
COMPARATIVE AND ONTOGENIC
PHYSIOLOGY

A Distantly Perceived Primer Pheromone Controls Diapause Termination in the Ant *Myrmica rubra* L. (Hymenoptera, Formicidae)

V. E. Kipyatkov

Department of Entomology, State University, St. Petersburg, Russia

Received December 15, 2000

Abstract—The worker ants *Myrmica rubra* kept at long (18–20 h) days (LD) for 2–3 weeks cause the diapause termination (reactivation) in larvae and queens even under short-day (12 h) conditions (SD). The experiments performed have shown this effect to be due to a distant action of an activating stimulus that has the chemical nature and able to be spread from one laboratory nest with ants to another by means of diffusion through a double perforated net-covered partition or with an artificial air flow. The transfer of this stimulus does not require tactile contacts or food exchange between workers. The chemical nature of the activating stimulus was confirmed by the experiments, in which reactivation was produced by killed LD workers that were ground in a mortar and added twice a day to the nest with diapause ants kept at SD. The detected pheromone is the first distantly perceived primer found in social insects.

INTRODUCTION

The seasonal life cycle of ants of the genus *Myrmica*, like in other insects, is controlled by photoperiodic responses that determine the moment of diapause onset at the end of the summer [1–4]. The effect of short-day photoperiods (12–13 h of light per day) on experimental colonies of *Myrmica* ants collected in nature in mid-summer and kept at optimal temperatures of 22–24°C results in induction of diapause in the last (third) instar larvae as early as in 2–3 weeks. This leads to the disappearance of pupae, and somewhat later, in 4–5 weeks, the diapause starts in queens that cease to lay eggs. Meanwhile, under conditions of long (>17 h) days the development and pupation of larvae and the egg-laying by queens continue for many weeks [2, 4–6]. A detailed study of photoperiodic responses in the *Myrmica rubra* L. population from “The Forest on Vorsk-la” reserve (Belgorod oblast) has allowed proving

that it was photoperiodic regulation that provided in queens and larvae the diapause onset in natural ant colonies at the ecologically appropriate time, i.e., about the mid-August [6–8].

In this connection, a natural question arises: how do the larvae and queens living permanently inside nests constructed in soil, moss hillocks or dead wood and thus insulated from natural light, manage to perceive the photoperiodic effects? It was not by chance that Brian [9, 10] believed the photoperiodic regulation of diapause in ants to be impossible for this particular reason. However, the experiments by Kipyatkov [11] carried out in formicaria carefully insulated from the light have allowed proving that perception of photoperiodic conditions by forager-ants walking out of dark nest to illuminated arena was quite sufficient for a normal photoperiodic regulation of the seasonal cycle of the *M. rubra* colony. Besides, it was possible to prove that *M. rubra* larvae themselves did not respond to photoperiodic conditions, if

even such a possibility existed, and their developments was entirely controlled by the nurse workers. The physiologically active workers kept under long days (LD) stimulated fast growth and pupation of larvae and terminated larval diapause if it had already been induced before. The workers exposed to short days (SD), i.e., physiologically inactive, were not able to cause the growth and pupation of larvae and induced their diapause [11, 12]. It was also established that queens themselves were able to perceive the photoperiod, however, their physiological state, i.e., the onset or the end of diapause, was strongly affected by their surrounding workers [11].

All these facts have allowed concluding that it were the *M. rubra* forager ants spending much time outside the nest, which perceived the photoperiodic signals and transferred further the photoperiodic information, i.e., in fact, some stimuli for development or diapause, to intranest nurse workers, queens, and larvae that live permanently in darkness. A hypothesis was also put forward that this process could be realized through a transfer of the released by workers, biologically active substances of the hormonal or pheromonal nature during trophallaxis (food exchange) [11]. The experiments reported in this paper were carried out to check this hypothesis.

In planning experiments it was decided to concentrate at the first stage on elucidation of the nature and the ways of transmission of hypothetical activating signals, i.e., of the stimuli for non-diapause development, which are transferred by forager workers perceiving the long photoperiods to intranest individuals: nurse workers, larvae, and queens. For this purpose, it turned out to be the simplest way to use the effect of photoperiodic termination of larval and queen diapause (reactivation). It was shown earlier that if groups of diapause *Myrmica* ants kept long at SD or collected in nature during autumn were put to the LD conditions at optimal temperatures, their diapause would soon end and the queens would start egg-laying as early as in 1–2 weeks and the first larvae would pupate in 2–3 weeks [5, 11]. The photoperiodic reactivation has appeared to be a suitable test, as it requires considerably less time in comparison with photoperiodic induction of diapause. Besides, there also was used the above-

mentioned ability of physiologically active LD workers to interrupt the diapause in larvae and queens even under SD conditions [11].

MATERIALS AND METHODS

Used in the experiments were the *M. rubra* colonies collected in mid-summer or in autumn in “The Forest on the Vorskla” natural reserve (Belgorod Province) or in the park of the Biological Research Institute of St. Petersburg University in Staryi Petergof. Ants were settled in plastic laboratory formicaria (Fig. 1) that consisted of two main parts, the overshadowed and wet (the relative humidity around 100%) nest chamber and the illuminated and dry extranest space (arena and food chamber), and were maintained in photothermostats at 24–25°C. The food (insects cut into pieces and 15% sucrose solution) was given twice a week.

The diapause workers, queens, and larvae obtained either from natural autumn colonies or from the colonies collected in summer and kept at SD (12 h) for 4 weeks and more were used in experiments. The worker ants from the nests, in which no egg laying and pupation had already occurred, were considered as diapause individuals [11]; also the term “SD workers” is used below to designate them. Reactivated workers or, otherwise, “LD workers” were obtained as a result of an exposure of the diapause ant colonies to LD (18 h for the Belgorod and 20 h for the Petergof populations) for 2–3 weeks. The experimental ant groups were composed of individuals taken randomly from base colonies: from 50 to 150 workers, one or two queens, and from 15 to 100 larvae of the third age in each group.

The experiments requiring transfer of queens from one nest to another are difficult due to aggression of workers towards individuals from alien colonies. Therefore, in all cases the queens were transferred only between related ant groups, i.e., those obtained by a division of the same natural colony.

During examinations of formicaria under a binocular microscope the presence of eggs, larvae, prepupae, and pupae was checked. As a rule, the eggs laid and larvae pupated were removed and counted once a week. To do this,

the ants were anesthetized by a short (less than 30s) exposure to carbon dioxide, which is shown to have no negative effect on ants [13]. The descriptions of more particular procedures and special constructions of formicaria used in individual experiments are presented below in corresponding sections.

The data were processed using the Quattro Pro 7.0 and Statistica 4.3 software applications. The statistical significance of the effects of factors and of the differences between the means was determined by the method of dispersion analysis (ANOVA) and a subsequent comparison of the means, using the *post hoc* test (the Tukey HSD test). Since the distribution of variables deviated significantly from normal in many cases, the non-parametric statistics (the Kruskal-Wallis H-test) was also used in each comparison. The *t*-test for independent samples was used for simple comparison of two means.

RESULTS AND DISCUSSION

Evidence for a possibility of distant perception of activating stimuli

The first series of experiments. To elucidate the possible role of trophallaxis in transmission of activating stimuli, the double formicaria were used, each divided with a thin partition made of aluminum foil into two compartments, each serving as a separate ant nest (Fig. 1). The *M. rubra* workers were able to easily put their heads through a slit, 0.87–0.88 mm wide, in this partition (as the ant head is flat in dorso-ventral direction), but were not able to pass into the adjacent nest chamber. Preliminary experiments showed that if ants from the same natural colony were settled in both halves of such a formicarium and a sugar syrup mixed with a supravital dye neutral red was placed to one compartment, the workers, indeed, would transfer the colored food through a slit into the partition to their neighbors.

Then an experiment was performed, in which one group of SD ants with diapause queens and larvae was put in one half of the double formicarium, while the same group but previously reactivated for 2–3 weeks of LD was placed in the adjacent compartment. In control variants, other formicaria were used, which were divided by a

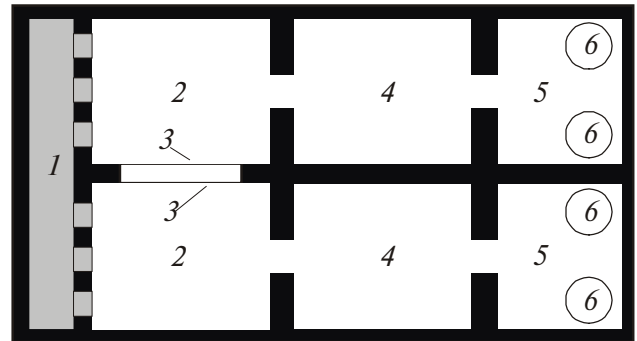


Fig. 1. Diagram of the double formicarium divided by one or two partitions. The size of the formicarium: 100 × 200 mm. (1) Wetting system, (2) nest chamber, (3) partition with net-covered round holes, (4) arena, (5) food chamber, (6) feeders.

partition with round holes, 0.8 mm in diameter, which did not allow workers to insert their heads and excluded completely the possibility of trophallaxis between neighboring ant groups. All experimental formicaria were kept at SD. The results have turned out to be rather unexpected: the photoperiodic reactivation of diapause ants occurred in formicaria of both types. From this, it followed that the food exchange between ants is not necessary for the transmission of activating stimuli from one ant group to another, while sufficient is only a penetration of some volatile substances with air flow or, probably, of some auditory or tactile signals (ants were able to touch their neighbors through the holes in the partition). Several experiments described below were carried out to check all these possible ways of transmission of activating signals.

The second series of experiments. The formicaria divided into two compartments by one or two partitions with round holes, 0.8 mm in diameter, were used (Fig. 1). In both cases a continuous exchange of air between two compartments took place due to diffusion. In formicaria with the single partition the tactile contact between neighboring ant groups was also possible; two partitions separated by a distance of 8–10 mm excluded completely such a possibility. In the second experiment a non-perforated opaque partition allowing only a little diffusion through thin slots along the perimeter was also used. A group of 70–75 SD workers with diapause queens and larvae was placed into one compartment of such a double formi-

Table 1. Results of rearing of diapause larvae by reactivated or diapause worker ants kept at short days in neighboring compartments of double formicaria, divided by a single or double partition with holes, during three weeks (the second experiment)

Variants of experiment and parameters measured	Number of groups (sample size)	Number of larvae in each group	The number of larvae							
			died							
			the number				per cent			
			Min	Max	Mean	St. Error	Min	Max	Mean	St. Error
Reactivated (LD) workers (the 1st variant)	6	25	8	14	11.2	0.95	32	56	45	3.8
Diapause (SD) workers under the effect of reactivated individuals (the 2nd–4th variants)	6	25	9	16	13.2	1.19	36	64	53	4.8
Student's <i>t</i> -test value	—	—	1.3				1.3			
The significance level (<i>p</i>)	—	—	≤ 0.22				≤ 0.22			

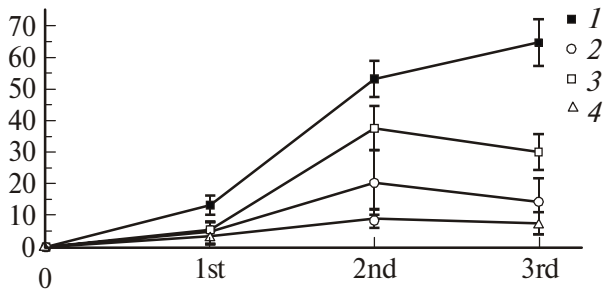


Fig. 2. Resumption of egg-laying by diapause queens in groups of reactivated and diapause workers kept under short days in adjacent compartments of double formicaria divided by one or two partitions with round holes or by a solid partition (the first experiment). The mean number of eggs laid by a queen per week is presented. Vertical bars show standard error of the means. *Abscissa*: the number of eggs laid per week, *ordinate*: weeks. (1) Reactivated workers. Diapause workers beyond the partition: (2) single, (3) double, (4) solid.

carium, while the other group of 100 reactivated LD workers that were given diapause queens and larvae was placed into the second one. All formicaria were kept at SD.

Two such experiments were carried out using ants collected in Petergof. In the first one, two formicaria with the single partition and two with

the double partition were used. Two queens and 35 larvae were placed into each compartment. In the second experiment the same number of formicaria with the single and double partitions as well as two formicaria with non-perforated opaque partition were used. One queen and 25 larvae were placed into each compartment. Thus, there were 4 variants in these experiments: (1) LD workers, (2) SD workers beyond the single partition, (3) SD workers beyond the double partition, (4) SD workers beyond the non-perforated partition. Two groups of diapause ants in separate formicaria served as control in each experiment.

The results on egg laying in both experiments turned out very similar and, therefore, were combined for the analysis (Fig. 2). The egg laying by queens began during the first week in the 1st variant, while in other variants, on average, somewhat later: in 9 groups during the first and in 3 groups, during the second week. The egg-production by queens was the greatest in the 1st, whereas the lowest, in the 4th variant. There was no oviposition in control. The effect of experimental conditions on the number of eggs laid was significant on the whole (ANOVA: $F = 18.9, p \leq 0.000001$; Kruskal-Wallis: $H = 28.1, p \leq 0.00001$),

Table 1. (Contd).

pupated								not pupated							
the number				per cent				the number				per cent			
Min	Max	Mean	St. Error	Min	Max	Mean	St. Error	Min	Max	Mean	St. Error	Min	Max	Mean	St. Error
11	17	<u>13.5</u>	1.05	44	68	<u>54</u>	4.2	0	6	<u>0.3</u>	0.21	0	4	<u>1</u>	0.8
0	3	1.3	0.49	0	12	<u>5</u>	2.0	9	14	<u>10.5</u>	0.85	36	56	<u>42</u>	3.4
10.4				10.4				11.7				11.7			
≤ 0.00				≤ 0.00				≤ 0.00				≤ 0.00			

but on *post hoc* comparison the differences appeared significant only between the 1st variant and all others (HSD test: $p = 0.002-0.03$). During the first three weeks of experiment, in total, the queens laid, on average, 130.7 ± 11.8 , 39.1 ± 13.1 , 73 ± 20.2 , and 19.8 ± 8.5 eggs in the 1st, 2nd, 3rd, and 4th variants, respectively (here and further the standard error of the mean is presented). The effect of experimental conditions on the total number of eggs laid was statistically significant (ANOVA: $F = 11.7$, $p \leq 0.0002$; Kruskal-Wallis: $H = 14.5$, $p \leq 0.002$); in *post hoc* comparison the differences are significant only between the 1st and the 2nd and 4th variants (HSD test: $p = 0.006-0.009$). Thus, although the quantitative differences in the intensity of reactivation between three variants with SD workers seem rather marked (Fig. 2), they are statistically non-significant. Only the 1st variant with LD workers differs statistically significantly from all others.

Now let us consider the pupation of larvae. In the first series of experiments, from 23 to 71% (on average, $51 \pm 8.5\%$) of larvae in 4 groups of the 1st variant and only 10 ± 4.3 and $1.4 \pm 1.43\%$ of larvae in groups of the 2nd and 3rd variants pupated during the first three weeks. The differ-

ences between the 1st variant and the 2nd and 3rd variants in the sum were statistically significant ($t = 3.52$, $p \leq 0.02$). There was no pupation in control formicaria.

From 44 to 68% (on average, 54%) of larvae in the 1st variant and only 2.8 and 6%, on average, of larvae in the 2nd, 3rd, and 4th variants, respectively, pupated during the first three weeks of the second experiment. There was no pupation in control. Due to their similarity the 2nd, 3rd, and 4th variants were combined for statistical analysis (Table 1). The larval mortality in variants with LD and SD workers was low and did not differ statistically significantly. At the same time, the differences in the number and proportion of the pupated and non-pupated (i.e., staying in diapause) larvae were highly significant.

Thus, physiologically active LD workers caused a rapid reactivation of diapause queens and larvae placed in the same compartment but, apart from it, they produced an evident effect on the SD ants in the adjacent compartment, in which the egg-laying also started and some larvae pupated. The reactivation of queens in the compartments with SD workers proceeded more slowly and their egg-production was statistically signifi-

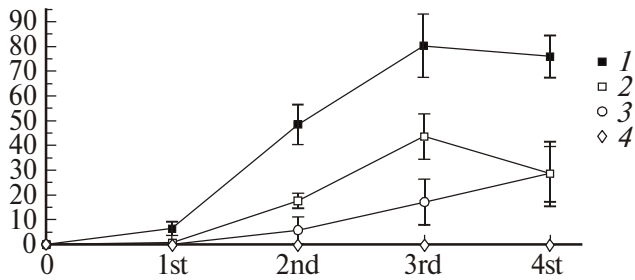


Fig. 3. Resumption of egg-laying by diapause queens in groups of short-day workers kept in adjacent compartments of light-proof formicaria divided by one or two partitions with round holes, when the exit to the long-day illuminated arena was opened or closed. The mean number of eggs laid by a queen per week is presented. Vertical bars show standard error of the means. (1) The exit is opened, (2) the exit is closed, double partition, (3) the exit is closed, single partition, (4) the exit is closed, control. Other designations as those in Fig. 2.

cantly lower (Fig. 2). The small number of larvae pupated in variants with SD workers is undoubtedly accounted for by that a much longer time is required for diapause termination in larvae as compared with queens [14], whereas the reactivated neighbor ants rapidly lost their physiological activity under SD conditions (see [11]); therefore, the activating stimuli produced by them soon disappeared, which was what stopped reactivation of larvae and probably also decreased the egg-production of queens as early as on the 3rd week (Fig. 2).

Nevertheless, the results of this series of experiments allow us to state that the activating stimuli emitted from LD workers penetrate equally easily both the single and the double partition with holes. In the second experiment, they passed even through the thin slots along the perimeter of the non-perforated partition to also produce reactivation, although evidently much weaker (with respect to queens). These facts allow us to exclude the tactile communication as well as trophallaxis from the list of possible ways of the photoperiodic information transmission by ants.

The third series of experiments was performed to achieve a more complete reactivation of larvae in response to the activating stimuli from LD workers present beyond the partition. The same double formicaria were used for this, but they were

also lightproof. The SD workers with diapause queens and larvae were placed in both compartments of the formicarium that was then kept at LD. The exit to illuminated arena was, however, open in only one compartment of each formicarium; it was there that the process began of the photoperiodic reactivation initiated by the workers entering the arena. In the adjacent compartment, where ants were kept in the constant darkness, the photoperiodic reactivation could not occur; this was shown earlier [14] and was now confirmed by control variants of the experiments (see below).

Two such experiments in the lightproof formicaria were performed in different years. In the experiment with ants from Petergof there were three formicaria with the single and two formicaria with the double partition. To increase the effect of reactivated ants on their diapause neighbors twice as many workers (100) were placed in each compartment with the opened exit to arena than in the compartments with the closed exit (50). There were one queen and 17–18 larvae in each experimental group. In the second experiment with ants from “The Forest on the Vorskla” two formicaria with the single and three formicaria with the double partition were used. There were 150 workers in each compartment with the opened exit and 100 workers in compartments with the closed exit. One–two queens and 100 larvae were in each experimental group. Two groups of diapause ants in the lightproof formicaria without exit to the illuminated arena and without the reactivated ant group in the adjacent compartment were used as control in both experiments. Thus, in the both experiments there were 4 variants: (1) the exit to arena is open, the photoperiodic reactivation is possible, (2) the exit to arena is closed, the photoperiodic reactivation is impossible, the reactivated ant group is in the adjacent compartment beyond the single perforated partition (3) the same as in 2, but the reactivated ant group is in the adjacent compartment beyond the double perforated partition, (4) the same as in 2, but there are no reactivated ants in the adjacent compartment (control).

In the first experiment the egg-laying by queens started already during the first week in the 1st variant (Fig. 3), as this is usually observed in earlier

Table 2. Results of rearing of diapause larvae in groups of short-day worker ants kept in adjacent compartments of light-proof formicaria divided by one or two partitions with round holes, with exit to the long-day illuminated arena only from one compartment (duration of experiment 4 weeks)

Variants of experiment and parameters measured	Number of groups (sample size)	Number of larvae in each group	The number of larvae											
			died				pupated				not pupated			
			Min	Max	Mean	St. Error	Min	Max	Mean	St. Error	Min	Max	Mean	St. Error
Exit to arena is open, forager workers are subjected to long-day conditions (the 1st variant)	5	100	6	56	32.8	8.33	41	94	62	9.16	0	9	5.2	1.65
Exit to arena is closed, effect from neighbor workers subjected to long-day conditions (the 2nd and 3rd variants)	5	100	11	41	23.6	4.88	51	88	71	5.94	1	9	5.4	1.50
Student's <i>t</i> -test value	—	—	0.95				0.82				0.09			
The significance level (<i>p</i>)	—	—	≤ 0.37				≤ 0.43				≤ 0.93			

studies on the photoperiodic reactivation [11, 14], whereas in the 2nd and the 3rd variants it occurred much later: during the first week in two groups and during the second week in three groups. Productivity of the queens rose much more rapidly in the 1st variant in comparison with the 2nd and the 3rd ones; there was no queen reactivation at all in the control, 4th variant. The effect of experimental conditions on the number of eggs laid was significant on the whole (ANOVA: $F = 25.9$, $p \leq 0.000001$; Kruskal-Wallis: $H = 23.0$, $p \leq 0.00001$); in *post hoc* comparison the differences turned out statistically significant only between the 1st variant and all others (HSD test: $p = 0.0002-0.001$). During the first four weeks of experiment the queens laid in total 198.0 ± 22.6 , 52.0 ± 20.0 , 90 ± 16 and 0 eggs, on average, in the 1st, 2nd, 3rd, and 4th variants, correspondingly. The effect of experimental conditions on the total number of eggs laid was statistically significant (ANOVA: $F = 16.9$, $p \leq 0.0013$; Kruskal-Wallis: $H = 8.9$, $p \leq 0.03$); in *post hoc* comparison the differences were also statistically significant between the 1st and 2-4 variants (HSD test: $p = 0.002-0.04$). It means that, although clear differences were observed in the intensity of

reactivation of queens between the variants with the single and double partition (Fig. 3), they are statistically non-significant. Only the 1st variant with the opened exit to arena differs statistically significantly from all others.

In the second experiment the photoperiodic reactivation of queens was very poor and most of them never began to lay eggs. This is most likely to be accounted for by the inclusion into experiment of many young queens that enter the already existing nests after the nuptial summer, in August, and begin to lay eggs only after overwintering [15]. Therefore, this experiment produced no results with respect to queens. However, the reactivation of larvae in the same experimental ant groups occurred quite normally.

In this series of experiments, unlike the series considered above, the formicaria were constantly kept under LD. Therefore, the ants reactivated with photoperiods in the compartments with the opened exits to arena did not lose their physiological activity and continued to transmit the activating signals to the adjacent compartment; hence, the corresponding process of reactivation did not cease there and much more larvae pupated.

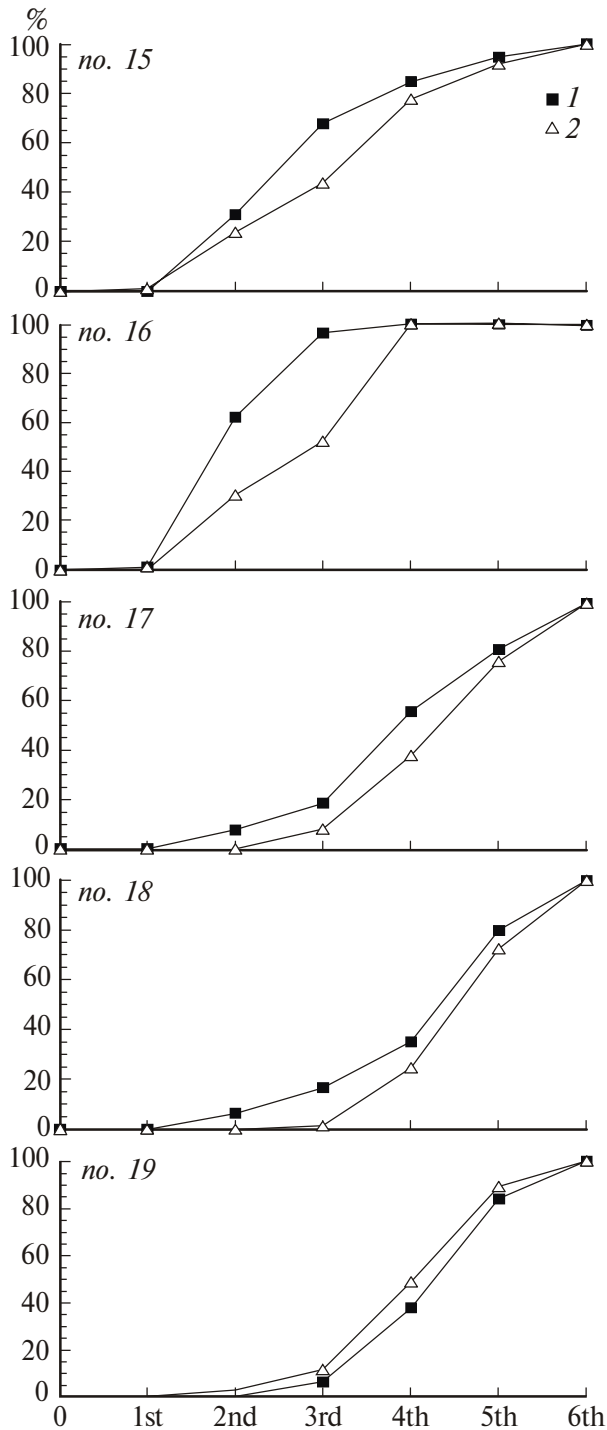


Fig. 4. Dynamics of reactivation and pupation of diapause larvae in groups of short-day workers kept in adjacent compartments of lightproof formicaria divided by one or two partitions with holes, when the exit to the long-day illuminated arena was opened only from one compartment. The cumulates of the numbers of pupation as percentage of the total number of pupated larvae for each experimental ant group are presented. (15–19) Numbers of formicaria. *Abscissa*: like in the Fig. 2, *ordinate*: weeks. (1) The exit is opened, (2) the exit is closed.

In the first experiment the pupation of larvae began during the 1st–2nd weeks in all groups of the variants 1–3. But later a significant mortality was observed among larvae, which was caused by insufficient air humidity due to inappropriate construction of the formicaria. Therefore, the number of larvae pupated for 4 weeks varied substantially, from 3 to 67%. On average, 34 ± 12.8 , 34 ± 19.3 , and 49 ± 1.3 larvae pupated in the 1st, 2nd, and 3rd variants, correspondingly. The rest of larvae died, therefore, the experiment was terminated. The differences in the proportion of pupated larvae between the 1st and 2–3 variants in sum are statistically non-significant ($p \leq 0.92$). There was no pupation at all in the control (4th) variant.

In the second experiment lasting for 6 weeks the formicaria with an improved construction were used, and, therefore, mortality of larvae did not exceed the norm (on average, 20–30%) characteristic of the process of photoperiodic reactivation [12, 14]. The differences in larval mortality between the 1st and 2–3 variants in sum are statistically non-significant (Table 2). The most part of survived larvae in 1–3 variants pupated, and only a few (0–9%) did not leave diapause. The differences in the number of pupated and not pupated larvae between the 1st and 2–3 variants in sum are statistically non-significant (Table 2). There was no pupation in the control (4th) variant.

Thus, the presence in the adjacent compartment of formicarium of ants that are in the process of photoperiodic reactivation turned out sufficient for the practically complete pupation of diapause larvae in the groups of workers that had no access to the illuminated arena. The sufficiently evident conclusion that this process was produced by activating stimuli from reactivated ants in the adjacent compartment is also confirmed by some delay in pupation of larvae in the compartments without the exit. This delay is especially evident on graphs of cumulative pupation, which are calculated as a percentage of the total number of pupated larvae for each experimental group (Fig. 4): in 4 out of 5 formicaria the proportion of pupated larvae increased more rapidly in the compartments with the opened exit to the arena. Apparently, this is explained by that first there had

occurred the photoperiodic reactivation in workers in the compartments with the opened exits and only after that these ants turned out to be able to stimulate the start again the activity in their neighbors.

On the whole, the results of this series of experiments allow coming to the conclusion that the activating stimuli penetrate equally easily through both the single and the double partition, as the completeness of reactivation in these variants did not differ statistically significantly. Thus, these experiments proved once again the possibility of the distant transmission of the stimulus to the non-diapause development in the *M. rubra* colony.

Evidence for the chemical nature of activating stimuli

A distant transfer of activating effect is possible in the case of its acoustic or chemical nature. To check the hypothesis that an activating stimulus is a volatile substance, a pheromone spreading in air, an experimental device was made. The air from one formicarium was pumped into another by a micropump at a rate of 0.01–0.02 ml/s. Both nests could be kept at the same time under different illumination conditions (Fig. 5). The diapause ants were placed to two such formicaria, one of which kept at SD, the other at LD, and the airflow was pumped between them. In the 1st variant of the experiment the air from the formicarium kept at LD, in which the photoperiodic reactivation occurred, was pumped into the formicarium kept at SD conditions; thereby, a pheromone that could be present in this air should be transferred to that formicarium. In the 2nd (control) variant, direction of the airflow was the reverse, so the hypothetical pheromone could not reach the formicarium kept at SD.

To increase concentration of the activating stimulus in the air pumped, considerably more workers (300–400) were placed in the formicaria at LD, as compared with those at SD (100 individuals). Each experimental group also contained many diapause larvae and one-two queens. Due to the necessity to provide the airflow through all nest chambers, the glass lids were tightly glued to formicaria, which prevented their removal and precise count of eggs and larvae. Therefore, merely approximate and graded estimations were used in

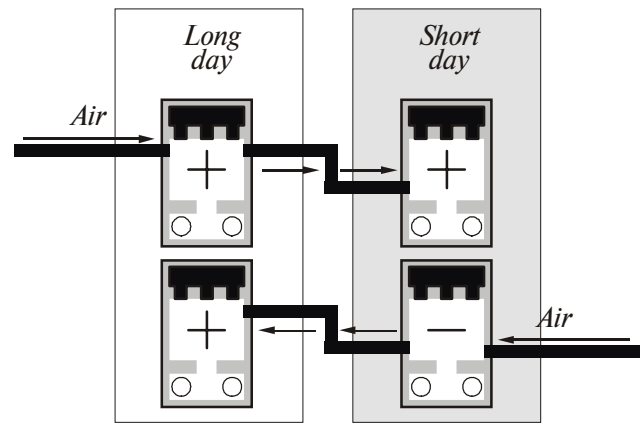


Fig. 5. A principal scheme of the experimental device for checking a possibility of the transfer of an activator stimulus with air flow. In the first variant of the experiment (*above*) the air from a formicarium, in which the photoperiodic reactivation occurred (designated by a plus sign) under the effect of the long-day conditions, was pumped into the formicarium kept at short-day illumination, which led to diapause termination (designated by a plus sign) in this formicarium. In the second variant (*below*) the direction of the airflow was the reverse, therefore, the reactivation in the formicarium kept at short day did not occur (designated by a minus sign).

counts. The experiment was repeated twice in different years with ants from “The Forest on the Vorskla” reserve.

In the first experiment, the 1st variant was doubled and the control (2nd) variant was single (the total of six formicaria). The experiment lasted for 3 weeks. In two out of three formicaria at LD the first eggs appeared already during the 2nd week and their number increased considerably during the 3rd week; the fast growth of larvae and their mass pupation was also observed on the 2nd–3rd weeks. In the third formicarium at LD the reactivation did not occur for some reason until the end of experiment that had to be stopped on technical grounds.

It is in the SD formicarium pumped with the air from the LD nest with the photoperiodic reactivation, in which the first eggs appeared on the 11th day. Later, their number increased, and the growth of larvae was also observed, so pupation of four larvae occurred at the very end of experiment. In the second formicarium pumped with the air from the nest with no reactivation,

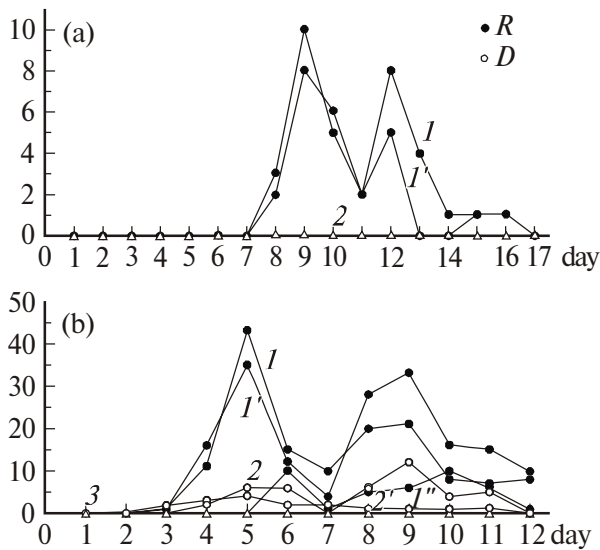


Fig. 6. Resumption of egg laying by diapause queens kept under the short-day conditions in groups of diapause workers as a result of everyday addition of reactivated (*R*) or diapause (*D*) workers killed and ground in a mortar. Each line—lying by one queen. (a) The first experiment, (b) the second experiment. Addition of reactivated workers: (*I*) the 1st group, (*I'*) the 2nd group, (*I''*) the 3rd group (only in b). Addition of diapause workers: (*2*) the 1st group, (*2'*) the 2nd group. Control (without any addition): in (a) 2, in (b) 3.

such changes did not occur, like in the control formicarium of the 2nd variant.

In the second experiment, the 1st variant was tripled and the control (2nd) variant was doubled (the total of 10 formicaria). The experiment lasted for 4 weeks. In all five formicaria at LD the normal photoperiodic reactivation was observed: the eggs appeared as early as on the first week and their number subsequently increased markedly; there occurred the fast growth of larvae that began to pupate on the 2nd week; by the end of the experiment, very many pupae were present in the nests.

Under SD conditions in all three formicaria that got the air from the nests at LD, there also occurred reactivation. Few first eggs appeared by the end of the 1st week, but their number increased slower than at LD. The larval growth also took place, but not so fast, and only some larvae start to pupate by the end of the 3rd week. In total 3, 7, and 9 larvae pupated until the end

of experiment in these three formicaria. At the same time, in two formicaria of the 2nd (control) variant, not only the eggs and pupae did not appear, but also the larval growth was not observed.

Thus, the activating stimulus has turned out to be a volatile substance, as it was spread with airflow but was unable to move in opposite direction.

Direct test for the chemical nature of activating signal. The experiment consisted in that 30–40 reactivated (the 1st variant) or diapause (the 2nd variant) workers were crushed in a mortar and added twice a day (in the morning and in the evening) to small groups of diapause ants composed of 100 workers, 30–50 larvae, and one queen that were kept at SD. The state of the experimental groups was evaluated every day. The experiment was repeated twice in different years with the ants from “The Forest on the Vorskla” reserve. In the first experiment there were two ant groups in the 1st and one group in the 2nd variant. In the second experiment there were three groups in the 1st and two groups in the 2nd variant. Two diapause ant groups kept at SD without an addition of any crushed workers were used as the control (3rd) variant.

On addition of reactivated workers the queens in all experimental groups started to lay eggs: in the first experiment on the 8th day, in the second experiment, on the 3rd day in two groups and on the 6th day in one group (Fig. 6). During the entire experiment the queens laid the total of 25 and 35 eggs in the first, and 39, 144, and 170 in the second experiment. After addition of diapause workers no eggs were laid in the first experiment, but a few eggs (the total of 17 and 41) appeared in the both groups in the second experiment (Fig. 6). In some experimental groups of the 1st variant, several larvae pupated: 4 larvae in one group of the first experiment and 1 and 8 larvae, in two groups of the second one. One pupa appeared in each of two groups of the 2nd variant of the second experiment. There were neither egg laying nor pupation in all control groups (3rd variant).

Thus, daily addition of crushed worker ants led to an evident diapause termination in all queens but only in a few larvae. This difference is quite

understandable. The diapause in queens at high temperatures is rather unstable and can be very quickly interrupted by the effect of LD or of reactivated workers, whereas diapause larvae need a much longer reactivating action [11, 14].

The results obtained allow stating that, indeed, the bodies of reactivated workers do contain some substance that is an activating signal for diapause ants. It might be suggested that the diapause workers also contain a small amount of this activating substance, but normally do not release it into the environment. However, after their grinding, this substance was released and produced a weak reactivating effect on the diapause ants.

CONCLUSIONS

The results of all the experiments carried out indicate, in their complex, that the role of activating stimulus, i.e., the agent that stimulates non-diapause development, is played in the *M. rubra* colony by some volatile substance, a pheromone secreted by the workers and perceived by distant chemoreceptors of other individuals. At present, nothing has been known about its chemical nature and the place of secretion in the body of the worker ant. To find this out, special investigations are required, with the use of quite different methods and equipment.

The revealed *activator pheromone* is undoubtedly one of the elements in the chain of social mechanisms regulating the development and diapause in the ant colony and serves for transmission of the information about the long-day conditions among the workers, first of all, from forager workers to intranest individuals. It is this mechanism that may provide the photoperiodic regulation of development in the lightproof experimental formicaria as well as in natural ant nests, into which the light does not penetrate. Probably, this pheromone might affect the queens directly. However, it has remained yet unclear, whether the larvae themselves also are able to respond to the activator pheromone. The results of our studies on the social control of larval development in ants of the genus *Myrmica* [16–19] allow us to suggest that only the workers are most likely to perceive this activator pheromone, whereas regulation of larval development is based on tactile and trophic

stimuli received by larvae from the nurse workers.

According to widely accepted classification of pheromones [20–22] the activator pheromone discovered in our studies should be ascribed to the group of primers, as it affects, first of all, the physiological state of individuals and processes of development. However, it should be noted that the primer pheromones distantly perceived by animals have not been found so far in ants and other social insects [23, 24].

Unfortunately, up to now we have yet failed to get any evidence for the possible existence in *M. rubra*, apart from the activator pheromone, also of an inhibitor that might transmit information about the short-day conditions and block the processes of development and oviposition by inducing diapause. The search for this inhibitor comes across significant methodical difficulties. Unlike reactivation under effects of the long day or of physiologically active LD workers, the process of diapause induction is rather long and cannot be completely performed under other photoperiodic conditions except for short days. Therefore, at present it is impossible to decide whether the diapause in the *M. rubra* colony appears as a result of action of some inhibitor pheromone, i.e., of an inhibitory stimulus, or it is, in fact, a syndrome of the absence or a deficit of the activator pheromone.

ACKNOWLEDGMENTS

This work was partially supported by The Council for Grants from the President of Russian Federation and for State Support of Leading Scientific Schools (grant no. 00-15-97934).

REFERENCES

1. Danilevskii, A.S., *Fotoperiodizm I sezonnoye razvitiye nasekomykh* (Photoperiodism and Seasonal Development of Insects), Leningrad: Leningrad University, 1961.
2. Kipyatkov, V.E., Mechanisms of Regulation of Developmental Processes in Ants, *Meetings in the Memory of N.A. Kholodkovski. Lectures of the 33rd Annual Meeting, April, 3–4, 1980*, Nauka, Leningrad, 1981, pp. 59–91.
3. Danks, H.V., *Insect Dormancy: An Ecological Perspective*, Ottawa: Biological Survey of Canada

- (Terrestrial Arthropods), 1987.
4. Kipyatkov, V.E., Annual Cycles of Development in Ants: Diversity, Evolution, Regulation, *Proceedings of the Colloquia on Social Insects*, vol. 2, Kipyatkov, V.E., Ed., St. Petersburg: Russian-Speaking Section of the IUSSI, 1993, pp. 25–48.
 5. Kipyatkov, V.E., Detection of the Photoperiodic Reaction in Ants of the Genus *Myrmica*, *Dokl. Akad. Nauk SSSR*, 1972, vol. 205, pp. 251–253.
 6. Kipyatkov, V.E., A Study of the Photoperiodic Reaction in the Ant *Myrmica rubra* L. (Hymenoptera, Formicidae). I. Basic Parameters of the Reaction, *Entomol. Rev.*, 1974, vol. 53, pp. 35–41.
 7. Kipyatkov, V.E., A Study of the Photoperiodic Reaction in the Ant *Myrmica rubra* (Hymenoptera, Formicidae). III. Peculiarities of Temperature Correction, *Vestn. Leningrad Univ.*, 1977, vol. 3, pp. 14–21.
 8. Kipyatkov, V.E., The ecology of Photoperiodism in the Ant *Myrmica rubra* (Hymenoptera, Formicidae). 1. Seasonal Changes in the Photoperiodic Reaction, *Entomol. Rev.*, 1979, vol. 58, pp. 10–19.
 9. Brian, M.V., Studies of Caste Differentiation in *Myrmica rubra* L. 6. Factors Influencing the Course of Female Development in the Early Third Instar, *Insect. Soc.*, 1963, vol. 10, pp. 91–102.
 10. Brian, M.V. and Kelly, A.F., Studies of Caste Differentiation in *Myrmica rubra* L. 9. Maternal Environment and the Caste Bias of Larvae, *Insect. Soc.*, 1967, vol. 14, pp. 13–24.
 11. Kipyatkov, V.E., A Study of the Photoperiodic Reaction in the Ant *Myrmica rubra* (Hymenoptera, Formicidae). 5. Perception of Photoperiodic Information by the Ant Colony, *Entomol. Rev.*, 1967, vol. 55, pp. 27–34.
 12. Kipyatkov, V.E., A Study of the Photoperiodic Reaction in the Ant *Myrmica rubra* L. II. Effects of Photoperiod and Physiological State of Worker Ants on Development of Larvae, *Vestn. Leningrad Univ.*, 1974, No. 9, pp. 17–24.
 13. Wardlaw, J.C., The Effect of Carbon Dioxide on Egg Production in *Myrmica rubra*, *Insect. Soc.*, 1995, vol. 42, pp. 325–328.
 14. Kipyatkov, V.E., A Study of the Photoperiodic Reaction in the Ant *Myrmica rubra*. 4. Photoperiodic Reactivation, *Zool. Zh.*, 1977, vol. 56, pp. 60–71.
 15. Elmes, G.W., The Social Biology of *Myrmica* ants, *Actes Colloq. Ins. Soc.*, 1991, vol. 7, pp. 17–34.
 16. Kipyatkov, V.E. and Lopatina, E.B., Food Transfer by Worker Ants *Myrmica rubra* L. to Larvae under Different Photoperiodic Conditions, *Vestn. Leningrad Univ.*, 1998, No. 17, pp. 14–21.
 17. Kipyatkov, V.E. and Lopatina, E.B., Quantitative Study of the Behavior of Ants *Myrmica rubra* L. (Hymenoptera, Formicidae) in Connection with Photoperiodic Regulation of Larval Development, *Entomol. Rev.*, 1990, vol. 69, pp. 31–41.
 18. Kipyatkov, V.E. and Lopatina, E.B., Behavior of Worker Ants *Myrmica rubra* (Hymenoptera, Formicidae) during Feeding of Larvae, *Zool. Zh.*, 1989, vol. 68, pp. 50–59.
 19. Kipyatkov, V.E. and Lopatina, E.B., Social Regulation of the Onset and Termination of Larval Diapause by Worker Ants of Three Species of the Genus *Myrmica* Latreille (Hymenoptera, Formicidae), *Entomol. Rev.*, 1999, vol. 79, pp. 1138–1144.
 20. Bossert, W.H. and Wilson, E.O., The Analysis of Olfactory Communication among Animals, *J. Theor. Biol.*, 1963, vol. 5, pp. 443–469.
 21. Wilson, E.O., The Social Biology of Ants, *Ann. Rev. Entomol.*, 1963, vol. 8, pp. 345–368.
 22. Wilson, E.O., *The Insect Societies*, Cambridge, Mass.: Belknap, 1971.
 23. Hüllendobler, B. and Wilson, E.O., *The Ants*, Cambridge, Mass.: Belknap Press of Harvard University, 1990.
 24. Vander Meer, R.K., *Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites*, Boulder, Colo.: Westview, 1998.