
COMPARATIVE AND ONTOGENIC
PHYSIOLOGY

**Study of Intrapopulation Variability of Duration
and Temperature Norms of Development of the Linden Bug
Pyrrhocoris apterus (Heteroptera, Pyrrhocoridae)**

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Received July 17, 2007

Abstract—The existence of significant variability in duration and temperature norms of development between families within insect populations has been shown for the first time. This variability is interfamily and therefore has genetic ground. Revealed for the first time is the statistically significant positive correlation between the regression coefficient of the development rate for temperature and the temperature threshold for development of eggs and larvae from different families. The greater the slope of the regression line of the development rate for temperature, the higher the temperature threshold value in this particular family. These results demonstrate for the first time the existence of genetic co-variation between the regression coefficient and the temperature threshold within the insect populations. It is suggested that the source of the interpopulational and interspecies changes in the temperature reaction norms of the insect development might be the intrapopulational hereditary variability of the development duration, regression coefficient, and the development threshold, this variability being an object of natural selection. It was shown that in all studied families and populations the values of the linear regression coefficient of development rates for temperature in eggs of the linden bug *Pyrrhocoris apterus* were markedly and statistically significantly higher, while the temperature threshold values—lower as compared with the corresponding parameters in larvae. These results obviously are in contradiction with the concept of the “isomorphism of development rates” (Jarosik et al., 2002), according to which the development threshold for all life cycle stages of a species should be the same, whereas only slopes of the regression lines of the development rate for temperature can differ. For the first time the absence of genetic covariation has been shown between the temperature norms of development of different life cycle stages of the species—eggs and larvae. This means that the regression coefficient as well as the sum of the degree-days and the development threshold in eggs and larvae are inherited independently and therefore they can be independently changed in evolution in correspondence with specific environmental conditions, under which these life cycle stages take place.

DOI: 10.1134/S0022093008060069

Key words: *Pyrrhocoris apterus*, temperature norms of reaction, temperature threshold, thermolability, variability, isomorphism of development rate.

INTRODUCTION

According to the theory of life cycles, physiological norms of reaction of poikilothermal organisms to temperature are to depend on the climate,

i.e., to have geographic variability, as the natural selection optimizes parameters of development and reproduction to provide their correspondence with the climate conditions in each local population [1–4]. It is to expect the presence of such in-

traspecies geographic variability also for the parameters characterizing the temperature norms of the insect development—for the thermolability coefficient of development (i.e., for coefficient of equation of the linear regression of the development rate for temperature) and for the development temperature threshold [5]. Indeed, this variability was revealed in several species (see reviews [5, 6]), including the linden bug [7].

There are many papers in the literature on study of temperature norms of the insect development from different populations of one species. A part of species demonstrate a significant stability of the development norms in the whole area, for example, the golden-eye *Chrysosopa oculata* [8], the seven-spotted ladybird *Coccinella septempunctata* [9], the oleander aphid *Aphis nerii* [5], another part—a variability involving both the temperature threshold (the ground beetle *Pterostichus nigrata* [10], the corn borer *Ostrina nubilalis* [11]) and the thermolability coefficient (the treehole mosquito *Toxorhynchites rutilus septentrionalis* [12]) or changes of both parameters (the ants *Lasius* and *Murmica* [13, 14], the grasshopper *Melanoplus sanguinipes* [15], the potato beetle *Leptinotarsa decemlineata* [16]). It is obvious that at least some insect species during distribution in the area were adapted to the environment conditions by changing the temperature norms of development.

The adaptive meaning of the intraspecies geographic variability of the temperature norms of insect development is not clear until now. There are no clear proofs that it appears as a result of natural selection. Groeters [5] is right to note that all studies carried out so far have demonstrated only the presence of interpopulational variability of the development temperature norms in several species, whereas in order to prove the presence of the genetic heritability of the thermolability coefficient and development threshold, it is necessary to study the intraspecies variability of these parameters as a possible object of selection. So far, nobody has carried out such studies.

Therefore, we posed a task to reveal the intrapopulational variability of the development temperature norms in the linden bug *Pyrrhocoris apterus*; it is a convenient object of study owing to simplicity of its collection in nature and easiness of breeding and maintenance in laboratory. This task

could be solved by comparing temperature norms of development of insects from different families, i.e., genetically similar siblings originated from different parent pairs. We performed such comparison in six linden bug geographic populations.

MATERIALS AND METHODS

The bugs overwintering in nature were collected in Kostroma, Moscow, Tula, Orel, Voronezh and the Borisovka Village of the Belgorodskaya District in spring 2006. They were kept in laboratory at 28°C and the daylight daily duration of 20 h. This daylight duration was chosen because it deliberately exceeds the critical one (17 h 30 min for bugs from the Belgorodskaya District according to data of Volkovich and Gorishin [17]) and, hence, does not produce diapause in imago. The food was seeds of the large-leaved lime. The water was given in test-tubes closed by a cotton-wool. The randomly chosen males and females were placed in pairs in plastic Petri dishes. Offspring of each pair, i.e., siblings, were maintained and studied separately from the same other families. To study the temperature norms of development, the insects were maintained in photothermostats with the daylight duration of 20 h and the temperature regimes of 20 ± 0.5, 22 ± 0.5, 24 ± 0.5, 26 ± 0.5, and 28 ± 0.5°C. The temperature higher than 28°C was not used, as at the elevated temperatures the development rate is too high, which needs more frequent recording for the precise measurement of the development duration, what is technically hard to accomplish. The temperature lower than 20°C was unfavorable for development of the thermophilic bugs. The temperature was measured by using Tinitalk^R autonomous microprocessor sensors that recorded temperature each hour. This was necessary for the more precise evaluation of the experimental temperatures.

Twice a day, in the morning and evening, the cages with bugs were examined and the laid eggs were exposed at different temperature regimes by keeping the randomization rules. The larva hatching from eggs also was recorded twice a day. The error of measurement of the egg development duration was ± 0.5 days. The larvae were provided by food and water and were kept at the same temperature regimes, as the eggs, with the daylight dura-

tion 20 h. To detect the moment of the imago appearance, the cages with insects were examined once a day. In this case the error of the measurement of the larva development duration was ± 1 day. The used intervals between the measurements, 0.5 and 1.0 day, did not exceed the standard errors of the mean development duration of eggs and larvae, respectively, which fits requirements of statistics and allows the adequate evaluation of variability of the measured parameters [18, 19].

As a result of the observations, the egg and larva development duration (D) was obtained and compared at the designed temperatures. Then the reverse values of the development duration were calculated—the development rates $R = 1/D$. By assuming linear dependence of the development rate on temperature within the limits of the used interval, the coefficient of equation of the linear regression of the development rates R was calculated at the temperature T for each family:

$$R = a + bT,$$

where a —constant and b —coefficient of the linear regression, which also is the thermolability coefficient. The value of the low temperature threshold (t_0) was determined by extrapolating the regression line until $R = 0$ from the formula: $t_0 = a/b$. The standard errors of constant and regression coefficient were obtained from regression analysis, while the threshold error was calculated by formulas from the special literature [20]. These calculations were performed by using special software DevRate 4.1 (©V. E. Kipyatkov, 2006) realized in the supplement Quattro Pro 9.0.

Statistical procession of data was performed using the supplement Statistica 6.0. The statistical significance of interfamily differences in development duration in individuals within the populations was determined by using dispersion analysis (ANOVA) and subsequent paired comparison of the mean values with aid of a posteriori (*post hoc*) criteria (the Tukey HSD test). To check the normal distribution, the Kholmogorov—Smirnov test was used. If the distribution of the development duration differed statistically significantly from the normal one, the logarithmic data transformation was used. The same transformation was used, if an essential correlation was observed between the means and dispersions. In the cases that taking log-

arithm did not eliminate the problem, non-parametric methods (the Kruskal—Wallis ANOVA) were used. The association between the regression coefficient and the temperature threshold was determined by calculating the Pearson coefficient of linear correlation. The individual values of the regression coefficient and the temperature thresholds were compared by using the t -test.

The development temperature norms were determined for 76 families originated from 6 geographic populations (Table 1). The number of the studied individuals in the families varied from 82 to 483 for eggs and from 48 to 358 for larvae (Table 1). In some families, as a result of unusually high death rate of the insects, it was impossible to obtain data for one, or more rarely, for two out of five used temperatures (Table 1). For these families, the regression lines of the development rate for temperature were calculated from data for four or three temperatures, respectively.

RESULTS

The dispersion analysis (ANOVA or Kruskal—Wallis ANOVA) performed separately for each geographic population showed the statistically significant effect of the family factor on duration of the development of eggs and larvae (Table 2). A comparison of the mean development durations in individual families was performed at two extreme temperatures—at 20°C and 28°C. The choice of these particular temperatures was due to that the majority of regression lines of the development rate at temperature, which were constructed for individual families, are crossing in the middle part of the temperature interval (see below) and therefore it is to expect the greatest differences between the families in the development duration at these extreme temperatures. The paired comparison using the a posteriori criterion Tukey HSD has shown that the majority or a considerable part of families differ statistically significantly in the mean duration of the egg and larva development at 20°C and 28°C (Table 2). The hierarchic dispersion analysis (Nested ANOVA) was performed for the whole mass of data at temperatures of 20°C and 28°C. It showed the statistically significant effect of the family factor on duration of development of eggs ($F_{107.5181} = 87, p < 0.01$ at 20°C; $F_{93.4399} = 202,$

Table 1. Regression coefficient values of the rate of development by temperature and the development threshold of temperature (t-re) for eggs and larvae of linden bug from different families and populations

Popula- tion	Fam- ily	Eggs						Larvae					
		omitt- ed tem- peratu- res (°C)*	the numb- er of indivi- duals	regressi- on coeffici- ent (day ⁻¹ grad ⁻¹), (× 10 ⁻³)	standard error of regressi- on coeffici- ent (× 10 ⁻³)	tempe- rature thresh- old (°C)	standard error of temper- ature thresho- ld (°C)	omitt- ed tem- peratu- res (°C)*	the num- ber of indivi- duals	regressi- on coeffici- ent (day ⁻¹ grad ⁻¹), (×10 ⁻⁴)	standard error of regressi- on coeffici- ent (×10 ⁻⁴)	tempe- rature thresh- old (°C)	standard error of temper- ature thresho- ld (°C)
Kostroma	A	—	235	12.78	0.121	14.9	0.09	—	87	39.7	1.35	16.7	0.33
	B	22, 26	119	11.51	0.078	14.2	0.07	—	—	—	—	—	—
	C	—	199	11.27	0.077	13.8	0.07	22	70	36.4	2.84	16.6	0.64
	D	—	206	11.97	0.146	14.7	0.12	—	109	40.4	1.93	18.0	0.36
	EK	28	115	12.00	0.457	14.5	0.35	—	—	—	—	—	—
	G	—	199	12.31	0.212	14.7	0.16	26	63	36.1	2.07	16.4	0.46
	H	—	269	13.96	0.148	15.8	0.10	24, 28	53	33.8	1.85	15.9	0.42
	I	—	224	12.90	0.146	15.0	0.11	—	98	39.5	1.89	17.4	0.34
	K	26	82	13.37	0.138	15.1	0.10	—	—	—	—	—	—
	L	24	149	12.63	0.227	14.8	0.19	20, 24	63	32.9	1.94	16.1	0.63
	M	—	299	14.06	0.155	15.8	0.10	20, 24	80	28.3	2.95	14.0	1.41
	P	—	212	12.64	0.153	14.8	0.12	28	119	31.8	1.43	16.2	0.33
	RF	22, 26	128	11.53	0.068	14.5	0.06	—	—	—	—	—	—
		the mean			12.53	0.248	14.8	0.15	—	—	35.4	1.37	16.4
	the mean (5 t-res)**			12.74	0.333	14.9	0.22	—	—	39.9	0.29	17.4	0.37
Moscow	A	—	373	12.60	0.156	14.5	0.12	—	112	35.7	1.52	16.8	0.37
	B	—	449	12.37	0.087	14.4	0.07	—	358	38.8	0.78	17.2	0.15
	D	20	165	14.19	0.110	15.7	0.07	—	126	52.3	0.85	18.8	0.12
	E	20, 24	116	12.95	0.059	15.4	0.05	20, 24	88	44.9	1.56	17.8	0.31
	F	—	333	11.59	0.064	13.9	0.06	22	115	36.8	1.59	16.7	0.39
	G	—	284	10.37	0.148	13.0	0.16	—	125	38.8	1.48	16.8	0.31
	H	—	217	12.93	0.120	14.9	0.09	—	140	38.2	1.07	16.8	0.23
	I	—	331	11.22	0.104	13.7	0.09	—	260	37.8	1.03	17.2	0.20
	K	—	483	12.01	0.144	14.6	0.12	—	272	34.0	0.84	16.5	0.20
	L	—	214	11.95	0.223	14.4	0.18	—	—	—	—	—	—
	M	—	415	12.96	0.091	14.9	0.06	—	298	36.9	0.77	16.7	0.16
	N	—	383	14.06	0.120	15.6	0.07	—	169	36.0	1.00	16.9	0.22
	O	26	99	10.96	0.103	13.7	0.09	—	—	—	—	—	—
	P	—	551	11.55	0.097	13.9	0.09	—	241	37.6	1.23	17.0	0.27
	R	—	413	10.76	0.085	13.4	0.08	—	167	36.4	0.82	17.0	0.17
	S	—	217	13.39	0.101	15.1	0.07	—	118	39.0	1.87	17.8	0.36
	T	—	422	12.22	0.091	14.3	0.08	—	213	33.6	1.14	16.2	0.29
	И	—	297	11.14	0.285	13.4	0.27	—	190	42.3	0.97	17.7	0.18
	V	—	230	11.31	0.107	13.7	0.10	24	120	39.7	0.92	17.2	0.19
W	—	457	12.07	0.140	14.4	0.11	—	183	39.5	0.66	16.4	0.13	
Y	—	397	13.27	0.167	15.3	0.12	—	269	33.8	0.98	17.0	0.25	
	the mean			12.18	0.232	14.4	0.17	—	—	38.5	1.00	17.1	0.14
	the mean (5 t-res)**			12.10	0.231	14.3	0.17	—	—	38.2	1.10	17.1	0.15
Tula	B	—	239	11.68	0.037	14.1	0.03	—	133	40.8	1.22	16.7	0.23
	DS	20	106	11.88	0.151	14.6	0.1.3	—	—	—	—	—	—

Table 1. (Contd.)

Popula- tion	Fam- ily	Eggs						Larvae					
		omitt- ed temp- eratu- res (°C)*	the numb- er of indi- viduals	regressi- on coeffici- ent (day ⁻¹ grad ⁻¹), (× 10 ⁻³)	standard error of regressi- on coeffici- ent (× 10 ⁻³)	tempe- rature thresh- old (°C)	standard error of temper- ature thresh- old (°C)	omitt- ed temp- eratu- res (°C)*	the numb- er of indi- viduals	regressi- on coeffici- ent (day ⁻¹ grad ⁻¹), (× 10 ⁻⁴)	standard error of regressi- on coeffici- ent (× 10 ⁻⁴)	tempe- rature thresh- old (°C)	standard error of temper- ature thresh- old (°C)
	E	—	298	11.41	0.071	13.9	0.07	20	205	47.2	0.91	18.1	0.14
	F	—	248	13.15	0.278	15.0	0.20	—	149	41.8	1.16	17.3	0.22
	G	—	217	9.90	0.230	13.1	0.27	—	92	2–7.4	1.41	15.1	0.41
	I	—	266	11.63	0.099	14.1	0.09	28	155	40.9	1.45	16.7	0.21
	K	—	207	10.22	0.118	13.1	0.13	26	111	35.1	1.29	16.8	0.30
	L	—	258	11.35	0.098	14.0	0.09	22	120	42.4	2.07	17.1	0.39
	MT	—	209	14.11	0.136	15.6	0.09	—	182	42.1	1.20	17.3	0.23
	N	28	183	14.19	0.150	15.4	0.09	28	112	40.9	2.09	17.3	0.34
	P	—	340	12.17	0.084	14.3	0.06	—	141	45.9	1.05	17.5	0.18
	the mean			11.97	0.418	14.3	0.24	—	—	40.4	1.77	17.1	0.27
	the mean (5 t-res)**			11.74	0.437	14.1	0.26	—	—	39.6	3.17	16.8	0.44
Orel	A	—	238	11.78	0.120	14.2	0.11	22	129	33.0	1.04	16.1	0.29
	B	—	298	11.54	0.117	14.0	0.11	—	141	39.8	1.20	16.8	0.24
	C	—	286	11.08	0.170	14.0	0.16	—	142	42.7	1.26	17.5	0.23
	D	—	295	10.50	0.084	13.3	0.09	—	94	30.7	1.55	14.4	0.49
	E	—	290	11.95	0.161	14.0	0.14	20, 26	82	37.7	1.62	16.9	0.33
	F	—	186	11.66	0.151	14.5	0.13	—	48	43.4	3.04	18.0	0.48
	G	24	147	10.48	0.184	13.4	0.21	20, 24	112	37.8	1.71	16.5	0.43
	H	—	327	12.03	0.095	14.2	0.07	—	154	39.6	0.79	16.7	0.15
	I	—	263	12.10	0.071	14.3:	0.06	—	226	39.0	0.68	16.4	0.16
	K	—	167	10.01	0.280	13.1	0.32	26	98	42.7	0.69	16.9	0.13
	L	—	304	12.32	0.136	14.2	0.11	—	177	46.9	1.15	18.0	0.18
	M	—	261	11.00	0.091	13.8	0.09	22	90	39.7	2.66	17.6	0.52
	O	28	161	11.59	0.123	14.2	0.10	22	72	28.6	1.91	14.1	0.70
	the mean			11.39	0.198	13.9	0.12	—	—	38.6	1.44	16.6	0.33
	the mean (5 t-res)**			11.45	0.218	14.0	0.13	—	—	40.3	1.91	16.8	0.47
Voronezh	E	—	252	9.88	0.097	12.9	0.11	26	88	31.0	1.37	15.2	0.32
	F	—	268	12.18	0.169	14.5	0.14	—	155	43.2	1.06	17.1	0.21
	I	26	84	9.96	0.121	13.1	0.15	—	—	—	—	—	—
	K	—	197	11.31	0.096	13.8	0.09	20, 24	142	42.0	1.73	17.0	0.36
	N	—	114	11.30	0.139	13.9	0.13	24	112	38.3	0.52	15.6	0.13
	the mean			10.92	0.440	13.7	0.28	—	—	38.6	2.74	16.2	0.49
	the mean (5 t-res)**			11.17	0.476	13.8	0.32	—	—	—	—	—	—
Borisovka	A	—	281	10.46	0.128	13.4	0.13	—	—	—	—	—	—
	B	—	196	13.48	0.350	15.2	0.23	22	75	39.8	1.91	17.8	0.36
	C	—	303	10.89	0.115	13.6	0.11	—	252	37.3	1.08	16.8	0.23
	E	28	165	11.77	0.139	14.3	0.10	22	93	27.5	1.68	15.3	0.61
	G	—	262	11.26	0.080	14.0	0.07	20	117	46.5	1.56	18.2	0.27
	H	28	158	9.36	0.058	12.0	0.07	28	83	5.0.4	2.60	18.4	0.35
	I	—	229	10.85	0.084	13.5	0.08	26	132	36.1	0.74	16.2	0.15
	K	22, 26	105	11.30	0.081	13.7	0.07	—	—	—	—	—	—

Table 1. (Contd.)

Popula- tion	Fa- mily	Eggs						Larvae					
		omitt- ed temp- eratu- res (°C)*	the numb- er of indi- viduals	regressi- on coeffici- ent (day ⁻¹ grad ⁻¹), (× 10 ⁻³)	standard error of regressi- on coeffici- ent (× 10 ⁻³)	tempe- rature thresh- old (°C)	standard error of temper- ature thresho- ld (°C)	omitt- ed temp- eratu- res (°C)*	the num- ber of indi- viduals	regressi- on coeffici- ent (day ⁻¹ grad ⁻¹), (×10 ⁻⁴)	standard error of regressi- on coeffici- ent (×10 ⁻⁴)	tempe- rature thresh- old (°C)	standard error of temper- ature thresho- ld (°C)
	L	24	108	13.30	0.093	15.1	0.07	—	—	—	—	—	—
	M	—	155	11.21	0.100	13.8	0.09	—	69	37.8	2.07	17.2	0.43
	N	—	186	11.01	0.211	13.8	0.19	—	—	—	—	—	—
	O	—	92	10.99	0.124	13.9	0.12	—	68	39.8	1.46	16.6	0.30
	P	—	349	10.49	0.160	13.1	0.17	—	205	43.0	0.93	17.7	0.17
	the mean			11.26	0.306	13.8	0.22	—	—	39.8	2.18	17.1	2.18
	the mean (5 t-res)**			11.18	0.302	13.8	0.20	—	—	39.5	1.30	17.1	1.30

Note: One asterisk indicates the temperatures at which the eggs and larvae of some families were not studied; therefore, the parameters of the linear regression of the rate of development for temperature in these families were calculated from data for four or, sometimes, three temperatures. The bold type identifies the mean values of the temperature threshold and of the thermolability coefficient and their errors; they are calculated either in all families or only in the families studied at five temperatures. Two asterisks indicate the mean values of regression coefficients and thresholds; they are calculated only for the families studied at all five temperatures.

$p < 0.01$ at 28°C) and of larvae ($F_{82,1880} = 6.28$, $p < 0.01$ at 20°C; $F_{84,2715} = 20.6$, $p < 0.01$ at 28°C) in all geographic populations.

The values of regression coefficient of the development rate for temperature and of the development threshold of temperature for eggs and larvae of the linden bug from different families and populations are presented in Table 1. Analysis of these data has shown that individual families within the limits of one population can differ significantly by the development temperature norms (comparison of populations was beyond the task of this work). Since the regression coefficients and thresholds in some families were calculated from data for 4 or 3 temperatures (Table 1), it was necessary to check whether a decrease of the number of temperatures affected the value of these parameters. It is known [21] that elimination of the extreme temperature values at calculating the regression of the insect development rate for temperature can lead to a significant increase of the slope angle of the regression line and of the temperature threshold value. We have calculated the mean values of the regression coefficients and thresholds for each population in all families as well as only in the families studied at all five temperatures (Table 1). Anal-

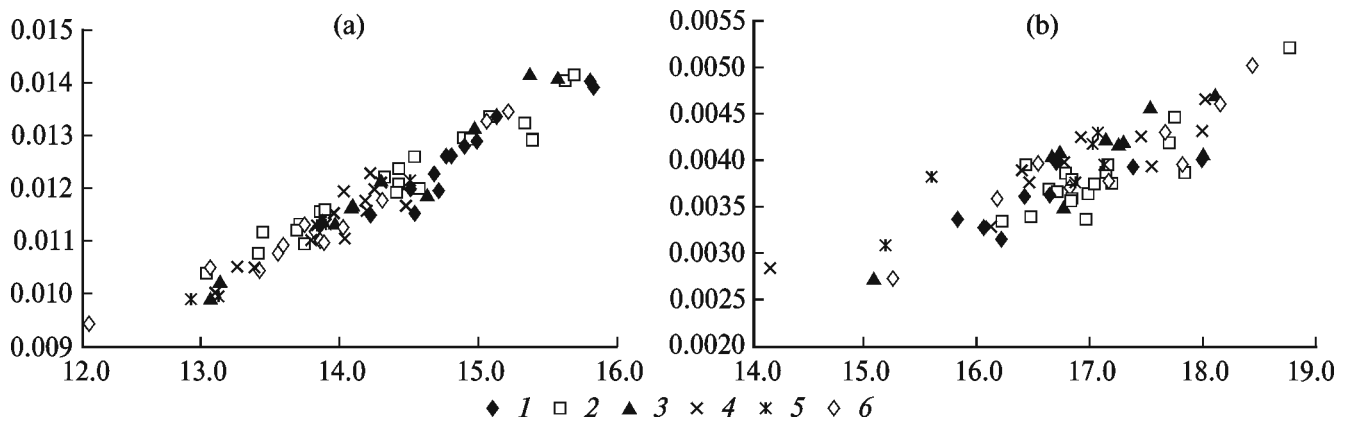
ysis of the obtained results has shown that elimination of the families studied at 3–4 temperatures leads to very slight and statistically non-significant changes of the mean values of the regression coefficient and threshold (Table 1). The absence of the effect of the number of temperatures seems to be explained by that the used temperatures are not outside the diapason favorable for development. This allowed us to use the data for families with the incomplete number of temperatures in the total analysis, which is done below.

The paired comparison of the regression coefficients and temperature thresholds of development of different families within the limits of each population was performed using the *t*-test. The differences at $p = 0.05$ were considered statistically significant. For eggs, the significant differences were obtained in 54 and 42 cases for the regression coefficient and threshold, respectively, from 78 comparisons (Kostroma), in 178 and 173 cases from 210 (Moscow), in 46 and 45 cases from 55 (Tula), in 58 and 40 cases from 78 (Orel), in 8 and 8 cases from 10 (Voronezh), and in 62 and 57 cases from 78 comparisons (Borisovka). For larvae, the significant differences were obtained in 14 and 9 cases for the regression coefficient and threshold, re-

Table 2. Results of dispersion analysis of effect of the family factor on the development duration of the linden bug eggs and larvae from different populations and results of the paired a posteriori comparison of families by duration of development at two contrast temperatures

Population	Stage of ontogenesis	Temperature (t-re, °C)	Statistic values F or H for ANOVA or Kruskal–Wallis ANOVA, respectively	Results of paired a posteriori comparison of families by Tukey HSD		
				the number of comparisons	the number of comparisons differing significantly	<i>p</i> values
Kostroma	Egg	20	$H_{15.676} = 586, p < 0.001$	–	–	–
		28	$F_{11.461} = 224, p < 0.01$	56	40	<0.02
		All 5 t-res	$F_{7.1793} = 150, p < 0.01$	56	52	<0.02
	Larva	20	$F_{10.138} = 11, p < 0.01$	55	21	<0.03
		28	$H_{7.228} = 92, p < 0.0001$	–	–	–
		All 5 t-res	$F_{2.279} = 7, p < 0.001$	3	3	<0.001
Moscow	Egg	20	$F_{32.1779} = 68, p < 0.01$	528	313	<0.02
		28	$F_{30.1438} = 200, p < 0.01$	465	366	<0.04
		All 5 t-res	$F_{16.6380} = 70, p < 0.01$	153	140	<0.01
	Larva	20	$F_{24.734} = 7, p < 0.01$	600	34	<0.047
		28	$H_{29.950} = 297, p < 0.001$	–	–	–
		All 5 t-res	$F_{14.3040} = 17, p < 0.001$	105	84	<0.01
Tula	Egg	20	$F_{12.644} = 86, p < 0.01$	78	63	<0.01
		28	$F_{11.516} = 427, p < 0.01$	66	45	<0.03
		All 5 t-res	$F_{8.2225} = 81, p < 0.01$	36	35	0 .004
	Larva	20	$F_{11.257} = 6, p < 0.001$	66	8	<0.03
		28	$F_{9.236} = 32, p < 0.01$	45	19	<0.03
		All 5 t-res	$F_{4.672} = 12, p < 0.00001$	10	10	<0.045
Orel	Egg	20	$F_{16.659} = 100, p < 0.01$	136	88	<0.02
		28	$F_{14.770} = 132, p < 0.01$	105	71	<0.01
		All 5 t-res	$F_{10.2861} = 347, p < 0.01$	3	–	–
	Larva	20	$F_{13.252} = 3, p < 0.0001$	91	4	<0.02
		28	$F_{15.445} = 29, p < 0.01$	120	46	<0.02
		All 5 t-res	$F_{5.858} = 8, p < 0.0001$	15	12	<0.007
Voronezh	Egg	20	$F_{14.505} = 477, p < 0.01$	105	90	<0.001
		28	$H_{9.456} = 381, p < 0.001$	–	–	–
		All 5 t-res	$H_{3.664} = 9, p < 0.02$	–	–	–
	Larva	20	$H_{10.199} = 76, p < 0.001$	–	–	–
		28	$F_{8.256} = 28, p < 0.01$	37	12	<0.001
		All 5 t-res	–	–	–	–
Borisovka	Egg	20	$F_{14.778} = 51, p < 0.001$	105	62	<0.001
		28	$F_{12.526} = 542, p < 0.01$	78	61	<0.001
		All 5 t-res	$F_{8.2003} = 116, p < 0.01$	36	34	<0.02
	Larva	20	$F_{11.260} = 5, p < 0.001$	66	5	<0.049
		28	$F_{10.492} = 24, p < 0.01$	55	12	<0.02
		All 5 t-res	$F_{3.574} = 6, p < 0.001$	6	4	<0.001

Note: The paired a posteriori comparison of families by the mean development duration of the eggs and larvae was possible only at use of the parametric dispersion analysis (ANOVA). In the cases that the character of the data distribution or the presence of correlation between the means and dispersions needed use of non-parametric methods (Kruskal–Wallis ANOVA), the a posteriori comparison of families turned out to be impossible.



Coefficient of regression of the development rate for temperature and the low temperature threshold of the *P. apterus* development. (a) Eggs, (b) larvae. *Abscissa*: the temperature threshold (°C), *ordinate*: regression coefficient of development rate for temperature (day⁻¹ grad⁻¹). (1) Kostroma, (2) Moscow, (3) Tula, (4) Orel, (5) Voronezh, (6) Borisovka.

spectively, from 36 (Kostroma), in 88 and 68 cases from 171 (Moscow), in 28 and 22 cases from 45 (Tula), in 48 and 47 cases from 78 (Orel), in 5 and 4 cases from 6 (Voronezh), and in 23 and 21 cases from 36 comparisons (Borisovka).

A high positive correlation was found between the values of the regression coefficient and temperature threshold (see figure). The value of Pearson correlation coefficient for the whole data massive was 0.97 ($p < 0.01$) for eggs and 0.84 ($p < 0.001$) for larvae. The correlation coefficients for individual populations varied from 0.90 to 0.99 ($p < 0.001$) for eggs and from 0.82 to 0.93 (p from 0.008 to 0.001) for larvae, except for the population from Voronezh, in which there were only four families and therefore the correlation turned out to be statistically non-significant.

Since in some families the regression lines were constructed from data for 4 or 3 temperatures, which might have led to an increase of values of the thermolability coefficient and temperature threshold [21], we performed additionally the correlation analysis in the following variants: (1) with elimination of families not represented at 20°C; (2) with elimination of families not represented at 28°C; (3) only with families represented at all five temperatures. As a result, the correlation coefficient values changed by no more than 0.01 and 0.05 for eggs and larvae, respectively, at the same level

of their significance. Thus, a decrease of the number of the temperature used for calculation of the regression parameters in some families had no effect on the whole character of the correlation between the thermolability coefficient and the temperature threshold.

We also compared the values of the regression coefficient and development threshold of larvae and eggs in each family by using the *t*-test. Out of 64 studied families, the regression coefficients differed statistically significantly in all cases ($p < 0.001$), while the threshold values differed statistically significantly ($p < 0.05$) in all families with exception of four. Thus, in linden bug eggs the development thermolability coefficients are markedly and statistically significantly higher, while the development thresholds are lower than the values of these parameters in larvae in all families (Table 2). We performed the correlation analysis to reveal a possible correlation between the temperature norms of development of eggs and larvae belonging to one family. It turned out that all coefficients of correlation between the regression coefficient values for eggs and larvae and between the threshold values for eggs and larvae in different families were statistically non-significant. The correlation coefficients varied in the different populations from -0.97 to 0.52 ($p = 0.10-0.49$) and from -0.62 to 0.61 ($p = 0.06-0.76$), while in the com-

bined sample they were -0.13 ($p = 0.33$) and -0.05 ($p = 0.72$) for the regression coefficients and temperature thresholds, respectively.

DISCUSSION

Our studies have shown for the first time the presence of a significant variability of the duration and temperature norms of development between the families inside populations. Based on general considerations, we cannot expect significant differences between all families in population; nevertheless, the presence of the considerable differences was convincingly demonstrated for a large part of families in all six studied populations. Since these differences exist between families, i.e., groups of closely related individuals, we can conclude that the intrapopulational variability of the duration, thermolability coefficient, and development threshold of linden bug has genetic ground. Thus, our study accomplished the task first formulated by Groeters [5].

The second important result is detection of the statistically significant positive correlation between the thermolability coefficient and development threshold of eggs and larvae, which also was earlier performed by nobody at the intrapopulational level. The presence of such correlation means that the greater the thermolability (a slope of the regression line of the temperature development rate) is characteristic of development of individuals of some family, the higher the temperature threshold value in this family. Hence, regression lines of the development rate by temperature are usually crossed in linden bug families. The obtained results have demonstrated for the first time the presence of the genetic interconnection (covariation) between the thermolability coefficient and the development threshold.

The direct correlation between the thermolability coefficient and the development threshold was revealed at the interspecies level by quite a few examples among invertebrates and plants [6, 21–24] as well as at the intraspecies level (interpopulation geographic variability) in several insects species [6, 13, 14, 25, 26] including linden bug. Now we can claim that the source of interspecies and interpopulation differences of the temperature norms of the insect development can be the intrapopulation

genetic variability of the duration, thermolability, and development threshold, which is the object of the natural selection. This conclusion is also confirmed by that by using artificial selection for shortening of the development duration, we have managed to obtain correlated changes of the temperature threshold and thermolability coefficient of development of *P. apterus* larvae [27].

An important result is the proof of the presence of marked and statistically significant differences between the temperature norms of development of the life cycle different stages, in this case—of linden bug eggs and larvae. It has been shown that in all studied families and populations in the linden bug eggs the thermolability coefficient of development are markedly and statistically significantly higher, while the development thresholds—lower, as compared with these parameter values in larvae (Table 1). These results obviously are in contradiction with the concept of the « isomorphism of developmental rates in insects and mites » [28], according to which the development thresholds of all life cycle stages of a species should be equal, while only slopes of the temperature regression lines can differ. We cannot rule out that this isomorphism is indeed characteristic of some insect and mite species. However, the linden bug as well as many ant species [23, 29] obviously do not fit this “regularity”, which makes it non-universal.

Moreover, our results have demonstrated the absence of the statistically significant correlation both between the thermolability coefficients of development and between the development thresholds of linden bug eggs and larvae. All correlation coefficients between these parameters are small, statistically non-significant, and have in different populations both positive and negative values, which indicates the absence of any certain correlation between the studied parameters. In other words, there has been shown for the first time the absence of genetic covariation between the temperature norms of development of different life cycle stages of the species, in this case—of eggs and larvae. This means that the thermolability and the development threshold of eggs and larvae are inherited independently and hence can change independently in evolution. Since the linden bug eggs are developed in a wet and cool ground litter (our observations), whereas the larvae choose dry, sun-

warmed areas [30], it can be suggested that the revealed absence of the genetic covariation between the temperature norms of development of eggs and larvae has the adaptive ecological grounds, as allows them to perform independent evolution.

These results also indicate the restriction of the “developmental rate isomorphism” concept. We are convinced that if the ecological conditions of habitation of different life cycle stages of the species are different, as this takes place in the linden bug, the natural selection should lead to a disturbance of the “developmental rate isomorphism” (even if it existed in ancient forms) and to formation of different norms of reaction to temperature in individuals at different stages of ontogenesis.

ACKNOWLEDGMENTS

The authors are grateful to E.B. Lopatina for valuable remarks allowing a significant improvement of the paper.

This work is supported by the Russian Foundation for Basic Research (project no. 06-04-49383) and Council for Grants of the Russian Federation President and the State Support of Leading Scientific Schools (project NSh-7130.2006.4).

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