C)		Day length (h)	Females	N	Males	N
set	real					
Early-season experiment (2016)						
20	19.7	12	46.9 ± 1.90	9	49.6 ± 3.51	7
	20.0	18	57.1 ± 5.85	12	54.8 ± 3.12	10
22	21.6	12	32.4 ± 2.29	25	33.4 ± 1.28	17
	21.9	18	38.3 ± 2.62	23	39.1 ± 1.83	15
24	22.8	12	30.0 ± 1.68	28	30.5 ± 1.85	35
	23.8	18	28.0 ± 1.49	24	28.1 ± 1.33	37
26	25.8	12	20.0 ± 1.07	34	20.0 ± 1.17	38
	26.0	18	23.2 ± 1.17	30	23.3 ± 1.45	17
28	28.2	12	17.4 ± 1.56	49	17.4 ± 1.28	30
	27.7	18	18.4 ± 1.05	56	18.6 ± 0.88	35
Late-season experiment (2015)						
20	19.9	12	46.2 ± 1.94	6	47.0 ± 1.41	2
	19.9	18	53.0 ± 1.22	5	53.5 ± 0.71	2
22	21.8	12	31.8 ± 1.28	8	32.5 ± 1.60	8
	21.9	18	36.5 ± 1.38	12	36.9 ± 1.55	8
24	24.0	12	23.8 ± 0.92	10	24.4 ± 0.84	14
	23.9	18	26.1 ± 0.83	15	26.6 ± 0.97	10
26	26.2	12	19.4 ± 0.83	15	19.3 ± 1.06	10
	25.8	18	20.8 ± 0.46	8	21.2 ± 0.41	6
28	28.1	12	16.6 ± 0.86	21	16.6 ± 1.16	14
	28.0	18	17.6 ± 0.65	14	18.5 ± 0.71	2

Durations are expressed as means ± SD. N is the number of individuals that successfully completed immature development.

DISCUSSION

Temperature is the primary factor affecting survival of *G. lineatum* bugs from the St. Petersburg and Bryansk populations. Nymphal survivorship is reduced at lower temperatures but bugs from the more northerly population turned out to be more tolerant to low rearing temperatures.

Interpopulation Variation of Thermal Requirements for Egg Development in the Bug G. lineatum (L.)

Photoperiodic conditions do not influence the duration and temperature sensitivity of egg development in the bugs originating from either St. Petersburg or Bry-

ansk, which is also known in other species (Kutcherov and Kipyatkov, 2011a; Lopatina et al., 2011c; Kutcherov et al., 2011, 2018; Lopatina et al., 2007). Temperature sensitivity of egg development is higher than that in nymphs, which is also typical of eggs and nymphs (or larvae) of most insects studied in this regard (Lopatina et al., 2011a; Kipyatkov and Lopatina, 2015; Kutcherov, 2015, 2016, etc.).

We discovered interpopulation differences in the thermal reaction norms for egg development. Temperature sensitivity and temperature thresholds for egg development are higher in the bugs from the more southerly Bryansk population. The lines of regression of egg developmental rate on temperature for the bugs from the
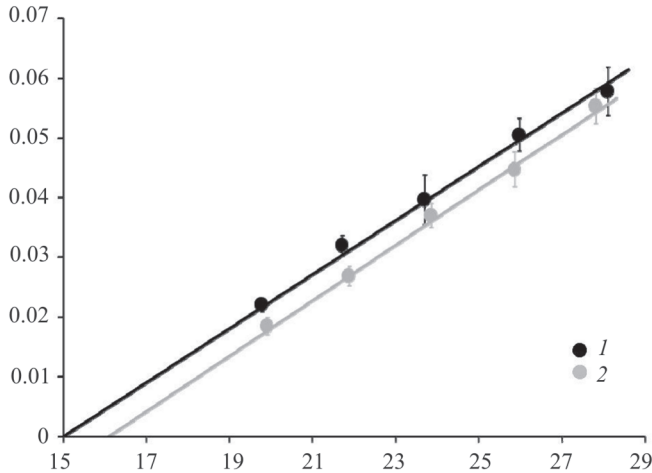
Fig. 7. Thermal reaction norms for nymphal development in the bug *Graphosoma lineatum* (L.) from the Bryansk population under different photoperiods (data from two experiments combined). Abscissa: temperature (°C), ordinate: developmental rate (d⁻¹). Experimental conditions: *1*, day length 12 h (data from two experiments combined); *2*, day length 18 h. The data shown are the mean values. Vertical bars denote standard deviations.
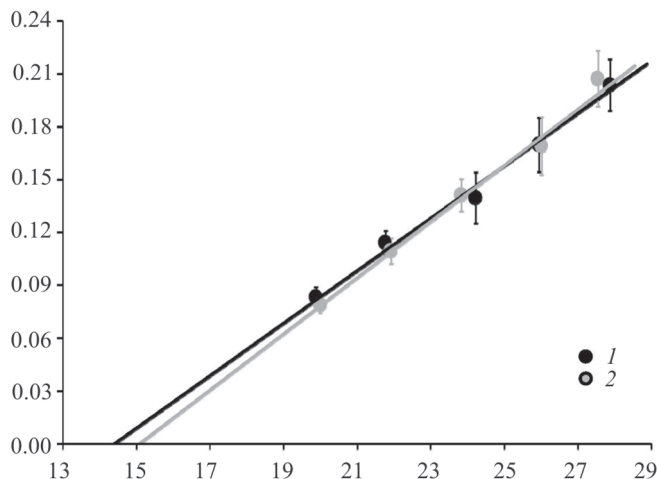
Fig. 8. Interpopulation differences in the thermal reaction norms for nymphal development in the bug *Graphosoma lineatum* (L.) (data from two photoperiodic regimens and from two experiments combined). Abscissa: temperature (°C), ordinate: developmental rate (d⁻¹). Populations: *1*, St. Petersburg; *2*, Bryansk. The data shown are the mean values. Vertical bars denote standard deviations.

two populations intersect at 25°C (Fig. 8). Eggs of the bugs from the more northerly St. Petersburg population develop more rapidly than eggs of the bugs from the more southerly Bryansk population at 20 and 22°C but slower than the latter at 26 and 28°C. Presumably, lower temperature sensitivity of egg development in the bugs of the St. Petersburg population, which would allow for their more rapid development at temperatures below

25°C, may be advantageous early in the summer when the weather in Leningrad Province is still rather cool. Bugs lay eggs openly on plant leaves, and so daily temperature fluctuations may have a considerable impact on their development. Comparison of still more remote populations would provide a more definite answer as to whether thermal reaction norms for egg development in *G. lineatum* exhibit interpopulation variation and what pattern this variation follows. Previously, well-defined interpopulation variation in thermal reaction norms for egg development was discovered in the linden bug *Pyrrhocoris apterus* (Kipyatkov and Lopatina, 2010). Eggs may have a less pronounced interpopulation variation in thermal requirements for development than nymphs do because eggs develop much faster and thus differences in the duration of the egg stage may be too small to be detected by daily censuses. Furthermore, in some insect species, egg development takes place in the leaf litter or soil where the temperature is more stable and more similar at different latitudes (Lopatina et al., 2011a, 2011b).

Interpopulational and Seasonal Variation of Thermal Requirements for Nymphal Development in the Bug G. lineatum (L.)

Photoperiodic conditions influence the rate of nymphal development so that bug nymphs from both populations develop faster under short-day photoperiodic conditions than under long-day ones at all the temperatures (Figs. 3 and 7). Under the short-day conditions, there is a modification of thermal reaction norms for development in late-season St. Petersburg bugs (the 2014 experiment) and in Bryansk bugs during both experiments. The temperature threshold is lowered but temperature sensitivity remains unchanged (and so does the sum of degree-days), i.e., the leftward parallel shift of the regression line for temperature-dependent developmental rate (Figs. 3 and 7). This modification pattern means that acceleration is disproportionate: it is more pronounced at a low temperature as compared with a high one with an average increase in developmental rate being from 15% at 20°C to 7% at 28°C in the bugs from the St. Petersburg population and from 16% at 20°C to 4% at 28°C in bugs from Bryansk.

It may be conjectured that emergence of such photoperiodic modification has ecological significance. Towards the fall season, as day length decreases and the mean daily temperatures drop, disproportional acceleration

of development enables the bugs of both populations to develop faster at relatively lower temperatures of late summer and early fall, successfully complete immature development, and enter dormancy at the adult stage.

Photoperiodic plasticity of thermal reaction norms for nymphal development in the late-season bugs of the St. Petersburg population (the 2016 experiment) is manifested in a different manner. The temperature sensitivity coefficient and the temperature threshold for nymphal development are lower under the long-day conditions than under the short-day ones (Table 3), i.e., the nymphal developmental rate is less temperature-dependent under the long-day photoperiodic conditions. Relative acceleration of nymphal development under the short-day ones in this case is more uniform across temperature and amounts to 11% at 20°C and 14% at 28°C.

The results of early- and late-season experiments did not differ from each other in the Bryansk population under either photoperiod and in the St. Petersburg population reared under the long-day conditions (Table 1). Diet and the rearing method were identical in all of the experiments, and the only aspects that differed were the time of collection of parental adults in the field and oviposition dates. In 2014, we were collecting eggs from 1.VII to 11.VII (late-season eggs) and in 2016, from 6.VI to 24.VI (early-season eggs), i.e., almost a month earlier. It may be hypothesized that differences in the physiological age of the adults somehow affected the rate of development of newly emerged nymphs kept under the long-day photoperiod. Under the short-day conditions, parental effects are masked by the influence of photoperiodic conditions. Early-season nymphs that emerge from the eggs laid in the first half of June would develop under the long-day conditions. It would be advantageous for them to develop more rapidly at relatively low temperatures of 20 and 22°C, which are typical early in summer in Leningrad Province. Early-season larvae are characterized by weaker temperature-dependence of development (a shallower slope of the regression line) and a lower value of the temperature threshold for development, compared with late-instar nymphs (Fig. 3, Table 3). Therefore, under the long-day conditions, the rate of development of early-season nymphs is less sensitive to temperature changes. Late-season nymphs develop relatively faster at higher temperatures of 26 and 28°C, which are typical of the first half of July, when the day length only

begins to decrease. Temperature sensitivity of their development and their temperature threshold increase, i.e., the rate of development of late-season nymphs gradually becomes more sensitive to temperature changes even under the long-day conditions. As the day length decreases, the thermal reaction norms for development change further. The nymphal developmental rate increases in the whole temperature range, while the degree of temperature sensitivity (the slope of the regression line) remains unchanged and the temperature threshold decreases (Fig. 3). Previously, a similar parental effect on the rates of nymphal growth and development rate was noted by us in the bug *Palomena prasina* from Leningrad Province (Gusev and Lopatina, 2018).

It may be hypothesized that parentally induced seasonal changes in the thermal reaction norms are more important for the bugs from the northerly St. Petersburg population than for those from the southerly Bryansk population as the period with temperatures favorable to development is shorter in the north. This is why the results of early- and late-season experiments under both photoperiods practically coincide for the bugs from Bryansk.

Thermal reaction norms for nymphal development practically do not differ between the two populations under the long-day conditions in the early-season experiments (Table 3). Late-season nymphs from the St. Petersburg population have both higher temperature sensitivity and a higher temperature threshold for development than those from Bryansk. While the southern bugs develop faster at the temperatures below 28°C than the northern ones do, the opposite may be true at temperatures above 28°C, which may occur in Leningrad Province in the second half of July. Under the short-day conditions, both temperature sensitivity and the temperature threshold for development also turned out to be higher in the bugs from the more northerly population. Thus, in the second half of summer and early in the fall season, high temperature sensitivity and high temperature threshold for development may enable the northern bugs to develop faster than those from the southerly population at the temperatures exceeding 28°C.

According to the results of our studies carried out in Sergievka Park (Table 4), the bugs are able to choose well-insolated biotopes and bask in the sun, reaching the temperatures over 30°C even late in August. Their ability for thermoregulation and plasticity of the thermal
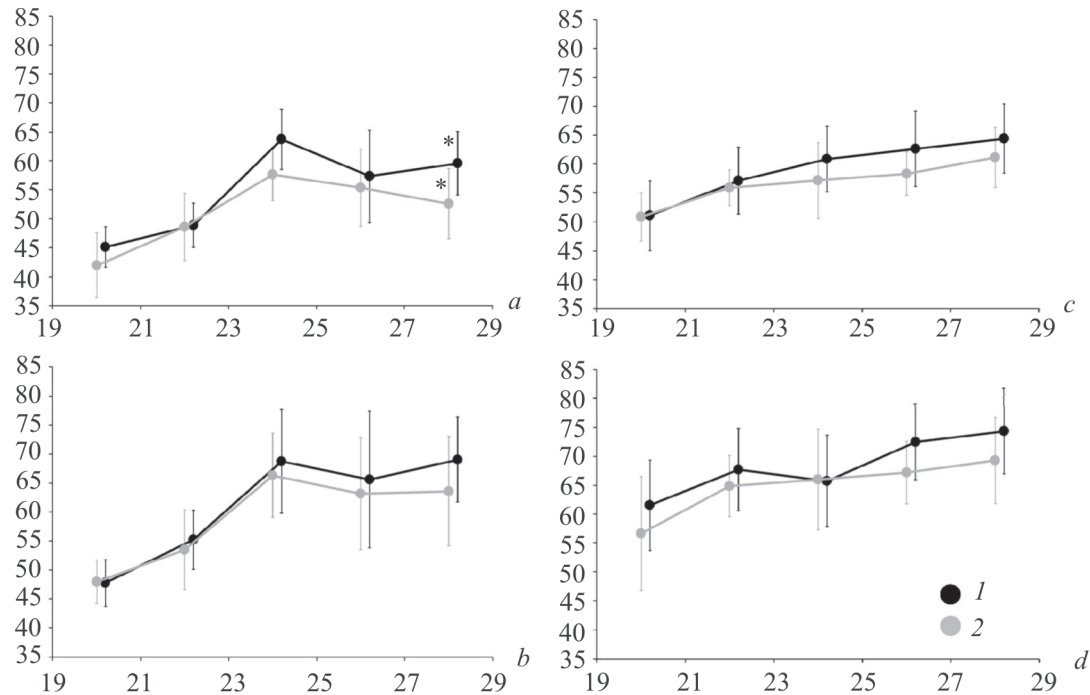
Fig. 9. Interpopulation differences in female and male body mass of the bug *Graphosoma lineatum* (L.) under various conditions of temperature and photoperiod (early-season experiment, 2016). Abscissa: temperature (°C), ordinate: body mass (mg). (*a*) Short-day males, (*b*) short-day females, (*c*) long-day males, (*d*) long-day females. Populations: *1*, St. Petersburg; *2*, Bryansk. The data shown are the mean values. Vertical bars denote standard deviations. Significant differences between populations are marked with an asterisk (Tukey's HSD test, *p* < 0.01). Symbols are slightly set apart for clarity.

reaction norms for development make it possible for *G. lineatum* bugs to thrive under the conditions of Leningrad Province. The ability to bask was earlier found by us in another bug species (Gusev and Lopatina, 2018). Thus, increased temperature sensitivity of nymphal development in this thermophilic species is arguably an adaptation to life in the north. Higher temperature thresholds and stronger sensitivity to temperature were also found in the northern populations of the linden bug *Pyrrhocoris apterus* (Kipyatkov and Lopatina, 2010).
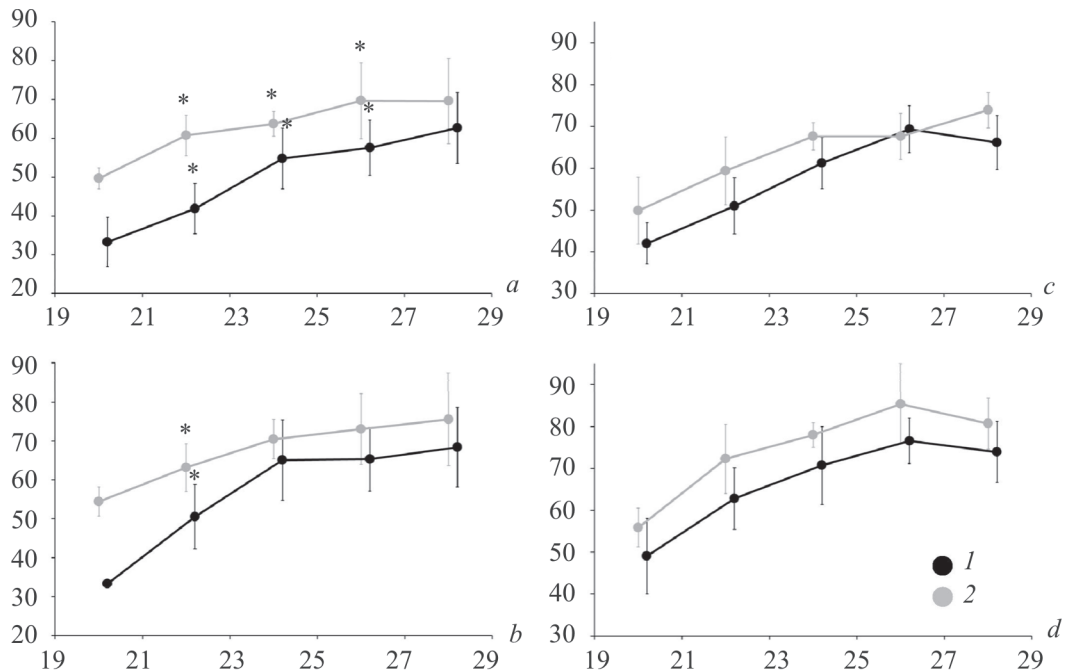
Interpopulation Differences in Body Mass and in the Pattern of Thermal and Photoperiodic Plasticity of Body Mass in the Bug G. lineatum (L.)

Two experiments, differing in collection dates, produced oppositely directed differences in body mass between the two populations. In the early-season experiments, bugs from the St. Petersburg population were slightly larger (Fig. 9), while the reverse was true of the late-season experiments: bugs from the Bryansk pop-

ulation turned out to be larger in all the experimental variants (Fig. 10). Therefore, without carrying out morphometric measurements of field-collected bugs, it is impossible to conclude whether the two populations of *G. lineatum* differ in body size.

We showed that neither temperature nor photoperiodic conditions affected the sex ratio and sexual size dimorphism.

Body mass in *G. lineatum* bugs from the Bryansk and St. Petersburg populations increased with temperature rise under both photoperiodic regimens (Figs. 2, 5, and 6), this tendency being more conspicuous in the bugs from the more northerly population. This pattern violates the "temperature-size rule" according to which ectothermic organisms attain a greater body size at higher temperatures (Atkinson, 1994). It is known that the thermal plasticity of body mass may be differently expressed in insects under the influence of abiotic factors (e.g., day length: Kutcherov et al., 2011; Kutcherov and Kipyatkov, 2011; Lopatina et al., 2011c; Ryzhkova

Fig. 10. Interpopulation differences in female and male body mass of the bug *Graphosoma lineatum* (L.) under various conditions of temperature and photoperiod (late-season experiment, 2014 and 2015). Abscissa: temperature (°C), ordinate: body mass (mg). (*a*) Short-day males, (*b*) short-day females, (*c*) long-day males, (*d*) long-day females. Populations: *1*, St. Petersburg; *2*, Bryansk. The data shown are the mean values. Vertical bars denote standard deviations. Significant differences between populations are marked with an asterisk (Tukey's HSD test, $p < 0.01$).

and Lopatina, 2015a, 2015b) and biotic factors (e.g., diet: Diamond and Kingsolver, 2010) and may also be dissimilar in individuals from different geographic populations (Lopatina et al., 2011a; Hassall, 2013; Kutcherov et al., 2015; Ryzhkova and Lopatina, 2015b, 2015c).

In *G. lineatum* bugs from both populations, more rapid nymphal development under a short day is accompanied by a reduction in body mass (Figs. 2, 5, and 6). The emergence of smaller adults under the short-day conditions, which also accelerate development, is widespread among the lepidopterans (Nylin et al., 1995; Gotthard, 1998; Ryzhkova and Lopatina, 2015a, 2015b). More often, however, individuals that have developed under the short-day conditions and are determined to overwinter have a larger body size (Danks, 1987) due to accumulation of reserves before diapause (Tauber et al., 1986). Presumably, bugs may benefit from completing immature development in time, even at the cost of not reaching the maximum body mass, as they still have opportunities to continue feeding and accumulate winter reserves at the adult stage before the onset of cold weather. In any case, nymphs that have failed to molt to the adult stage will perish, whereas smaller adults will

have a chance to overwinter and to reproduce during the next year.

Under all the experimental regimens, *G. lineatum* females from both populations are larger than males (Figs. 2, 5, and 6), which is typical of most arthropod species (Stillwell et al., 2010).

There are no interpopulational or sexual differences in the degree of photoperiodic plasticity of body mass in *G. lineatum*. The relative difference in female and male body mass between the two photoperiodic regimens in the St. Petersburg population varies from 4 to 25% and from 6 to 26%, respectively, depending on the rearing temperature. In female and male bugs from the Bryansk population, this difference varies from 6 to 25% and from 5 to 22%, respectively. Previous studies have shown that the photoperiodic plasticity of body mass may be expressed to a different degree in the individuals belonging to the opposite sexes or originating from different geographic populations. Body mass was more phenotypically plastic in female leaf beetles *Gastrophysa viridula* (Kutcherov and Kipyatkov, 2011b), and in the St. Petersburg population of the but-

terfly *Inachis io* (Ryzhkova and Lopatina, 2015a.) In the butterfly *Inachis io* from the Bryansk population, male body mass turned out to be more plastic (Ryzhkova and Lopatina, 2015b).

CONCLUSIONS

(1) A novel form of plasticity of thermal reaction norms for development in insects is described for the first time, namely, a disproportional acceleration of development under the short-day photoperiodic conditions. This developmental acceleration is more pronounced at lower temperatures as compared with warmer conditions. Thereby, temperature sensitivity of development is not changed but the temperature threshold for development decreases.

(2) It is hypothesized that nymphal development in the St. Petersburg population of the bug *G. lineatum* is modified by a parental effect that may cause a seasonal change in the thermal reaction norms for development under long-day photoperiodic conditions, namely, an increased temperature threshold and stronger sensitivity of development to temperature in late-season larvae.

(3) Interpopulation variation in the thermal reaction norms for development is discovered in eggs and nymphs of *G. lineatum*: lower values of the temperature sensitivity coefficient and temperature threshold for development in eggs and higher values of these parameters in late-season nymphs from the St. Petersburg population compared with those from the Bryansk population. The thermal reaction norms for development in early-season nymphs from these populations hardly differ.

(4) Bugs from both populations studied attain a greater body mass at higher temperatures. There are no interpopulational or sexual differences in the degree of photoperiodic plasticity of body mass in *G. lineatum*.

(5) The results obtained confirm our view that the thermal reaction norms for insect development can be phenotypically plastic in the course of the season, depending on the oviposition date, photoperiodic conditions, food quality, and other factors, as well as exhibit interpopulation variation within the species' distribution range.

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