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COMPARATIVE AND ONTOGENIC PHYSIOLOGY

Temperature and Photoperiodic Control of Diapase Induction in the Ant *Lepisiota Semenovi* (Hymenoptera, Formicidae) from Turkmenistan

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Abstract—The ants *L. semenovi* has been found to belong to species with endogenous-heterodynamic seasonal life cycles with the obligate diapause induced predominantly by factors internal for a colony, whereas external ecological factors (photoperiods and temperature) produce merely modifying effects by accelerating or delaying the diapause onset. The photoperiodic and temperature regulation of diapause induction in larvae and queens is shown. Under effect of short days and low temperature the periods of larval pupation and queen oviposition in a colony are shortened markedly, i.e., the diapause of larvae and queens occurs earlier. The daily rhythms of temperature 15/25°C and particularly 20/30°C as compared with constant temperatures 20 and 25°C that correspond to the mean circadian temperatures of the thermorhythm, inhibit manifestations of the short day effects by stimulating the non-diapause development and increasing duration of the seasonal development cycle of ant colonies. The *L. semenovi* photoperiodic reaction is quantitative, as development and pupation of larvae and egg-laying of queens cease sooner or later under both the short and the long days, but in the latter case significantly later. Thus *L. semenovi* is one more example among very rare ant species that are revealed to have the photoperiodic regulation of the colony development seasonal cycle.

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Key words: insects, ants, temperature, thermorhythms, photoperiod, seasonal development cycle, diapause.

INTRODUCTION

Seasonal changes in the climate conditions play a certain role in the ant life cycle, especially in regions with moderate and cold climate. For the ant colony to be able to use maximally the heat resources of the summer period larvae feeding and growing the sufficient number of worker and winged reproductive individuals, it should have regulatory mechanisms synchronizing processes of the brood growth, onset and arrest of larva and imago diapause with the climate seasonal rhythm.

The seasonal cycles of the most ant species living in the moderate climate are endogenous-heterodynamic and are characterized by obligatory onset of diapause in the end of the warm season; a decrease of the mean day temperature, and in some species also a shortening of day, are the regulatory factors accelerating completion of development and diapause induction [1—3]. In laboratory, with the long day and favorable temperatures, diapause begins all the same, although significantly later [1—3]. The photoperiodic reactions were detected only in some ant species—Myrmica and Aphaenogaster.

The shorting of the day duration at the summer end produces in these species a diapause formation in larvae and queens; in the ant colony the ant larvae stop pupation and queens stop the egg-laying [4–7]. The effect of the short photoperiods has also been reported in the ant *Pristomyrmex punctatus* from Japan [8]. The photoperiodic ant reactions are of quantitative character, as the colony development is terminated sooner or later under any conditions, even at the long light day and optimal temperatures, but the shorter the photoperiod and the lower temperature, as a rule, the faster the beginning of diapause [1, 2, 5, 9].

It was established in our studies that the circadian temperature rhythms (thermoperiods) provide the considerably more favorable conditions for the ant brood development and decrease statistically significantly its duration, as compared with constant temperatures [10]. It was demonstrated in the experiments on some ant species that the circadian temperature rhythms stimulated the development without diapause and increase duration of the endogenous seasonal cycle of the colony development, as compared with the constant temperatures equal to the mean circadian temperature of the thermorhythm [11–13]. Taking into account the fact that the ant nest is a natural incubator, in which, owing to the sun heat accumulation, a vertical gradient and clear circadian temperature rhythm forms used by ants for formation of the most comfortable conditions for their offspring growth [14], the circadian temperature rhythms are to be considered as the most important element of the abiotic ant life environment. Therefore, it is worth studying better the physiological aspects of effect of thermoperiods on the ant colony life.

A very convenient object for such study is *Lepisiota semenovi*—one of the common ant species in Turkmenistan. These ants make more frequently the nests in the Kopetdag mountains and foothills under the stones that are for them accumulators of the sun heat. The circadian temperature rhythms are the most obvious in these nests; therefore, we could rely on a possibility of detection of the thermoperiod physiological effects in this species. The ants construct at early spring the warming-up cameras under the stones; the ants transfer there the overwintering larvae from low horizons of soil nests and the queens are moving-in there. Therefore, on overturning the stone, it is sufficiently easy to collect the necessary number of ants together with queens and brood, i.e., the colonies of the complete composition, which is necessary for laboratory experiments. This is also promoted by polygeny, i.e., the presence of many reproductive queens in each *L. semenovi* nest.

MATERIALS AND METHODS

The *L. semenovi* colonies were collected in the mid-April in Turkmenistan and in the Kopetdag foothills. The preliminary phenological observations have allowed establishing that *L. semenovi* ant colonies overwinter with the larvae of all ages at diapause (by our data, the larvae of this species have 5 ages) and with diapausing queens ceased oviposition. The largest larvae are developing into the winged females and males, while the rest of larvae—into the worker individuals.

The collected colonies were divided in the laboratory into the experimental groups composed of 2–3 queens, 200–300 workers, and 100–150 overwintered larvae; they were placed into plastic laboratory formicaries that were placed into a photothermostat at the 25°C temperature and long light day (18 h). The ants were fed twice a week with the 10% sugar solution and with pieces of the cockroach *Nauphaeta cinerea*.

For two months of observations the complete development occurred in the ant groups: all overwintered larvae were pupated, the queens laid eggs, appearing from them were larvae that were growing and pupated. Then, on July 7, i.e., around the middle of the natural seasonal development cycle, when under the natural conditions the day begins shortening and the insects are affected by the shorter-days photoperiods, the ant experimental groups were distributed randomly to two photoperiodic and four temperature regimes that formed together 8 photometric regimes. Two photoperiods were used the long (18 h) and the short (12 h) day, two constant temperatures—20 and 25°C, and two circadian Π-like thermorhythms—15/25 and 20/30°C with 12-h periods of high and low temperature and the mean circadian 20 and 25°C temperatures, respectively. The scotophase of the long-day photoperiodic cycle of the 6-h duration was in the middle of the thermorhythm cryophase, and at the short day the scotophase of the photoperiodic cycle and the thermorhythm cryophase coincided in time. Transition from the high to the low temperature and back took about 1 hr. Maintained at each photothermic regime were 3–5 ant experimental groups.

The presence of the eggs and larvae prepared for pupation in cocoons was fixed using a binocular microscope twice a week at the nest examination. The larvae prepared for pupation are characterized by that they do not eat and construct a silk cocoon, through wall of which the larva can be viewed until the walls become sufficiently dense. After completion of the cocoon construction the larva excretes after a while the excrements accumulated during the life as a dark clot—meconium remaining in the cocoon back end and well seen from outside; the larva is transformed into the immobile pre-pupa, then into the pupa that is impossible to be seen from outside. If there is no meconium there, this means that the cocoon has been constructed quite recently. Therefore, the presence in the nest of larvae constructing the cocoon and/or cocoons without meconium was considered as a sign that the larvae continue to pupate. The absence of these signs means the absence of pupation. Besides, once a week, the number of the present pupae was counted without removing them, as narcosis using as a rule the carbon dioxide, produces a very high mortality of workers in this species.

In most regimes and in most groups the oviposition and pupation sooner or later stopped; therefore the eggs and the pupated larvae disappeared. This indicated onset of the queen and larva diapause. For each experimental ant group there was determined duration of the period of the egg presence—from beginning of the experiment to the day of the egg disappearance and of the period of pupation—from beginning of the experiment to the day when cessation of pupation (i.e., the absence of the pupated larvae) was fixed. The day of the egg disappearance or the pupation cessation was calculated as the mean between the date of this and the previous examination, during which the eggs and the pupated larvae still were present in this group. The disappearance of eggs in the group indicates that queens some time ago ceased the oviposition and all eggs were developed already in the larvae, which allows determining the time of onset the queen diapause. The cessation of larva pupation indicates directly their diapause onset. Besides, there was calculated the mean amount of the pupae presented in one experimental group at each regime per each date of counting. This parameter allows estimating and comparing intensity of larva pupation in different variants of the experiment.

The experiment was completed on January 31, i.e., 208 days after its beginning, when the queen oviposition and larva pupation stopped at the most regimes, which indicated onset of diapause in queens and larvae. Only in some groups, the queens did not yet complete the oviposition by this moment, while larvae continued to pupate (in thee out of four groups at the 20/30°C thermorhythm and the long day). In such cases we agreed conditionally that duration of the periods of the presence of eggs and pupae amounted to 208 days, thereby to decrease somewhat the possible real duration, but this has allowed us to calculate the means of these periods and to compare them by using Student's criterion.

RESULTS

The data in the table indicates duration of periods of the presence of eggs and pupation, while the figure shows dynamics of the number of pupae in experimental groups of *L. semenovi* at various photothermic regimes.

The appearance of diapause in queens. In most experimental groups maintained at the constant 20°C temperature and the 15/25°C thermorhythm, eggs have disappeared as soon as after several weeks, which indicates onset of the queen diapause (see table). Action of the short day led to a statistically significant decrease of the period of the egg presence only under the thermorhythm conditions, but not at constant temperature. At the long day, the later queen diapause onset is clearly detected under the thermorhythm conditions (29 and 27 days at the constant temperature and the thermorhythm are statistically significantly different, respectively at $p \leq 0.05$); at the thermorhythm, the oviposition was not yet completed in one out of three groups until the end of the experiment.

The constant 25°C temperature and even to the greater degree the 25/30°C thermorhythm prevented onset of the queen diapause: at the 25°C temperature, eggs disappeared in 3 out of 5 groups

Duration of development in experimental groups *Lepisiota semenovi* under different photoperiodic and temperature conditions

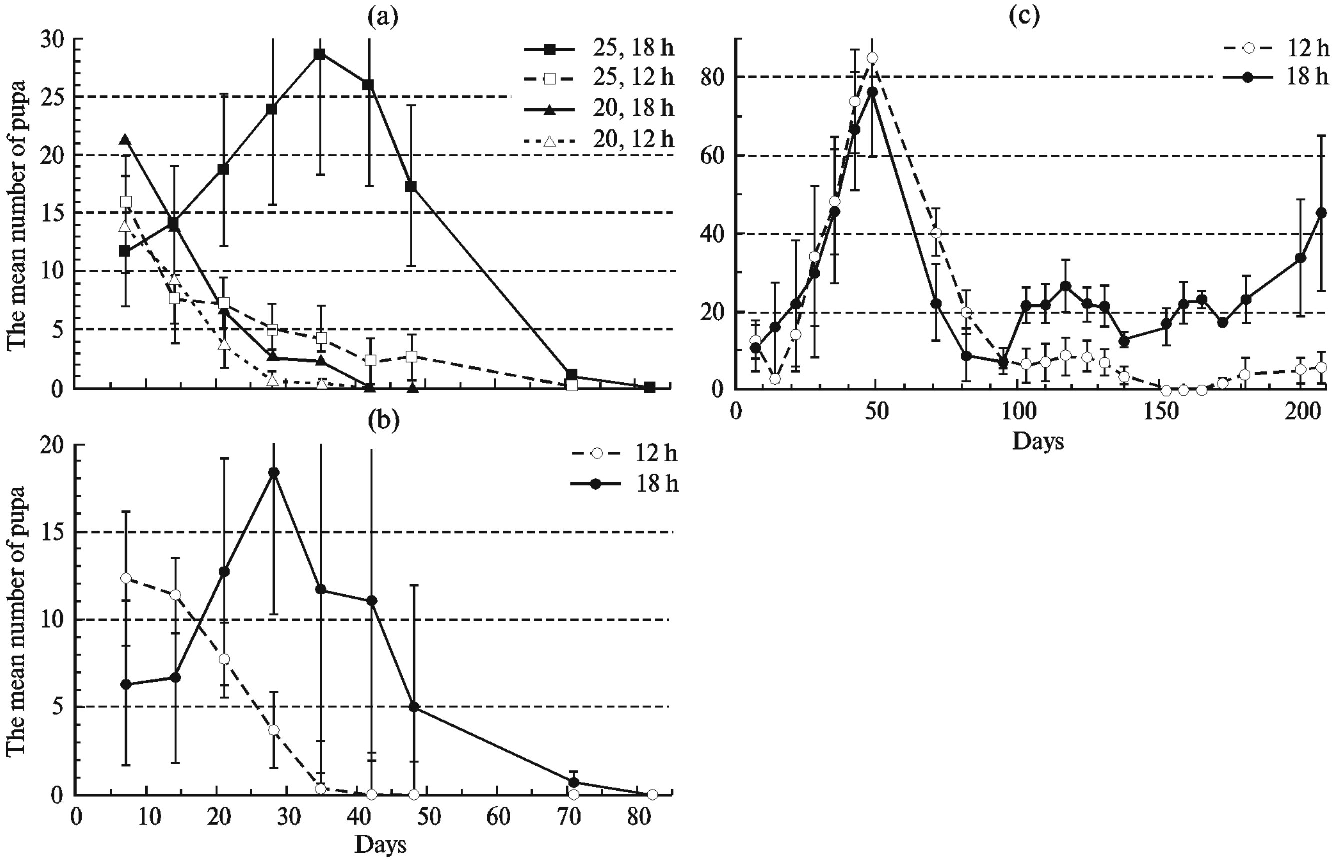
Temperature regime (°C)	Day duration (h)	The number of experimental groups			Duration of the period (days)							
		the total	with beginning of diapause		The presence of eggs				The presence of pupation			
			queens	larvae	min	max	mean	error of the mean	min	max	mean	error of the mean
25	18	5	3	5	45	208	112	1.7	39	55	48^{abc}	2.9
25	12	5	4	5	11	208	74	9.1	4	25	16^{ad}	4.1
20	18	3	3	3	25	32	29^{a}	2.3	11	18	15^{be}	2.3
20	12	3	3	3	18	32	25^{ab}	4	4	11	6^{e}	2.3
20/30	18	4	0	1	208	208	208	–	71	208	174^{cf}	34.3
20/30	12	5	0	5	208	208	208	–	39	128	95^{dg}	15.9
15/25	18	3	2	3	11	208	79	2.9	11	39	20^{f}	9.3
15/25	12	3	3	3	10	25	20^{b}	4.7	4	25	13^{g}	6.2

Note: Comparison of the means by Student's criterion was performed between the following variants: (1) 18 and 12 h at each temperature regime; (2) 25 and 20°C at 18 and 12 h separately; (3) 25 and 20/30°C at 18 and 12 h separately; (4) 20 and 15/25°C at 18 and 12 h separately; (5) 20/30 and 15/25°C at 18 and 12 h separately (the total of 12 comparisons). For duration of the period of the presence of eggs, all means except for those marked by the same letters differ statistically significantly (at $p \le 0.05$). For duration of the period of pupation, the means marked by the same letters differ statistically significantly (at $p \le 0.05$).

at the long day and in 4 out of 5 groups at the short day, while at the thermorhythm, eggs did not disappear at all till the end of experiment regardless of the photoperiod (see table). We observed at 25°C a significant photoperiod effect on the egg presence period duration, which was, on average, 74 days at the short day and 112 days at the long day. Thus, at the 25/30°C thermorhythm the photoperiod effect on the oviposition was significantly less expressed than at the constant 25°C temperature equal to its main circadian temperature. It can also be easily seen that the egg presence period duration is much longer in the ant groups at 25°C than at 20°C, both at the short and at the long day (see table).

The appearance of diapause in larvae. The short day action at the constant 20 and 25°C temperature and the 15/25°C thermorhythm produced a significant and sufficiently fast decrease of the number of pupated larvae until its complete cessation and the disappearance of pupae, whereas under conditions of the long day the larva diapause occurred more later (see table) (see Figs. 1a, 1b); at 25 and 15/25°C, intensity of pupation first even increased and only then began to decrease to reach zero sooner or later (see Figs. 1a, 1b). The photoperiodic induction of the larva diapause was the most obvious at constant temperatures: the larva pupation period decreased at the short day nearly three times as compared with the long day and amounted at 20°C, on average, 6 and 15, while at 25°C, 16 and 48 days at the short and long day, respectively (see table). The differences between the short and long day are statistically significant at $p \le 0.05$. At the 15/25°C thermorhythm, effect of the photoperiod on the mean pupation period also took place, but the differences were not statistically significant. It is also to be noted that duration of the pupation period was markedly and statistically significantly longer in the ant groups at 25°C than at 20°C, and at 20/30°C than by 15/25°C, both at the short and at the long day (see table).

Action of the 20/30°C thermorhythm was essentially different than of the constant 25°C tempera-



Dynamics of the number of pupa in experimental groups of ants *Lepisiota semenovi* under different photoperiodic and temperature conditions. (a) At constant temperatures 20 and 25°C, (b) at the 15/25°C thermorhythm, (c) at the 20/30°C thermorhythm. *Continuous line*—at the 18-day duration; *broken line*—at the 18-day duration. *Abscissa*: days of experiment; *ordinate*: the mean number of pupa. *Vertical lines*—the standard error. Beginning of experiment on July 7.

ture equal to, similar to the thermorhythm mean circadian temperature. The larva pupation at the thermorhythm was significantly longer than at the constant temperature: the pupation period at the long day was, on average, 174 and 48 days at 20/30° and at 25°C, while at the short day—95 and 16 days, respectively (see table). Under conditions of thermorhythm, during the experiment, much more larvae was pupated than at the constant temperature, both at short and at the long day, what is clearly seen at comparison of Figs. 1a and 1b. It is also to be noted that at the thermorhythm at 20/30°C under the long day conditions, the larva pupation lasted in three out of four groups until the end of the experiment and only in one group it stopped and pupae disappeared at the 71st day.

Action of short-day photoperiods at the 20/30°C thermorhythm also occurred: duration of the pupation period was, on average, 174 days at the long day and 95 days at the short day (see table). However, the larva diapause appearing at the short day and the 20/30°C thermorhythm turned out to be unstable: the larva pupation was resumed after a while in two out of five groups and continued until the end of the experiment ending, although it was not massive (see Fig. 1c).

Effect of the 15/25°C thermorhythm effect was not so different from action of the constant 20°C temperature equal to the mean circadian thermorhythm temperature. The larva pupation continued at the thermorhythm, on average, only by 5—7 days longer than at the constant temperature both at the long (on average, 20 and 15 days at 15/25° and 20°C, respectively) and the short day (on average, 13 and 6 days at by 15/25 and 20°C, respectively) (see table). However, the total number of pupated larvae was significantly higher at the thermorhythm than at constant temperature, which follows from comparison of Figs. 1a and 1b.

DISCUSSION

Results of the performed experiments allow ascribing with certainty *L. semenovi* to species with endogenous-heterodynamic seasonal life cycles, in which the appearance of diapause is obligate and is induced first of all by factors intrinsic of the colony, whereas external ecological factors (photoperiod and temperature) produce merely modifying effects by accelerating or delaying the onset of diapause. This is also confirmed by that we observed in this species the spontaneous rhythms of oviposition and development at maintenance of ants for several years under the constant temperature conditions [3], which also is characterized of endogenous-heterodynamic species [1, 2].

The results of the performed experiments also indicate the presence of the distinct photoperiodic and temperature regulation of the diapause onset in *L. semenovi.* Under effect of the short day and low temperature, duration of periods of the larva pupation and queen oviposition decreases essentially, i.e., the diapause of larvae and queens begin earlier. The photoperiodic reaction in *L. semenovi* is of the quantitative characteristic, as the larva pupation and queen oviposition stop both at the short and the long day, although much later in the latter case. Earlier, similar quantitative photoperiodic reactions were revealed in ants *Myrmica rubra, M. ruginodis*, and *Aphaenogaster sinensis* [3, 4, 6, 7].

The temperature conditions produce an essential effect on peculiarities of the photoperiodic reaction of *L. semenovi.* The queen and larva diapause began considerably earlier at 25°C at the short-day regime, as compared with the long day. The photoperiodic reaction at 20°C was similarly pronounced, but differences of duration of the larva pupation and queen oviposition between the colonies maintained at the long and short day were significantly lower than at 25°C. Hence, the 20°C temperature itself stimulates fast formation of the larva and queen diapause.

Of special interest are physiological effects of the temperature circadian rhythms as compared with action of the constant temperatures corresponding to the mean circadian thermorhythms temperatures. The performed experiments have shown that the 15/25°C and particularly the 20/30°C thermorhythms inhibit the effect of the short day by stimulating development without diapauses and increasing duration of the seasonal cycle of the ant colony development. Under the 20/30°C thermorhythm conditions, the queen oviposition did not cease at all both at the long and at the short day, i.e., the queen diapause did not occur. At this thermorhythm, the larva diapause although did occur, by but was unstable: the larva pupation at the long day was practically uninterrupted, while it ceased at the short day only for small time, but soon was resumed, although not as intensively as at the long day. This means that the high thermophase temperature of the 20/30°C thermorhythm produces a strong stimulating effect on oviposition, although the mean circadian temperature of this thermorhythm corresponds to 25°C. Thus, the variable temperatures promote an increase of duration of the seasonal development cycle, efficiency of the brood growth, and an increase of the *L. semenovi* colony production.

Earlier we demonstrated a similar effect of thermorhythms on the seasonal development cycle also in other ant species. Thus, an increase of the queen oviposition period duration was detected in *Formica cinerea* and *F. fusca* at the 15/25°C and especially at the 16/30°C thermorhythms, as compared with the constant 20 and 23°C temperatures equal to the mean circadian temperatures of these thermorhythms [11]. An increase of duration of the endogenously limited seasonal development cycle and of colony productivity at thermorhythms, as compared with the constant temperatures equal to the mean circadian temperatures, is detected in *Camponotus herculeanus* (14/32 and 16/30°C thermorhythms, 23°C constant temperature), *C. xerxes* (16/30 and 20/30°C thermorhythms, 23 and 25°C constant temperatures, respectively), *Myrmica rubra* and *M. ruginodis* (10/25 and 15/25°C thermorhythms, 17.5 and 20°C constant temperatures, respectively) [12, 13]. The uninterrupted oviposition and larva pupation occurred in the colonies of three species of the genus Tetramorium studied at 15/25°C thermorhythm; at the same time, we observed their cessation and formation of queen and larva diapause at the 20°C constant temperature [15]. The constant development occurred in *Monomorium ruzskyi* colonies at 16/30°C thermorhythm and in *Tapinoma karavaievi* at 16/30° and 20/30°C thermorhythms, whereas at 23 and 25°C constant temperatures, the queen oviposition and larva pupation stopped [15].

It is also to be noted that much more time is necessary for formation of the queen diapause than of the larva diapause, the temperature conditions being more important than the photoperiodic ones. Therefore, in the Kopetdag foothills, in the nests under stones warmed by sun, queens can long continue making oviposition until the temperature conditions allow, even when the short day begins and the larva diapause is formed. As a result, a great amount of brood is accumulated in the colonies. Since the *L. semenovi* larvae overwinter at all ages, the larvae of the first age hatched from the eggs positioned in autumn do not develop, if the environmental temperature does not allow, but start their diapause and overwinter successfully.

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