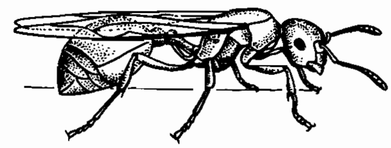


Myrmica Prepares for Winter

a New Ant Pheromone has been Identified

Ants are ubiquitous. They are to be found everywhere: in forests, swamps, fields, or asphalt pavements of our cities, and even in our apartments. There are over 9000 species of ants and almost 80 percent of them inhabit the tropics. Not without reason, the first white settlers called them 'the Kings of Brazil' and considered them 'the real conquerors of the country'. Undoubtedly, the family of ants originated from the tropics. When ants had developed the specific social *modus vivendi*, they spread to the North and South and inhabited regions of moderate and even very cold climate. The ants had to adapt themselves to seasonal changes and, primarily, to survive the winter to which they were quite unprepared. Nonetheless, some species of ants now live successfully in the northern forest-tundra and feel fine even at the pole of cold in the central Yakutia.

WHAT HELPS ANTS WITHSTAND WINTER FROSTS?

Wintering ants, like many other representatives of fauna, have been found to possess a remarkable capacity of hibernation, or falling into a diapause in winter. During the diapause, all vital functions in an organism are slowed down and its resistance to unfavorable environmental factors, such as cold, dryness or absence of food, increases. The diapause, however, requires long and timely preparation.

During summer ants accumulate substantial reserves of nutrients in their bodies, which permits them to survive without food the winter and early spring. The insects build special wintering quarters deeper in the soil where it is not as cold as on the surface. After the first frosts in the fall, ants gather together in underground chambers in dense masses and surround the fertile female (queen) and larvae. If this cycle is disturbed by placing a nest of ants in midsummer into a refrigerator with a temperature below 10°C, the insects will begin to perish: first pupae and eggs, then larvae and adults. They die because metabolic processes have not been suppressed at the right time and no nutritive reserves have been accumulated in their bodies. Under such conditions, the insects spend too much energy and substance for breathing and therefore, suffer from exhaustion, as well as from dessication, since they loose water during breathing and do not restore it with food (it has been found experimentally that insects cannot eat at low temperatures even if the food is abundant).

At the end of summer or during the fall, when ants have prepared themselves properly for winter, they can live in a refrigerator for several months. In spring, after such an artificial hibernation, they will be as lively as before.

For most insects living in the moderate climate, the leading role in adaptation to seasonal change is played by what is called photoperiodic reactions: their vital functions depend on the ratio between the light and dark part of a day. When days are long and nights short, the processes of reproduction and development of insects occur actively. When the days grow shorter at the end of the summer, a diapause begins. During a year, days become longer or shorter in a perfectly definite manner notwithstanding all the whims of weather and other variable factors of the environment. Because of this, the length of

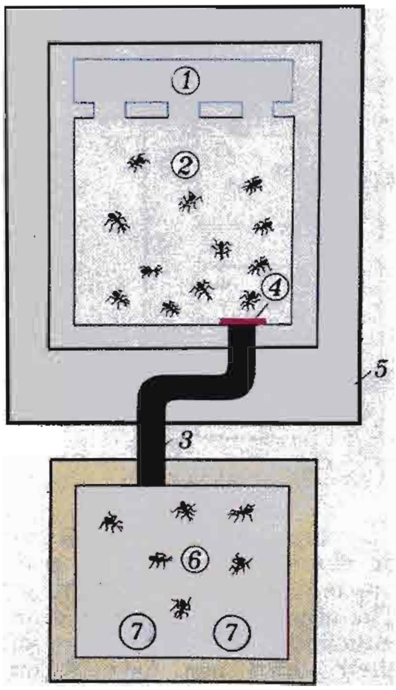


Fig.1. A formicarium with light-insulated nest section.
1 – water reservoir; 2 – nest chamber where a humid atmosphere is formed by water-impregnated gypsum; 3 – S-bent black tube through which light cannot penetrate; 4 – perforated partition with holes sufficiently large to pass worker ants, but impassable for queens; 5 – light-impermeable box; 6 – arena; 7 – feeding pans. In this formicarium, only the insects which can visit the illuminated arena could perceive the day length, whereas all others stayed always in darkness and were deprived of this possibility.

daytime is an almost unique indication for the beginning of a diapause. Many insects have adjusted this universal factor for controlling their annual development cycle.

Do ants exhibit photoperiodic reactions? In order to answer the question, we carried out special experiments with one of the commonest ant species in the European territory of the USSR, the red ant (*Myrmica rubra L*).

ACQUAINTANCE WITH THE SUBJECT OF EXPERIMENT

In the 'Forest on Vorskla' reserve in the Belgorod Region where the experiments were carried out, red ants are common inhabitants of oak forests. They build their nests mostly in rotten branches, twigs, stumps, and dead tree trunks lying on the soil surface. The insects carve out intricate cavities and passages under the bark and in the wood where their queens, eggs, larvae and pupae, surrounded by a numerous suite, live in summer time. If the rotten wood dries because of the absence of rains, ants transfer the brood (which does not stand dryness) into the underground portion of the nest at a depth of 15-20 centimeters below the soil surface. They also hibernate there.

Having awakened in spring from winter torpidity, ants bring again their larvae into the sun-heated portions of the nest above the ground. Queens (which are always numerous in Myrmica nests) also come into these portions and start laying eggs. Thousands of worker

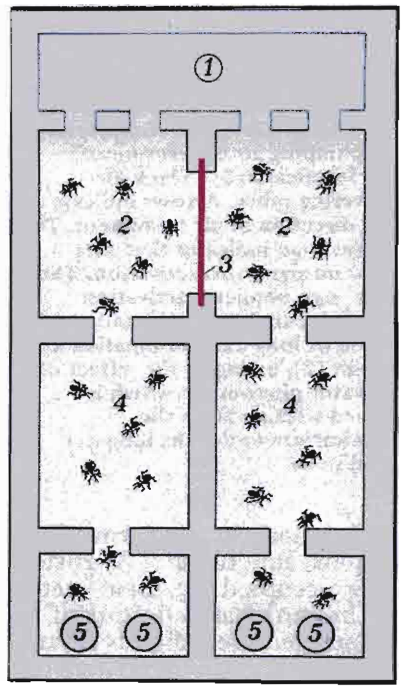
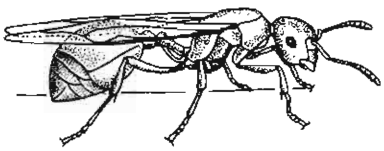


Fig.2. A double formicarium.
1 – water reservoir; 2 – nest chambers in which humid atmosphere is formed by water-impregnated gypsum; 3 – partition with a slit or net-covered holes for separating two groups of ants; 4 – arenas; 5 – feeding pans. The experiments have proved that information on the length of days is transmitted by food-gathering ants to the nest neither with food nor by means of feelers.



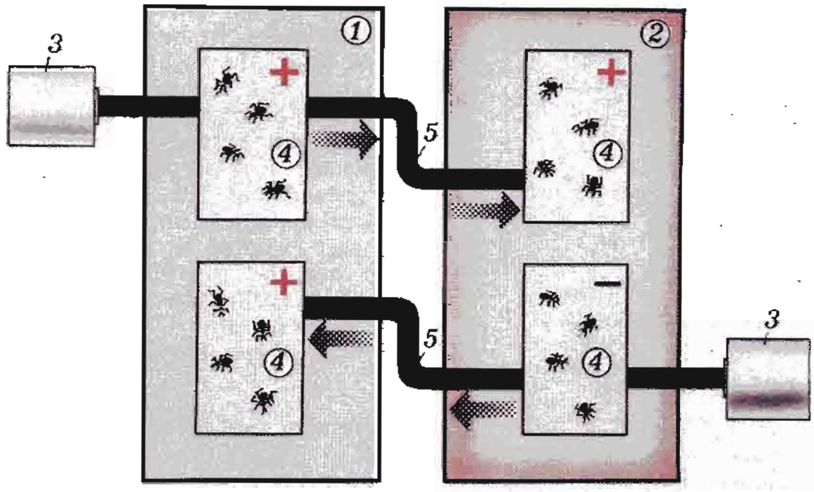


Fig.3. An experimental unit in which air could be blown from one formicarium to another.
1 – artificial nest with long-day illumination; 2 – nest with short-day illumination; 3 – compressors for pumping air into formicaria; 4 – formicaria; 5 – black air-delivering tubes. Arrows indicate the direction of air movement. The 'minus' sign indicates that ants show no signs of reactivation. The 'plus' sign implies reactivation which occurs in nest (1) under the action of long-day illumination and in nest (2), owing to the effect of activator pheromone which is carried with air from the formicarium under the long-day conditions.

ants begin to perform their various functions*. A rotten twig occupied by them seems to be uninhabited, but if it is damaged even slightly, many infuriated defenders emerge immediately, ready to use their stings at any moment.

During the period from May through July, active development of the young takes place in Myrmica nests. Small worm-like larvae hatch from the eggs that have been deposited by queens. Entomologists have established that larvae feed on eggs during the initial period which is called the first age**. After a certain time, the larvae cast off their skins and pass on to their second and then third age. Adult ants separate the larvae of the two last stages from the eggs, lick them thoroughly, and feed them mouth to mouth. A grown larva ceases feeding and soon ejects from its intestines the undigested food accumulated from its birth, which is removed by adults. Its body is now of an ivory color and from that moment on it is called prepupa. In a few days, it throws off the skin and becomes a pupa, i.e., a full resemblance of an adult ant but with its legs and feelers tightly pressed to the body. In two-three weeks, the white pupa becomes brown, its eyes darken, and finally, an adult ant is hatched. The whole cycle from egg to adult takes around two months at a temperature of 23-24°C and an appreciably longer time at lower temperatures.

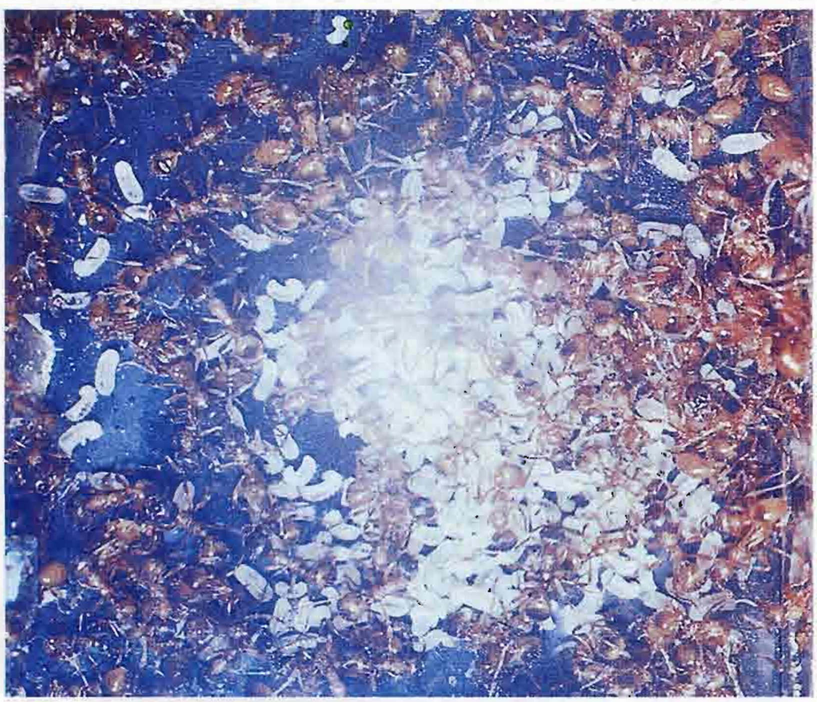
All larvae which have wintered in the nest pupate and transform into adults in June and July. Many of the larvae which have appeared from the eggs deposited by queens after wintering also pupate at that time. In the first half of August, however, i.e., when it is still quite warm, new pupae cease to form. At that time, all stages of insect development, except for prepupae, can be found in a nest. The absence of prepupae is an indication that the larvae in their third age have fallen into a diapause.

Soon after that, the queens stop laying eggs, and the ant colony starts preparations for wintering. Worker ants hatch gradually from the remaining pupae and larvae hatch from the remaining eggs. The larvae develop only to the third stage and fall into a diapause. By mid-September, only adult ants and third-stage larvae remain in a nest; they have an ample reserve of nutrients accumulated in their bodies.

The laboratory experiments to clarify the problem of how ants get to know in due time that winter is approaching were carried out in specially designed artificial ant nests, formicaria. A formicarium consisted of a number of chambers covered at the top by glass plates and connected with one another by apertures which could be closed easily by shutters. There was neither

* See: A. Zakharov. "Ant Communities", *Science in the USSR*, 1987, No. 1 (*Ed.*).

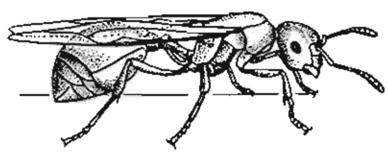
** The phenomenon is quite common with ants, but has not yet been explained satisfactorily by scientists (Author's note).



Typical oak forest in the 'Forest on Vorskla' reserve where *Myrmica rubra* ants are especially numerous. They often inhabit old stumps, dead tree trunks, and rotten twigs lying on the soil surface.

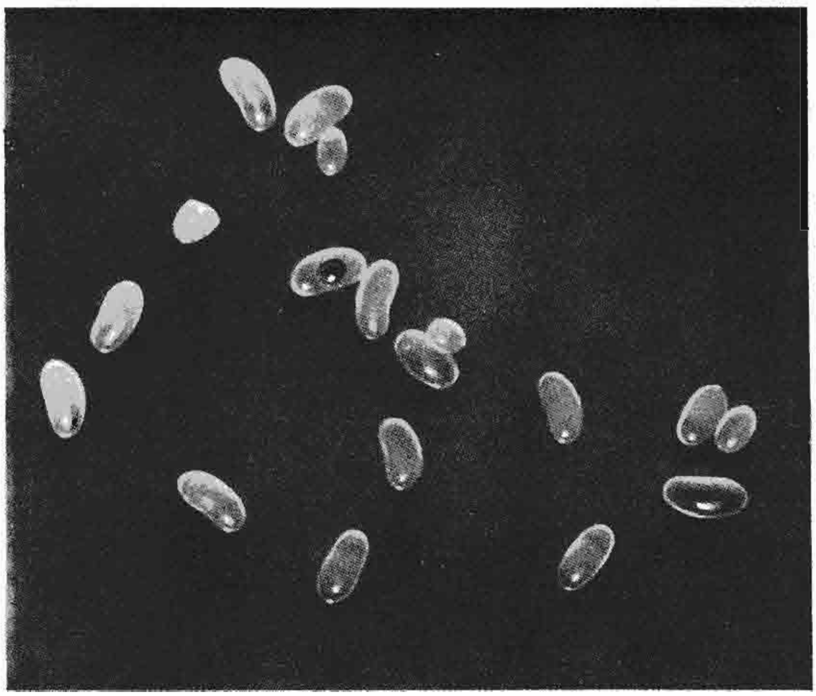
Red ants and their larvae in a laboratory formicarium.

soil nor any other building material in a formicarium, so that all insects and the brood were always in sight. One of the chambers had a higher humidity which was essential for the normal life of the brood. It was closed at the top with a black cloth, since ants dislike bright light in their nests. Forager ants had



Eggs deposited by red ant queens (magnification 15x).

Stages of development of a worker ant. From left to right, top row: two eggs, first-age larvae, two second-age larvae, and five third-age larvae; bottom row: two prepupae, four pupae at different stages of development, and an adult ant (magnification 8x).



the possibility for hunting in a specially constructed 'arena' with feeding pans. Such a formicarium was then placed into a laboratory thermostat in which the specified temperature and length of the day were maintained automatically.

PHOTOPERIODIC REACTION

We found that the best temperature for the existence of ants of the given species is 23-24°C. The length of the longest day (June 22) at the latitude of the 'Forest on Vorskla' reserve is around 18 hours. The short days (12 hours) were at the end of September when the development of Myrmicae in nests has already ended.

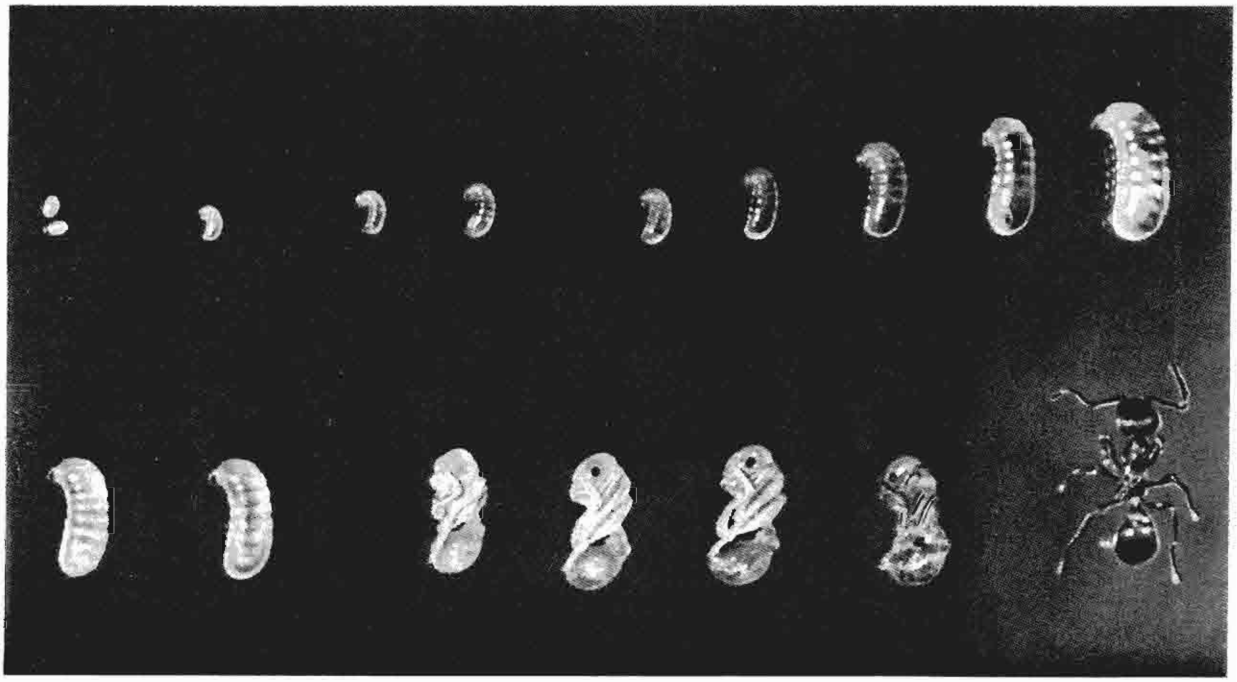
In two-three weeks from the beginning of the experiment, the number of newly hatched pupae and laid eggs in the formicaria with a shortened day illumination (12 hours) drops sharply compared to the nests in which there is a longer (18 hour) day. Under the shorter-day conditions, prepupae disappear in three or four weeks and third-stage larvae cease to pupate and fall into a diapause. In five or six weeks, queens stop laying eggs and begin hibernating as do worker ants.

In the formicaria with a longer day, normal life still continues. Ants may live here for several months and their development continues, though their fellows in natural conditions are already hibernating. Thus, continuous development of Myrmicae is only possible when the day is long, whereas a shortened day invariably causes a diapause.

If the length of the day is gradually reduced, just as in natural conditions, ant larvae cease to pupate in about eight weeks' time if the nest is illuminated for 16 hours a day. This occurs in six weeks' time if the daytime equals 15 hours, in four-five weeks with a daytime of 14 hours, and in three-four weeks with a daytime of 13 or 12 hours (a further reduction of the daytime does not accelerate substantially the onset of diapause).

The photoperiodic reaction of ants is strongly influenced by temperature: with a decrease of temperature, the diapause starts sooner. Under the conditions of a 12-hour day, larvae cease pupating in three-four weeks' time at 23-24°C, in two-three weeks at 20°C, and in one-two weeks at 17°C. This relationship is quite clear. The weather is very variable, and the rate of development of the insects is reduced substantially with a decrease of temperature. If the summer was rather cool, a cold spell in the fall begins earlier. Accordingly, the diapause of larvae and queens sets in earlier, since otherwise the diapausing, i.e., prepared for hibernation, larvae will not develop from the laid eggs, nor will worker ants from the pupae and they will not survive. The eggs and younger larvae will perish in winter.

Young ants which hatched too late from pupae and also larvae which fell into diapause quite recently will not be capable of hibernating successfully, since they have not



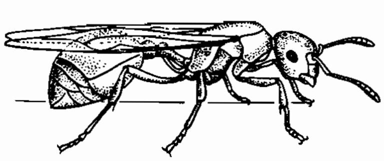
stored enough nutrients. On the other hand, if the summer was hot, it is reasonable to postpone hibernation and rear more worker ants and larvae.

In order to prove that the development of ants in nature is actually controlled by photoperiods and temperature, we measured during the summer the temperature in a number of natural anthills and checked periodically the presence of all development stages in dozens of ant nests. After that, the average dormancy periods of larvae and queens were calculated on the basis of the data obtained in laboratory experiments, with due account of seasonal variations of the day length and temperature. It has turned out that the calculated time of onset of diapause is quite close to that observed in nature (the deviations are not more than 5-7 days).

WHO DETERMINES THE DAY LENGTH?

It should be recalled, however, that larvae and queens live always in darkness. Only some worker ants, in particular foragers, come out of the nest onto sunlit places to gather food. How then can the photoperiod control the development of larvae and laying of eggs by queens if it has no direct effect on them? May be forager ants which come onto the surface can transmit this information to larvae and queens?

In order to prove this supposition, special formicaria with a light-insulated nest section were constructed (Fig.1). The brood and queens were kept constantly in darkness, whereas worker ants could hunt on an illuminated arena with feeding pans. They could pass to the arena through an S-bent black light-impermeable tube. The exit from the nest section was closed by a perforated partition with holes of a diameter that was passable for workers and impassable for queens. In that case, only the insects which visited the illuminated arena could see the length of days, whereas all other lived in constant darkness. In the experiments, the development soon ceased in the nests where the arena was kept under short-day conditions and proceeded in those where the arena was under long-day conditions. This suggests the conclusion that the nests were informed on the length of days by foragers.



If ants are collected in fall, i.e., when they are in diapause, and placed at the optimal temperature into the conditions of long and short day, egg deposition and pupation will be resumed in the former case already in one or two weeks, whereas in the latter case this will not occur. This resumed development is called photoperiodic reactivation.

It turned out later that larvae are actually insensitive to photoperiods and their development is controlled by worker ants. Diapausing larvae were placed into a short-day nest with worker ants which had been passed through photoperiodic reactivation. In two weeks, some of these larvae pupated, though were living all the time under short-day conditions. They were awakened from hibernation by reactivated worker ants. In order that all larvae could pupate, they should be periodically (say, every week) transferred into another nest with worker ants just taken from a 'long-day' nest (if transfered into short-day conditions, worker ants gradually fall into diapause and cannot induce larvae to further development).

Worker ants also control rather effectively the deposition of eggs by queens. If a diapausing queen is placed to reactivated workers and the nest is kept under short-day conditions, the queen will start egg laying already in a week. It should be noted however that queens are themselves sensitive to photoperiods, but are under a very strong influence of worker ants.

How forager ants can inform the nest on photoperiodic conditions? They only bring the gathered food to the nest, but do not participate in feeding of larvae and queens. This role is played by 'rearing' ants which never come out of the nest and do not know the day length. Therefore, the information is first transmitted from foragers to rearers and then from the latter to queens and larvae.

WHAT ENSURES INFORMATION TRANSMISSION?

In colonies of ants and other social insects, a continuous food exchange takes place between adult individuals. It is quite probable that some chemical substances which can control the behavior and development of insects are exchanged together with food. These substances are called pheromones. Do they participate in controlling the development of ants?

In order to study this problem, a new formicarium was constructed. It consisted of two halves separated by a partition with a slit. An ant could put its head into the slit, but could not pass through (Fig. 2). One half of the nest was kept under short-day conditions and contained a group of diapausing ants with larvae and queens, while reactivated ants were placed into the other half. The insects could transfer food through the slit to one another. Reactivated ants were replaced every week. The results of the experiments were always the same: in one or two weeks, eggs and pupae appeared in the group of diapausing ants, i.e., reactivation started in the group.

It could be supposed that the information on day length is transmitted with food, but the supposition must be rejected. When groups of ants were separated not by a partition with slit, but by a simple net through which they could not exchange food, reactivation was initiated all the same. This was utterly unexpected!

Maybe ants use tactile signals? They actually pass their feelers through the net and can touch worker ants at the other side. Science knows many examples of information transmission by social insects, including ants, exactly in this way.

Groups of ants in our double formicarium were separated by two nets spaced at a distance of 7-8 mm from each other, so that ants could not 'reach' their neighbors by the feelers. Notwithstanding this arrangement, information transmission did take place.

At this stage, a new experiment was needed, which could distinguish between acoustical and chemical signalization, i.e., between sound and odor. For this purpose, air from a formicarium with a long day was pumped by a micropump at a rate of 0.01-0.02 ml/s through a black S-bent tube into a formicarium kept under short-day conditions (Fig. 3). In a check experiment, air was pumped in reversed direction. In the long-day nests, where diapausing ants with larvae and queens were lodged, reactivation began in a short time, i.e., eggs and pupae appeared. A few days later, signs of reactivation were discovered in the short-day formicarium which was supplied with air from long-day nests. In the check experiment, however, where air was pumped from short-day to long-day nests, no changes occurred. This meant that signals were transmitted exactly with air, since sound could propagate even against a weak air flow.

For the final proof of the conclusion that the information on day length is transmitted by odor, another experiment was undertaken. Reactivated ants were killed and ground and placed into the nest sections of formicaria with diapausing insects. The result was that in 7-10 days the queens started egg deposition and some larvae even pupated.

UNDERSTANDING THE CONTROL MECHANISM

Most of the pheromones being studied by science belong to the class of releasers: chemical substances whose perception causes immediate changes in the behavior of an animal. For instance, on encounter with an enemy or a large prey, a forager ant releases a particular gland secretion, an alarm pheromone which signals danger. Other ants happen to be nearby, smell the odor, become excited and aggressive, move quickly in the direction of the source of odor, and attack the enemy.

Much less known are primer pheromones which can influence the physiological state of animals and control their development. Examples of such substances in social insects are very few, though many specialists believe that primers can also play an important part. For instance, a queen of honey bee can secret a pheromone that makes the percepting worker bees sterile and prevents the production of new queens in the hive. Similarly, the pheromones of the queen and king of termites prevent the appearance of new fertile insects in the termitarium, but instead stimulate the growth of new soldiers, defenders of the colony.

The pheromone of honey bee is active only in direct contact: worker bees in the 'suite' touch from time to time the queen and carry off a certain quantity of the pheromone on their bodies; after that they come into contact with other bees, so that all individuals in the hive periodically perceive the scent of their queen. With termites, king pheromones are transferred from one termite to another together with food and in the final result reach every member of the colony. Up to the present time, no primer pheromones have been known in social insects which could be perceptible at an appreciable distance from the secreting individual, as is the case with many releaser pheromones.

The pheromone that we have discovered in *Myrmica rubra* ants is a typical primer (we have called it an activator). It controls the processes of development in a colony, stimulates pupation of larvae and egg deposition of queens, but does not change the external signs of behavior of ants and relates to a new class of pheromones: remotely perceptible primers.

With solitary insects, each individual must for itself adapt to seasonal changes and correct respectively its development. On the contrary, social insects possess intrinsic 'collective' forms for controlling the living activity, which are based on the interaction of individuals in a community. As we have established for myrmica, photoperiodic conditions can be perceived and estimated only by forager ants which hunt outside the nest. They can transmit the acquired information to 'rearing' ants in the form of a specific signal, an activator pheromone. As days become shorter, forager ants release less of the pheromone. This evidently explains the above-mentioned influence of day length on the terms of cessation of development. 'Rearing' ants receive signals from foragers and accordingly control the development of larvae. Queens probably can perceive foragers' signals independently. The absence of activator pheromone causes the queens to cease egg deposition and larvae to cease pupation. They start preparing to wintering, i.e., fall into diapause.

It may be supposed that ants possess an inhibitor pheromone which informs on shorter days and causes hibernation. The science still has however no experimental evidence on the existence of this pheromone.

Neither do we know where the gland (or glands) for secretion of activator pheromone is located in the ant's body. The chemical nature of the pheromone is also unknown. Further experiments are needed to answer these questions. If it were possible to synthesize the activator pheromone in laboratory, the science and practice would acquire an utterly new tool for controlling the development not only of ants, but probably of some other insects, including those of economic importance.

It is of no less interest to understand how 'rearing' ants in turn 'command' the development of larvae: whether by means of the activator pheromone or in a different way? They can probably 'add' to the food of larvae some substances which can stimulate or, on the contrary, suppress their development. We are now working on the problem and have already obtained some interesting, though preliminary, results. These studies will certainly be helpful in better understanding the remarkable world of ants and in studying the intricate forms of 'collective' organization of their communities.

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Photos by the author