

**45**

**MEMORABILIA  
ZOOLOGICA**

**Wojciech Czechowski, Bohdan Pisarski**

**Laboratory methods for rearing ants  
(*Hymenoptera, Formicoidea*)**

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Ants, the superfamily *Formicoidea* within the order *Hymenoptera*, constitute a fairly numerous group. There have been recorded a dozen or so thousand species of ants. In Poland, 100 species (Pisarski, 1988) are known. Ants are highly social insects. They live in family groups (colonies, societies), internally differentiated into castes and functional groups (workers and females (queens), gynes, males, etc.). They perform various functions and due to this in many cases they exhibit polymorphism and, periodically, a new generation of sexuals (gynes and males). Particular castes and functional groups are interdependent — not only through reproductive processes, but also as a result of a system of intrasocial physiological and psychological self-regulation that forms the structure of a given society according to the current requirements imposed by the environment. That is why both the development of a colony and of an individual as well as the conditions of their life are interdependent. An isolated ant lives very short, several times shorter than one in a society. In ant colonies many processes may take place only when the abundance of the swarm is high enough. The higher is the abundance usually reached by colonies of a given species, the higher is the minimum abundance of the group (the so called social minimum) indispensable for the performance of various processes such as the production of offspring or sexual forms, building of nests etc. The social minimum, by the way, is different for each of these processes. The behaviour of workers — undertaking various functions, the degree of aggressiveness, territorial behaviour, etc. — also depends on the abundance of a colony. Ant societies are long-lived, existing for many years. They inhabit specially built nests whose construction is greatly varied, depending on the ant species and often also on the local topographic conditions. Ants live in small colonies (over one dozen or several hundred individuals) or in large colonies (up to several thousand individuals) in natural shelters or in broken

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Wojciech CZECHOWSKI, Bohdan PISARSKI

**Laboratory methods for rearing ants (*Hymenoptera*, *Formicoidea*)**

INTRODUCTION

Ants, the superfamily *Formicoidea* within the order *Hymenoptera*, constitute a fairly numerous group. There have been recorded a dozen or so thousand species of very diverse bionomics. In Poland, there merely are about 80 species (PISARSKI 1975) but even these show a considerable ecological and ethological differentiation.

Being social insects, ants live in family groups (colonies, societies), internally differentiated morphologically and functionally. They consist of fecund females (queens), generally a great number of neuters (workers) performing various functions and due to this in many cases their demonstrate polymorphism and, periodically, a new generation of sexuals (alate males and females). Particular castes and functional groups within a colony are interdependent — not only through reproductive processes and the division of labour, but also as a result of a system of intrasocial physiological and psychical self-regulation that forms the structure of a given society according to the current requirements imposed by the environment. That is why both the life and the development — of a colony and of an individual as well — depend on the social conditions. An isolated ant lives very short, several times shorter than one in a society. In ant colonies many processes may take place only when the abundance of the swarm is high enough. The higher is the abundance usually reached by colonies of a given species, the higher is the minimum abundance of the group (the so called social minimum) indispensable to the commencement of various life processes such as the production of offspring or sexual forms, building filial nests etc. The social minimum, by the way, is different for each of these processes. The behaviour of workers — undertaking various functions, the degree of aggressiveness, territorial behaviour, etc. — also depends on the abundance of a colony.

Ant societies are long-lived, existing for many years. They inhabit specially built nests whose construction is greatly varied, depending on the ant species and often also on the local topographic conditions. Ants living in small colonies (over one dozen or several dozen individuals) often take advantage of natural shelter under stones, in rock crevices, under patches of moss, within litter, in broken

bark etc., doing no or practically no construction work of their own. Such is, for instance, the way of nesting of *Ponera coarctata* L. ants and of many species of the genus *Leptothorax* MAYR.

Ants that make more numerous colonies (hundreds, thousands of individuals) build nests which are appropriately bigger and far more complex. These are, in most cases, underground structures, consisting of a network of chambers and a system of connecting corridors. Underground nests may be horizontally spread just under the surface of the ground (e.g. in *Lasius niger* L.), vertically extended deep into the soil (in *Formica fusca* L.) or formed as a combination of these two types (in *Tetramorium caespitum* L., *Lasius alienus* FOERST.). Underground nests are frequently supplemented by mounds made of soil or plant matter, and inside these there also are systems of chambers and corridors. Small mounds with a simple structure in the form of small plant remnants loosely arranged are built over nests in tufts of grass by several species of the genus *Myrmica* LATR. Mounds made of the same material, but bigger and more solid, are constructed by ants of the subgenus *Coptoformica* MÜLL. (e.g. *Formica exsecta* NYL., *F. pressilabris* NYL.). Compact ground mounds overgrown with plants are home to the underground ants *Lasius flavus* F. Similar mounds, yet without vegetation, are often an extension to the nests of *Lasius niger* L., *Tetramorium caespitum* L. or *Formica cunicularia* LATR. Mounds that are most imposing and have the most complex structure are those built of plant matter by red wood ants of the subgenus *Formica* s. str. (e.g. *F. truncorum* F., *F. pratensis* RETZ., *F. rufa* L., *F. polycytena* FOERST.) and these colonies comprise, now and then, over a million inhabitants while the capacity of their nests reach a few cubic metres. The design of such nests makes it possible to effectively regulate the temperature and humidity according to the current colony requirements and the external conditions (the difference between the temperatures outside and inside the nest may even reach 20°C).

Nests built in dead wood constitute a separate group. Many species, normally nesting in the ground, also inhabit rotten tree stumps, but there even are certain ants, for instance those of the genus *Camponotus* MAYR or *Lasius brunneus* LATR. that live almost exclusively in wood where they bore corridors also in dead parts of living trees. Some of them, for instance *Lasius fuliginosus* LATR. use, for building their chambers inside tree trunks, a kind of paper mass they themselves prepare from wood.

Nests serve not only as shelter. They are constructed in such a way that they provide the right microclimatic conditions for the development of offspring — eggs, larvae and pupae. Brood at particular developmental stages are placed in different zones of a given nest where they are ensured the required optimum temperature, humidity, etc. A nest also makes it possible for ants to survive winter. This purpose is generally best served by the deepest parts, but certain ants, for instance *Formica sanguinea* LATR., use special winter nests.

Ants forage mainly outside their nest — most frequently on the surface of the ground but also in the soil, among herbaceous plants or in trees. Large areas are sometimes penetrated in search of food; foraging areas can extend from a few

square decimetres to several hectares, depending on the abundance of colonies, on the size and mobility of their workers. The basis of ant forage consists of protein food, usually in the form of small arthropods. This is indispensable to their reproduction and development. To a large extent, ants also feed on carbohydrate food, mainly on honey-dew from aphids. Carbohydrate food, however, serves only for energetic purposes. Some species feed on the seeds of certain plants.

#### AIMS AND GENERAL PRINCIPLES OF REARING

Myrmecological investigations have recently entered a stage of a hitherto unprecedented boom. This is connected with the development of ecological studies — in many habitats ants are the main components of the zoocoenoses. Their occurring in great numbers makes them play a vital role in ecosystems where they have impact both on the biocoenoses and on the physical and chemical conditions of the sites. At the same time they are, due to the type of their diet and the place of occurrence, either an important assistance to man, especially in the protection of forests, or a dangerous pest in crops. Moreover, certain ant species are beginning to occupy the main position among synanthropic animals and this is due to the fact that they are spreading all over the world because of the mass exchange of commodities and also they find favourable conditions for numerous occurrence in the developing urban agglomerations. Thus, their sanitary-epidemiological impact is increasing.

Independently of the ecological factor, another important stimulus to the development of myrmecology was found in the growing interest in general sociobiological questions. The systems of communication, the organization and functioning of animal societies, the social evolution — these are problems in solving that ants are especially convenient material. Therefore they are frequent objects of such studies.

Due to a nest, partly hidden mode of life many aspects of ant biology may be studied only with the help of properly constructed artificial nests (formicaria). Moreover, laboratory rearing, with its guaranteed constant environmental conditions, provides possibilities of carrying out many observations and experiments that are very difficult or utterly impossible to make under natural conditions.

Laboratory rearing of ants (meant properly, not as merely keeping the insects) is more difficult than rearing arthropods which live individually. This is brought about not so much by the sensitiveness of the very ants as organisms (they adapt fairly easily to new conditions or even to those that are not entirely typical for them) but by the necessity to provide them with conditions indispensable to social life and nesting. In addition to this, the ecological and ethological variety of ants makes it impossible to give a universal formula for an artificial nest and how to deal with the colony settled there. Almost every single species requires

different rearing conditions, and with the ultimate objective in mind a suitable variant must be chosen. The most general principles that must be observed while undertaking ant rearing are as follows. A colony must have its proper social structure, i.e. the number of fecund queens suitable for a given species, offspring and the number of workers exceeding the social minimum indispensable to a given experimental variant. The dimensions of an artificial nest or a container with natural nest material should correspond to the size of a natural nest with that particular abundance of a swarm. Only certain species of very small ants that form colonies of low abundance may, even for a long time, be kept in simple and small containers (dishes, test-tubes).

Ants find wood to be the best material for nest building, but this quickly warps under damp conditions. It is far more practical to use glass or Plexiglass pieced together with putty or glue used for making aquaria. Glue containing soluble toxic components must be avoided. Plastic containers are convenient and therefore used frequently, but they do not deserve to be recommended because many kinds of plastic emit toxic substances.

A typical formicarium should have two separate sections: a nest part and a feeding one (the latter being a substitute for the foraging area) — each with a specific microclimate. The nest part should be blacked out (at least at the time when no observations are being made) or lit with a dim red light, it should have a constant temperature appropriate for a given species (e.g. about 20°C for most ants of the genus *Myrmica* LATR., 25°C for the genera *Lasius* F. and *Formica* L., 30°C for *Monomorium pharaonis* L.; BRIAN 1977, KRETZSCHMAR 1978) and optimum humidity (60—80%; LE MOLI, PARMIGIANI 1981) — so that the nest will neither dry out nor go mouldy. The nest chamber does not require strong ventilation but if it is in a closed container there must be some means of providing it with fresh air (a wire gauze window, many small openings or cracks). The foraging part (arena) of the nest should be full of light and dry, permanently directed towards the source of light (e.g. a window), well ventilated and always provided with a supply of the right food (protein, carbohydrates) and water. Ants that hibernate in their natural environment must be provided with conditions allowing them to overwinter in this way.

Cleanness is essential to laboratory rearing because if the refuse, which is normally carried outside and far from the nest, starts to decay mould may appear, being lethal to the colony.

In all types of laboratory rearing it must be made sure that ants will have no possibility of escaping.

## METHODS OF REARING

### Artificial nests

As it has already been mentioned, all methods of ant rearing must be adapted to the bionomics of particular species, to their structure and social organization.

At the same time however, they should be selected according to the objective of the rearing itself. Four main types of rearing may be distinguished on the basis of the purpose assigned:

- 1) rearing in which natural conditions are imitated and no permanent observations of ants is required;
- 2) rearing that makes it possible to study the inside of a nest;
- 3) rearing aimed at experiments and observations of ants within their foraging area;
- 4) mass rearing of certain forms (castes) for practical or experimental purposes.

#### Rearing imitating natural conditions

In many studies, although it is necessary to provide ants with conditions as close to the natural ones as possible, there is no need to make any special observation of individuals either in the nest or the foraging area. In such cases, rearing is technically the easiest. Ants may simply be placed in large containers or insectaria. Containers with solid walls are more suitable because here humidity is maintained better than in cages made of a thick metal net. Aquaria or containers that have a wide opening at the top are particularly useful because they make free access to the nest possible. This is especially significant when experiments require periodic inspections of the composition of a given colony (e.g. in order to determine the changes in the social structure of the swarm). The size of a container must be properly chosen since there must be room for the nest and also some space serving as the foraging area. It is of course possible to prepare the arena in a separate container provided that the ants have a possibility to move freely. The bottom of the container should be covered with a few-centimetre layer of sand (sea sand is the best because it is resistant to moulding), but a bottom left uncovered is more convenient in rearing involving periodic reviews of a colony.

The material provided for nest building should be suitable for the species; the best situation is when it is taken, together with the ants, from the natural maternal nest. In cases when ants constructing soil mounds need additional building material, they should be provided with soil deposited by workers near a natural nest of the same species (such soil is resistant to moulding and contains no harmful bacteria; KONDOH, MATSUMOTO 1977). Ants that build mounds from parts of plants are given material taken from such a mound. Building material must not be collected from deserted mounds — recently deserted mounds may contain parasites that have caused the removal, and old ones are a seat of fungi and bacteria. Species nesting in wood should be given a piece of wood placed on a layer of sand — best if this wood can be taken from the trunk with the natural nest, but pieces of bamboo or hollowed out sticks (e.g. alder ones) can also be used. In order to facilitate access to internal part of such a nest, sticks may be broken into two sections temporarily held together (e.g. by means of an elastic

band). If the wood is to maintain humidity it must lie striking directly to the layer of wet sand. Ants that normally build their nests under stones or in rock crevices must be provided with a few flat stones, stone slabs, etc. placed on the layer of sand. Synanthropic ants (e.g. *Monomorium pharaonis* L.), for which artificial material is basically natural, can build their nest in a pile of empty glass test-tubes with loosely fitting stoppers. Each test-tube may contain a piece of paper that will give the ants some additional area and make it easier for them to move. In each case the amount of the building material provided must be concordant with the abundance of the colony reared.

Another rearing method, where natural nest material plays only a supplementary role, is recommended by CHAUVIN (1972) for large ants of the genus *Formica* L. The main part of a nest consists of several dozen wooden drawers covered with transparent tops made of Plexiglass. Each drawer, with the dimensions 11.5 x 11.5 x 0.4 cm, has in its side an entrance hole in the form of a 2-centimetre crack. The drawers are arranged in tiers, one on top of another, and they form a pile in a container which acts as the arena (Fig. 1). Ants readily settle in such a nest and build it over with natural nest material provided. The main advantage of such nests is the fact that their contents can easily be controlled.

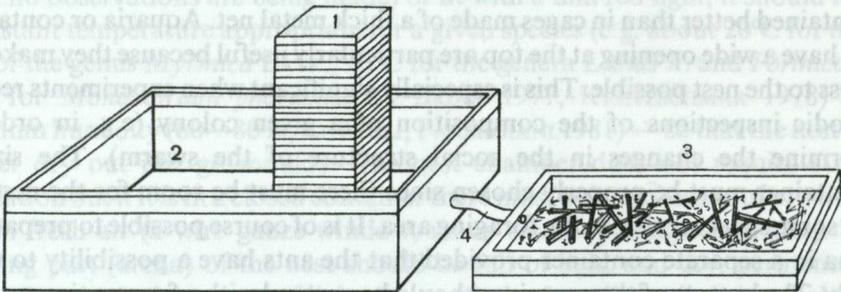


Fig. 1. A set of formicaria for rearing ants of the *Formica rufa* group; 1 — summer nest in the form of tiers of shallow boxes; 2 — arena; 3 — winter nest; 4 — connecting tube (after CHAUVIN 1972).

Constant humidity in nests is maintained by means of a moistener (a piece of blotting-paper, cloth or fine-porous sponge) with one end inserted into the nest and the other connected in the source of water. Best for this is a glass tube with the part near the nest ending in a capillary or stopped with a loose swab of gauze or cotton wool (the swab must be trimmed evenly at the opening of the tube) — and water is dropped in at the other and from a pipette. The moistener should be firmly secured at a wall of the rearing container because an unsteady one could destroy the nest. Moistening a nest through spraying water over it is not correct since with this procedure water does not easily permeate into the nest material, but generally forms a film on its surface. Moreover, spraying does not ensure that the humidity gradient in the nest is properly directed.

Ants should be prevented from leaving their rearing containers by means of one of the ways given further on.

### Observation nests

The most difficult question posed by ant rearing is how to construct an artificial nest of ideally matched parameters thanks to which the life of a given colony could be studied and a fairly successful existence of the inhabitants would be possible. There are a lot of more or less positive solutions and particular authors either promote or criticize the same models. This divergence of opinion probably arises from the fact that a particular type of formicaria is used for different ants and for various purposes. In order to choose the most suitable variant it is necessary to have great experience and knowledge of ants.

Two types of observation nests are generally distinguished: with soil or some other nest material (natural or its substitute<sup>1</sup>), and entirely artificial constructions which deprive ants of any building material.

The construction principle of an observation nest consists in the fact that the thickness of the layer of the nest material is so restricted that ants are forced to bore corridors and chambers directly along the transparent walls of the formicarium. Thus, a kind of cross-section of the nest is formed.

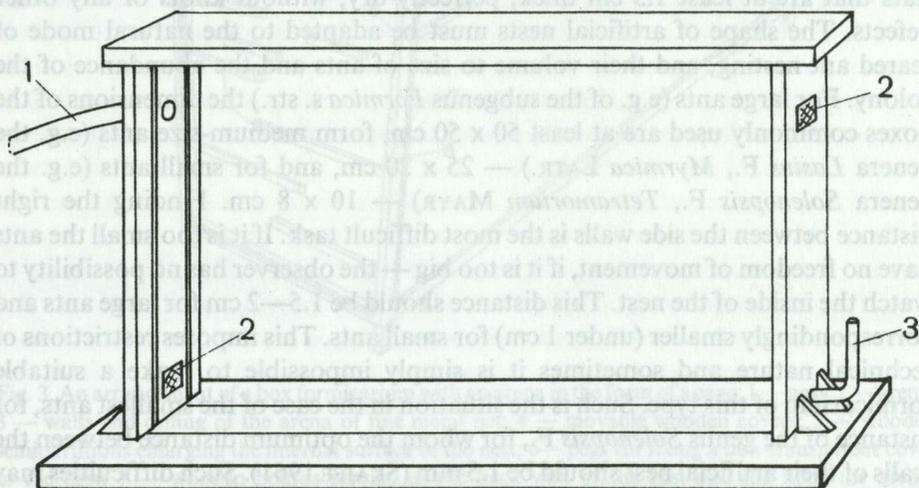


Fig. 2. A box formicarium after HUBER's model: 1 — tube connecting the nest with the arena; 2 — ventilation holes; 3 — moistening tube (after SZCZERBAKOW 1957).

A closed box formicarium based on a XIX-century prototype by HUBER is the most common solution of this type (WHEELER 1926, SKAIFE 1961). This structure looks like a standing double window frame — the glass side walls are set in a wooden frame. The top of such a formicarium is closed by a tight fitting opaque lid with sliding doors thanks to which slight manipulations at the nest are

<sup>1</sup> In some countries there already are on sale ready-made formicaria in which the nest material has been prepared out of perlite, that grain diameters are different to meet the requirements of ants of different size (KONDOH, MATSUMOTO 1977).

possible without undue disturbance to the ants. The glass walls must have tightly-fitting non-transparent blinds of black cardboard, pasteboard, cloth, etc. that are taken off, raised or pushed aside only at the very time of observations. It should be easy to remove them without shaking the box. One of the narrow wooden walls of the nest has two holes in it — entrance (at the top) and a ventilation hole (at the bottom) the latter with a thick metal net. The opposite wall has a ventilation hole at the top but in its lower part is inserted a moistening tube bent at an angle of 90° (Fig. 2). If the formicarium is not directly placed in a vessel (e.g. a cuvette) serving as the foraging area, the entrance hole must be supplied with a tube leading to the arena. If the tube is made of glass, therefore slippery, it is advisable to put into it a strip of rough paper or glue some sand all over the inside (liquid glue, e.g. arabic gum, may be used). The outside surface of the tube must be protected, of course, for instance by a ring saturated with a substance deterring ants. Prior to placing any nest material in the formicarium its bottom must be covered with a layer of sand reaching a little above the point where the moistener is inserted.

As wood has a tendency to warp, one must use, while building box formicaria, slats that are at least 1.5 cm thick, perfectly dry, without knots or any other defects. The shape of artificial nests must be adapted to the natural mode of reared ant nesting, and their volume to size of ants and the abundance of the colony. For large ants (e.g. of the subgenus *Formica* s. str.) the dimensions of the boxes commonly used are at least 50 x 50 cm, for medium-size ants (e.g. the genera *Lasius* F., *Myrmica* LATR.) — 25 x 20 cm, and for small ants (e.g. the genera *Solenopsis* F., *Tetramorium* MAYR) — 10 x 8 cm. Finding the right distance between the side walls is the most difficult task. If it is too small the ants have no freedom of movement, if it is too big — the observer has no possibility to watch the inside of the nest. This distance should be 1.5—2 cm for large ants and correspondingly smaller (under 1 cm) for small ants. This imposes restrictions of technical nature and sometimes it is simply impossible to make a suitable formicarium of this type. Such is the situation in the case of the smallest ants, for instance of the genus *Solenopsis* F., for whom the optimum distance between the walls of their artificial nest should be 1.5 mm (SKAIFE 1961). Such difficulties may be partly overcome if a box made is twice as wide as required and then a strip of perforated sheet metal is put inside as a partition (HUBER'S prototype was constructed in this way). Unfortunately, this diminishes the observation area in relation to the volume of the nest. The use of box formicaria (and others based on the same principle) is further limited by the fact that they are not fit for ants whose queens are inordinately big in comparison with their workers. Yet this type of artificial nest is nevertheless used very often because it is serviceable for a great many ant species. Of course, it is modified in various ways, but this depends on the ingeniousness of research workers.

For instance, worthy of note is a variant in which the box nest is directly connected to the arena in the form of a cage. Such structures were successfully used by FREELAND (1958) for rearing Australian ants of the genus *Myrmecia* F.

An additional element applied in these formicaria, and worth recommending, consists of level semi-partitions made of wooden slats which add to the internal surface of the nest (Fig. 3). Thanks to such a framework ants bore larger, more noticeable chambers. These partitions must be easy to insert so that they can be put in one by one as the nest is being filled up with the nest material and taken out as it is being emptied.



Fig. 3. An arrangement of a box formicarium with an arena in the form of a cage; 1 — nest; 2 — arena; 3 — walls and ceiling of the arena of fine metal net; 4 — movable wooden covers; 5 — wooden semipartitions enlarging the internal surface of the nest; 6 — pegs for fixing a non-transparent cover to the nest; 7 — wooden boards serving as the back wall of the nest and the floor the arena; 8 — wooden lath determining the width of the nest (after FREELAND 1958, simplified).

An open formicarium is another variant of an artificial nest built on the same principle as the one used in a box formicarium. This one is made from a transparent cylindric or cuboidal vessel of suitable capacity (minimum 10 l for large ants). In this case, the inside of the nest can be watched because a wooden block of appropriate dimensions and shape is placed within the container (on a layer of sand). The space between the block and the walls has the required width and this is filled up with nest material. The nest is hidden under a non-transparent cover shaped like a case. A moistening tube is pushed through the nest material right to the bottom of the vessel or (better still) it is inserted in a hole made in the wooden core. Since this formicarium is open at the top its edges must be

protected against ant escape, but there must be a platform leading to the arena (SZCZERBAKOW 1957).

Similar, as far as the principle is concerned, but situated horizontally, are flat nests built after a model designed by LUBBOCK (LUBBOCK 1882, STITZ 1914, WHEELER 1926, SKAIFE 1961, BRIAN 1977). The XIX-century prototype consisted of two glass panes of 10 square inches separated by border slats of optional width, depending on the size of the ants reared. One of the slats, a loosely inserted one, made it possible to fill the nest with soil; a small opening in one corner served both as the entrance for the ants and a ventilator as well. These nests were placed in flat box-arenas covered with a glass pane lying on cloth. This arrangement ensured that the arena was closed off tightly, but the same time an exchange of air was possible.

Such horizontal nests are obviously best suited for ants nesting just under the ground, under stones and patches of moss. Therefore, they are used for very small ants that require a very small space between the walls of their formicarium (or rather between the floor and the ceiling). In such cases wooden slats separating the glass panes are replaced by leather belts of appropriate width. In this type of nest it is altogether good to replace wood by some other material that does not warp, and to use cramps to hold the structure (Fig. 4). The whole thing is then easy to dismount. The floor need not be of glass — as a bedding for ants this is too cold and slippery; it should not be transparent either. An opaque cover should be put on the top of the nest. Slits in the side walls of the nest serve ventilation and moistening purposes; if the walls are very thin then holes must be made in the cover. Since the space between the floor and the ceiling is very narrow, horizontal nests cannot be filled with ants and nest material at the same time. First, a formicarium must be filled with soil — previously sifted if very flat nests are to be used — and then ants can be introduced in a way described further on. The nest is connected with the arena by means of a tube leading from the entrance hole in a side wall. Some other solution may also be applied (Fig. 4).

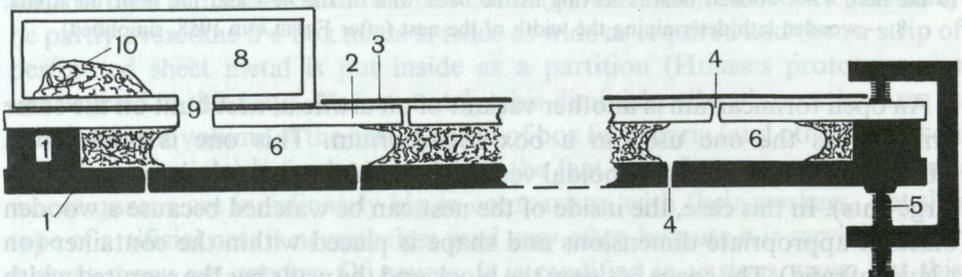


Fig. 4. Modified LUBBOCK's horizontal nest; 1 — non-transparent floor and side walls; 2 — transparent lid; 3 — non-transparent cover for the nest; 4 — ventilation holes in the lid and floor; 5 — cramps fixing the structure; 6 — nest chamber dug out by ants; 7 — soil (or some other nest material); 8 — arena; 9 — holes connecting the nest with the arena; 10 — food (after BRIAN 1977).

The second group includes observation formicaria with no nest material where the inside is a more or less exact copy of the internal structure of a nest. Of course, ants are far better visible in such nests than in those with natural building material, but it is more difficult to achieve success in rearing them. Such formicaria are best suited for small ants which nest in nature in rock crevices rubble, etc., and also for species with synanthropic tendencies. They are used in short-term experiments involving groups of ants selected from a colony. This is why the arena of such nests is usually limited to a small space that is an integral part of the formicarium.

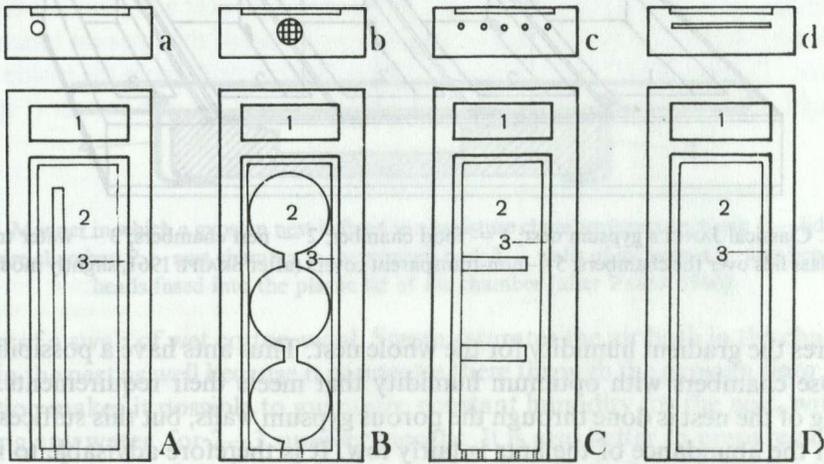


Fig. 5. Small gypsum nest after JANET's pattern with a different system of chambers (A, B, C, D) and different entrance holes in the front wall (a, b, c, d; b — large opening with a allowing the workers to pass, but stopping the queens); 1 — water trough; 2 — nest chambers; 3 — projection supporting the glass lid (after SKAIFE 1961).

JANET's model from the end of the XIX century is the oldest, yet still widely used type of such a nest (STITZ 1914, WHEELER 1926, SKAIFE 1961, BRIAN 1977). It is in the form of a horizontally placed gypsum slab (limestone or a similar soft material have also been used) with a few hollows bored out and joined by a small corridor. These hollows serve as nesting chambers. At present, such nests are made from gypsum cast in a previously prepared mould made of any material (SKAIFE 1961). Glass (or plastic) slabs make the bottom and the top. It is better if each chamber has a separate lid — microscopic slides serve this purpose best. But non-transparent covers are also necessary, of course. The size of a given nest and the depth of the chambers must be adapted to the number and size of the ants reared. Generally, these nests are small; in the case of the smallest ants they can even be studied by means of binoculars. For instance, when the aim is to study the initial stages of colony founding by queens of the smallest ants (e.g. of the genera *Solenopsis* F., *Leptothorax* MAYR, *Tapinoma* FOERST., *Monomorium* MAYR) gypsum nests of about 2 x 3 cm are used (but nests hollowed out in balsa wood are

more convenient for this purpose). The number and system of the chambers are optional — these are left to the resources of experimentators (Fig. 5). A linear system is the most practical because of the manner of moistening applied in nests of this type: along one edge of the gypsum slab there is a longish hollow nowhere connected to the other chambers and this serves as a small chute for water (Figs 5 and 6) refilled as water evaporates. Water permeating through the gypsum

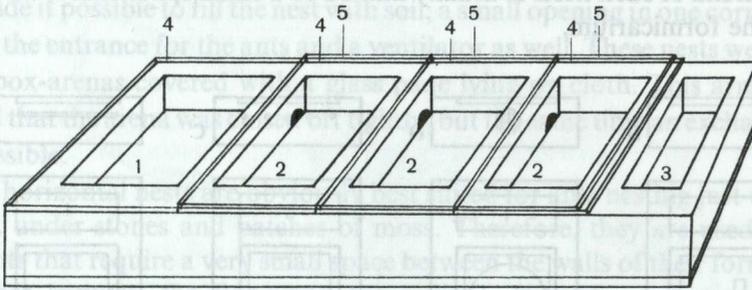


Fig. 6. Classical JANET's gypsum nest; 1 — food chamber; 2 — nest chambers; 3 — water trough; 4 — glass lids over the chambers; 5 — non-transparent covers (after SKAIFE 1961, slightly modified).

ensures the gradient humidity for the whole nest. Thus ants have a possibility to choose chambers with optimum humidity that meets their requirements. The airing of the nest is done through the porous gypsum walls, but this suffices only when the abundance of the ants is fairly low. It is therefore advisable to make small holes in the walls or to replace one of the upper glass panes by a cover made of thick metal net.

In its original form, JANET'S nest had no arena. Its substitute was in the last, most dry and best lit chamber serving as a bait (Fig 6). However, since ants react very badly to being completely cut off, one should give them access to some open space.

The gypsum nests used now have many improvements in comparison with the prototype; these mainly being various kinds of frames or adaptations that allow for adding standard units to accommodate a growing colony.

The main drawback of gypsum nests is their tendency to dry out too quickly but apart from this they are difficult to keep clean and therefore they easily go mouldy. It is very difficult to keep the constant humidity of a gypsum block, but it is easy to let the nest dry out. This difficulty may be overcome if the system of moistening is improved. A gypsum nest is best for species that are particularly hygrophilous (e.g. *Stenammina westwoodi* WESTW., *Myrmecina graminicola* LATR., *Ponera coarctata* LATR.). These ants require a permanent and high saturation of the walls of their nest with water. To have this done, there must be a constant inflow of water. The simplest way to do this is to fix a wick of cotton wool hanging over the nest with one end dipped in a dish with water dripping continually off the other end onto the gypsum. The amount of water supplied in

this way is regulated by the thickness of the wick (FABER 1966). An automatic feeder would be a more dependable appliance.

The situation is easier with moderately hygrophilous ants for which it is enough when the air in their nest is saturated with steam. Their gypsum nests may be placed in hermetic containers that play the role of moisture chambers (FABER 1966, BRIAN 1977). The model suggested by FABER (1966) is especially useful. The moisture chambers are in the form of polystyrene containers (glass ones would be better) with a transparent lid. A gypsum nest is hooked on to the underside of the lid (Fig. 7). On the bottom of the chamber (and under the nest) there is a dish with

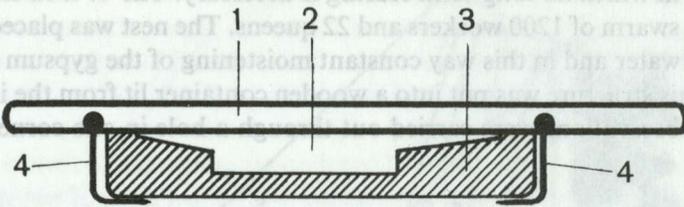


Fig. 7. Manner in which a gypsum nest is fixed in a moisture chamber (cross-section): 1 — lid of the moisture chamber; 2 — nest chamber in the gypsum nest; 3 — walls of the nest; 4 — pins bent, with heads fused into the plastic lid of the chamber (after FABER 1966).

water of a swab of wet cotton wool. Steam saturates the air both in the chamber and in the nest as well because it permeates there through the gypsum floor. This solution makes it possible to guarantee constant humidity for the nest, without adding any water, for 2—3 summer months. It is also better to prepare nests in this way for overwintering; then the supply of water is enough for the whole winter. Moisture chambers have one disadvantage, though, and this is lack of gradient-controlled moistening of the nest which is possible in the classical

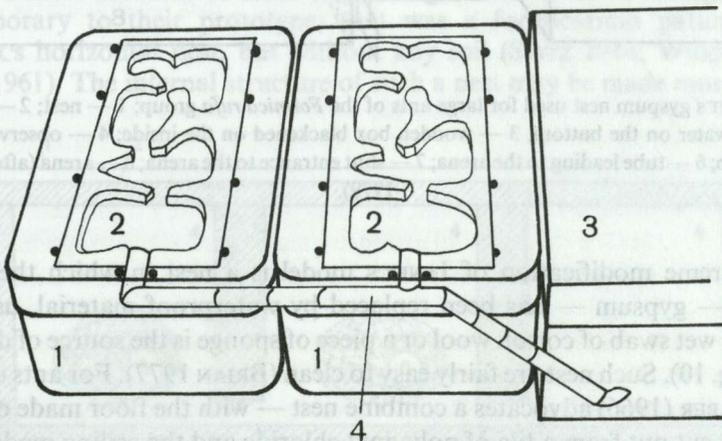


Fig. 8. Manner of arranging the moisture chambers and connecting them with the arena: 1 — moisture chambers; 2 — gypsum nests; 3 — container serving as an arena; 4 — connecting tube (after FABER 1966).

model. However, the nests in the chambers may be connected with one another (and with the arena) by means of tubes sticking outside (pushed through the walls) (Fig. 8) and in this way the ants have a possibility to choose a suitable living quarter. The chamber must be opened from time to time in order to air it. Apart from this, CO<sub>2</sub> absorbents may be used, for instance sodium hydroxide (BRIAN 1977).

A solution used by MABELIS (1979) for studying *Formica polyctena* FOERST. ants is worthy of note. The fact that a gypsum nest was used for such big ants must be considered an exceptional case, and the nest served its purpose in an experiment in which no long-term rearing is necessary. The 47 x 12 cm nest was settled by a swarm of 1200 workers and 22 queens. The nest was placed aslant in a dish with water and in this way constant moistening of the gypsum block was ensured. This structure was put into a wooden container lit from the inside with red light. Observations were carried out through a hole in one corner (Fig. 9).

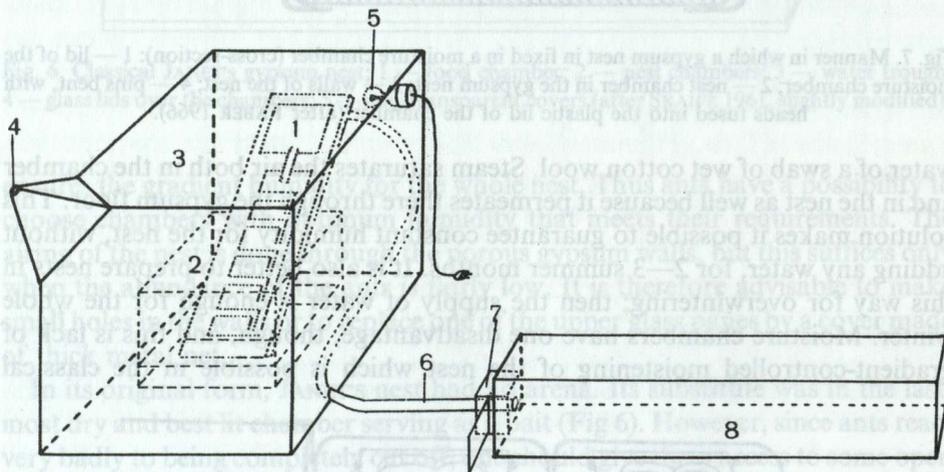


Fig. 9. JANET'S gypsum nest used for large ants of the *Formica rufa* group; 1 — nest; 2 — container with some water on the bottom; 3 — wooden box blackened on the inside; 4 — observation hole; 5 — red bulb; 6 — tube leading to the arena; 7 — shut entrance to the arena; 8 — arena (after MABELIS 1979).

An extreme modification of JANET'S model is a nest in which the original material — gypsum — has been replaced by waterproof material, usually by plastic. A wet swab of cotton wool or a piece of sponge is the source of dampness there (Fig. 10). Such nest are fairly easy to clean (BRIAN 1977). For ants nesting in wood, FABER (1966) advocates a combine nest — with the floor made of veneer, chambers cut out from a tile of polyvinyl chloride and the ceiling made of glass (Fig. 11). These ants do not require high humidity, they are satisfied with the amount that permeates through the wooden floor of their nest placed in the moisture chamber. CZYŻEWSKI (pers. comm.) has used nests made entirely of

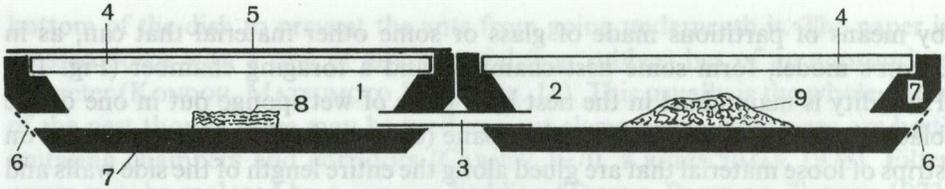


Fig. 10. Modified JANET'S nest made of waterproof material; 1 — nest chamber; 2 — food chamber; 3 — connecting tube; 4 — transparent lids; 5 — non-transparent cover; 6 — ventilation holes; 7 — non-transparent floor and side walls; 8 — wet sponge; 9 — food (after BRIAN 1977).

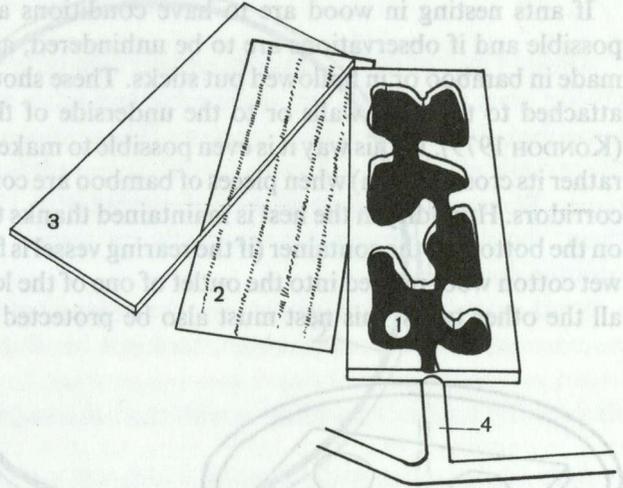


Fig. 11. Modified JANET'S nest for ants nesting in wood; 1 — nest chambers cut out in a polyvinyl chloride plate; 2 — floor of veneer; 3 — glass lid; 4 — tube connecting the nests and arenas (after FABER 1966, modified).

wood (covered with a glass pane), with its chambers and corridors bored in a small wooden board with a dentist's drill.

JANET'S nests modified in these ways bring to mind FIELDE'S design that was contemporary to their prototype; that was a formicarium patterned after LUBBOCK'S horizontal nest, but without any soil (STITZ 1914, WHEELER 1926, SKAIFE 1961). The internal structure of such a nest may be made more complex

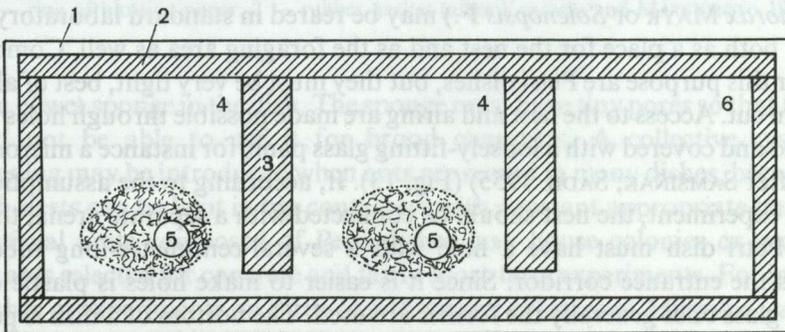


Fig. 12. Classical FIELDE'S a nest (top view); 1 — glass base; 2 — glass side walls; 3 — glass partitions; 4 — nest chambers; 5 — wet pieces of sponge; 6 — chamber (after WHEELER 1926).

by means of partitions made of glass or some other material that can, as in JANET'S model, form some nest chambers and a foraging chamber (Fig. 12). Humidity is maintained in the nest by a piece of wet sponge put in one of the blacked out chambers. The top glass pane (or several of them) is supported on strips of loose material that are glued along the entire length of the side walls and on the partitions — this gives air free access to the nest. But formicaria of this type have a drawback — it is difficult to supply the ants with food and water — but this difficulty may be overcome if an arena, not found in the prototype, is added to the nest and the moistening system is modified.

If ants nesting in wood are to have conditions as close to natural ones as possible and if observations are to be unhindered, appropriate nests should be made in bamboo or in hollowed out sticks. These should have their cutting plane attached to the glass walls or to the underside of the lid of the rearing vessel (KONDOH 1979). In this way it is even possible to make a copy of a natural nest (or rather its cross-section) when pieces of bamboo are connected to form a system of corridors. Humidity in the nest is maintained thanks to a piece of wet sponge put on the bottom of the container (if the rearing vessel is fairly hermetic) or a swab of wet cotton wool pushed into the outlet of one of the lowest lying "corridors". As all the other types, this nest must also be protected from light.

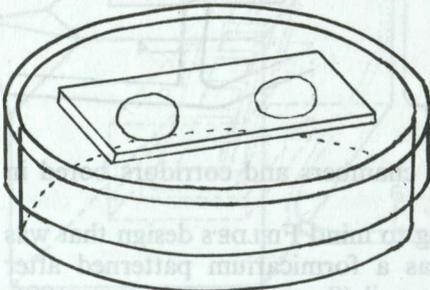


Fig. 13. Petri dish as a formicarium; holes in the lid covered with a slide (after SAMŠIŇAK acc. SADIL 1955).

As it has already been mentioned, very small ants (e.g. of the genera *Leptothorax* MAYR or *Solenopsis* F.) may be reared in standard laboratory ware serving both as a place for the nest and as the foraging area as well. Commonly used for this purpose are Petri dishes, but they must be very tight, best of all with their rim cut. Access to the nest and airing are made possible through holes bored in the lid and covered with a loosely-fitting glass pane, for instance a microscopic slide (after SAMŠIŇAK; SADIL 1955) (Fig. 13). If, according to the assumptions of a given experiment, the nest should be connected with a separate arena, the side of the Petri dish must have a hole with a several-centimetre-long tube that imitates the entrance corridor. Since it is easier to make holes in plastic dishes than in glass ones generally the former are used. The bottom of a dish is padded with filter paper that absorbs ant excrement; this paper must be changed from time to time (the ants must be inactivated prior to that, and this can be achieved by lowering the temperature). The paper must stick very closely to the walls and

bottom of the dish to prevent the ants from going underneath it. The paper is pressed down with a ring of any material (e.g. rubber) but of an appropriate diameter (KONDOH, MATSUMOTO 1977) (Fig. 14). This usually is the whole outfit of the nest though there may be used various elements (wooden ones are best) imitating chambers and corridors (CARNEY 1970, KRETZSCHMAR 1978). Filter paper may be replaced by a gypsum bedding (TRICOT, PASTEELS, TURSCH 1972, SORENSEN, MIRENDA, VINSON 1981). Humidity in the nest is maintained thanks to

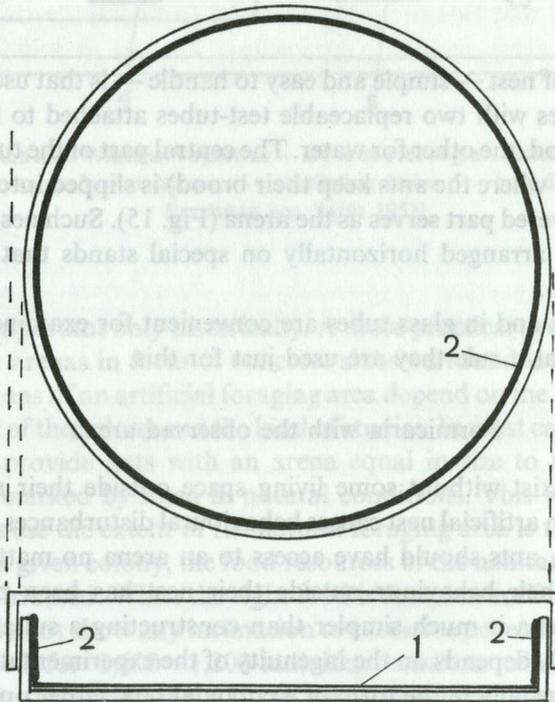


Fig. 14. Petri dish as a formicarium — the way a ring of filtration paper is fixed (top and side view):  
1 — ring of blotting-paper; 2 — rubber gasket (after KONDOH and MATSUMOTO 1977).

a piece of wet sponge in the dish. The sponge must have tiny pores so that the ants should not be able to use it for brood chambers. A collective system of moistening may be introduced when ants are reared in many dishes, because sets of dish-nests can be kept in big containers with constant appropriate humidity.

Artificial nests composed of Petri dishes may house colonies or groups of larger ants selected for concrete and fairly short-term experiments. For instance, a number of experiments on three-five-thousand colonies of *Myrmica laevinodis* NYL. were conducted in 14-centimetre dishes (TRICOT, PASTEELS, TURSCH 1972). Similar dishes ( $\varnothing$  15 cm) were used in tests on groups (25 individuals) of big ants of the genus *Camponotus* MAYR (CARNEY 1970).

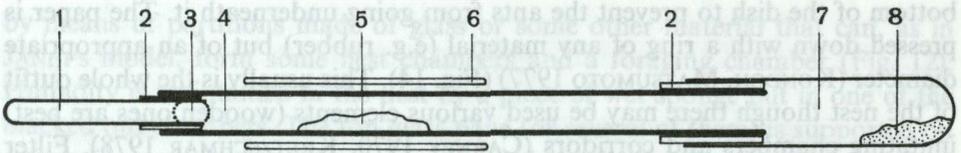


Fig. 15. Formicarium of a glass tube and test-tubes: 1 — test-tube with water; 2 — paper gaskets; 3 — swab of cotton wool; 4 — glass tube (the central part of the formicarium); 5 — place where the ants keep their brood; 6 — non-transparent cover of the nest; 7 — test-tube-bait; 8 — food (after PLATEAUX 1960).

Another type of nest — simple and easy to handle — is that used by PLATEAUX (1960): glass tubes with two replaceable test-tubes attached to both ends. One test-tube is for food, the other for water. The central part of the tube with the nest (or rather a place where the ants keep their brood) is slipped into a case of black paper. The uncovered part serves as the arena (Fig. 15). Such nests, whole sets of them, should be arranged horizontally on special stands that ensure a fixed position for them.

Nests in dishes and in glass tubes are convenient for examination under the microscope and as a rule they are used just for this.

#### Formicaria with the observed arena

Ants cannot exist without some living space outside their nest. Prolonged confinement in an artificial nest causes behavioural disturbances in them (SKAIFE 1961). Therefore, ants should have access to an arena no matter whether any observation of their behaviour outside their nest has been planned or not. Arranging an arena is much simpler than constructing a suitable observation nest, and it mainly depends on the ingenuity of the experimenter. An artificial foraging area is usually in the form of a cuboidal box, either open at the top or covered with a transparent lid, connected with the nest in one way or another. The nest can of course be placed within the arena, but in such a case it limits the field of observation. The inside of the vessel-arena is left open and either it is made more complex in some way or an imitation of the natural area is created — as the particular studies require. The vessel must not be too deep, because it may be necessary to watch the ants at short range; if it is open its rim must have some protection to prevent the ants from leaving, but it is better to have it covered with glass as this allows the observer to behave more freely (his breathing may alarm the ants). In such a case the lid should have a sliding window to make giving food and water or some minor manipulations possible without unnecessary disturbance to the ants.

Another type of arena worth mentioning is that used by WASMANN (STITZ 1914, WHEELER 1926, SKAIFE 1961) — a system of glass vessels that can be connected with an artificial nest (Fig. 16). This structure, however, is inconvenient to use

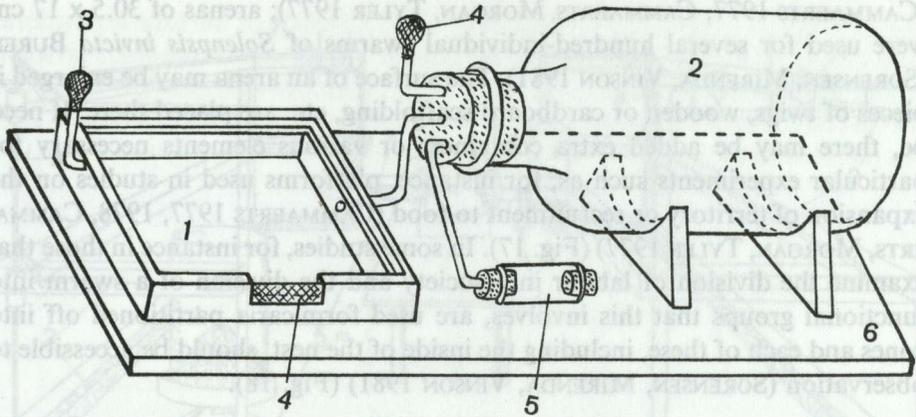


Fig. 16. Formicarium after WASMANN's pattern — arena as a set of glass vessels: 1 — nest (LUBBOCK's model); 2 — bottle-arena; 3 — moistener; 4 — ventilating device; 5 — bait; 6 — wooden base (after SAMŠIŇAK acc. SADIL 1955).

and now it is important only historically. A more practical proposition is that of SKAIFE (1961): arenas in form of concrete slabs surrounded by water.

The dimensions of an artificial foraging area depend on the ant species reared, the abundance of the colony and the kind of studies. In most cases it is technically impossible to provide ants with an arena equal in size to the range of their foraging area marked by them in natural conditions. This would not even be necessary, because the extent of the natural foraging area is the resultant of the abundance of a given colony, the food resources of the habitat and the way food sources are distributed. When the right food is found in sufficient amounts near the nest, ants do not show any inclination to excessive penetration. For instance, the arenas used for 1,000–1,200-individual swarms of *Formica polyctena* FOERST. had the dimensions 65 x 20 cm (MABELIS 1979), those for 3,000–5,000 strong colonies of *Myrmica laevinodis* NYL. — 80 x 60 cm (TRICOT, PASTEELS, TURSCH 1972) and those for smaller colonies of this species — 43 x 28 cm

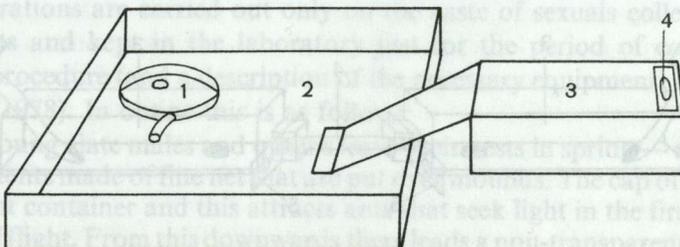


Fig. 17. Arena with a movable platform — a set for studies on the recruitment to food; 1 — nest (Petri dish); 2 — main arena; 3 — platform; 4 — bait (after CAMMAERTS 1978).

(CAMMAERTS 1977, CAMMAERTS, MORGAN, TYLER 1977); arenas of 30.5 x 17 cm were used for several hundred-individual swarms of *Solenopsis invicta* BUREN (SORENSEN, MIRENDA, VINSON 1981). The surface of an arena may be enlarged if pieces of twigs, wooden or cardboard scaffolding, etc. are placed there. If need be, there may be added extra containers or various elements necessary for particular experiments such as, for instance, platforms used in studies on the expansion of territory or recruitment to food (CAMMAERTS 1977, 1978, CAMMAERTS, MORGAN, TYLER 1977) (Fig. 17). In some studies, for instance in those that examine the division of labour in a society and the division of a swarm into functional groups that this involves, are used formicaria partitioned off into zones and each of these, including the inside of the nest, should be accessible to observation (SORENSEN, MIRENDA, VINSON 1981) (Fig. 18).

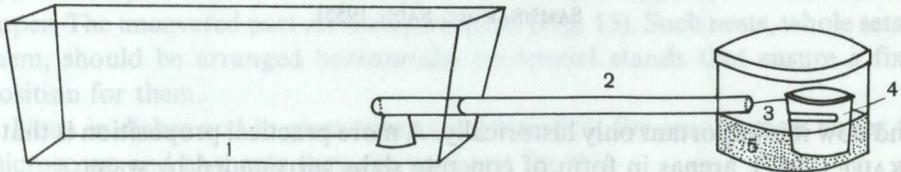


Fig. 18. Zonal space outside the nest — a set for studies on the intracolony division of labour; 1 — main arena (foraging area); 2 — transparent inlet tube; 3 — peripheries of the nest; 4 — container with the nest chamber; 5 — layer of gypsum (after SORENSEN, MIRENDA and VINSON 1981).

Arenas designed for investigations on the inter- and intraspecific competition in ants should have an adaptation for connecting them with two (or more) artificial nests (MABELIS 1979) (Fig. 19). This type of experiment requires fairly big arenas, where ants have a possibility to manifest their full territorial reaction. Even two-level structures filling the whole laboratory are sometimes used for large colonies (e.g. *Formica polyctena* FOERST.) (MABELIS 1979) (Fig. 20).

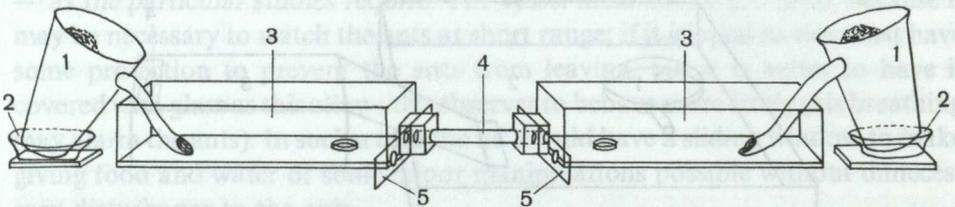


Fig. 19. Set of devices for studying the relations between alien colonies (species): 1 — containers with nests; 2 — vessels with water (preventing the ants from escaping); 3 — arenas; 4 — tube connecting the arenas; 5 — closing (sliding) entrances (after MABELIS 1979).

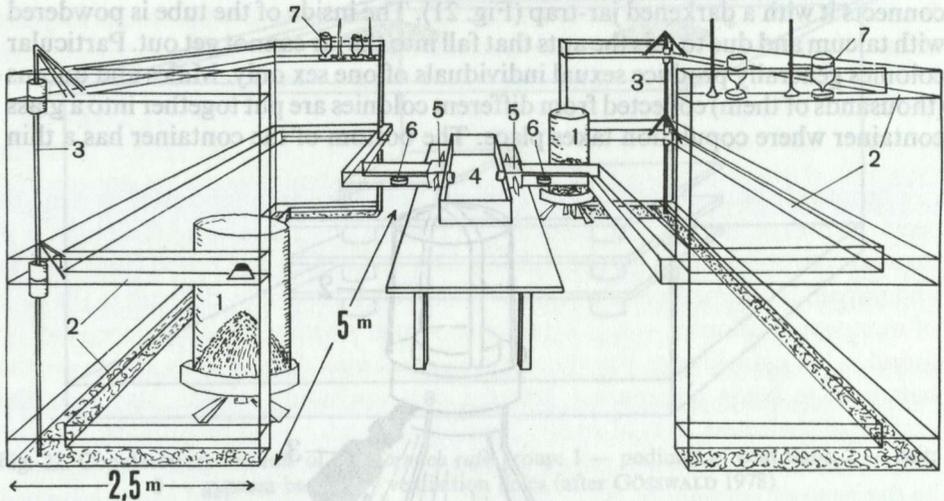


Fig. 20. Laboratory equipment for studying relations between large ant colonies; 1 — containres with nests; 2 — platform-arenas (the lower one with nest material strewn on it); 3 — connecting links between the platforms; 4 — border zone; 5 — sliding entrances; 6 — baits with protein food; 7 — baits with carbohydrate food (syrup) and water (after MABELIS 1979).

The reactions of ants are largely dependent on the weather conditions. All ethological and ecological experiments should therefore be conducted in a room with as stable parameters of temperature, humidity and light as possible. An air conditioned room with established photoperiodism would be ideal (MABELIS 1979).

### Mass rearing of sexual forms

This type of ant culture is mainly connected with the use of ants in the biological method of forest protection. The objective here is to obtain a great number of inseminated queens of polygynic species of red wood ants belonging to the *Formica rufa* group. These queens are later used for reinforcing populations that are dying out in endangered areas or young colonies that have developed through artificial colonization. The procedure is fairly complicated though operations are carried out only on the caste of sexuals collected from natural nests and kept in the laboratory just for the period of copulation<sup>1</sup>. A detailed procedure (and a description of the necessary equipment) is given by GÖSSWALD (1978). In outline this is as follows:

Ants — young alate males and queens leave their nests in spring — are caught into special tents made of fine net that are put over mounds. The cap of the tent is a transparent container and this attracts ants that seek light in the first stage of their nuptial flight. From this downwards there leads a non-transparent tube that

<sup>1</sup> For that reason, this is not ant culture in the full sense of the word.

connects it with a darkened jar-trap (Fig. 21). The inside of the tube is powdered with talcum and due to this the ants that fall into the jar cannot get out. Particular colonies generally produce sexual individuals of one sex only. Males and queens (thousands of them) collected from different colonies are put together into a glass container where copulation takes place. The bottom of the container has a thin

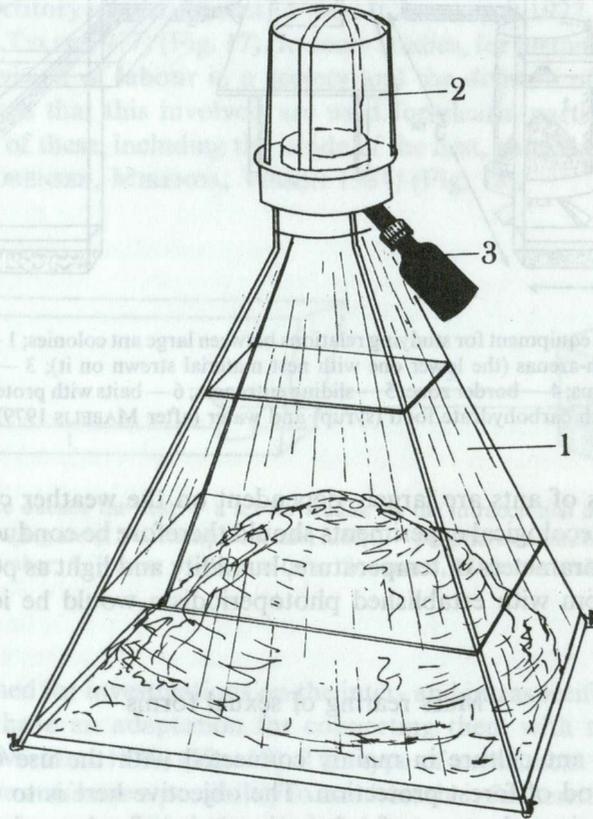


Fig. 21. Device for collecting alate sexuals from nests of ants of the *Formica rufa* group: 1 — tent of thick net; 2 — transparent luring container; 3 — darkened vessel-trap (after PAVAN 1981, modified).

layer of gypsum for absorbing formic acid. The surface of the copulatorium is enlarged thanks to tiers of peat plates. The food — diluted honey and soft larvae of insects (e.g. meal worms) — is served on a platform placed there (Fig. 22). The inside is lit by an infrared radiator to keep the temperature of 22—25°C. The moment the males begin to die and the queens to lose their wings (usually after 3 days) the ants are caught<sup>1</sup> and segregated into inseminated and uninseminated ones. The segregation occurs spontaneously in a glass vessel that is partly blacked out, partly lit with an electric light (inseminated females show negative

<sup>1</sup> All manipulations are made by means of an electric aspirator.

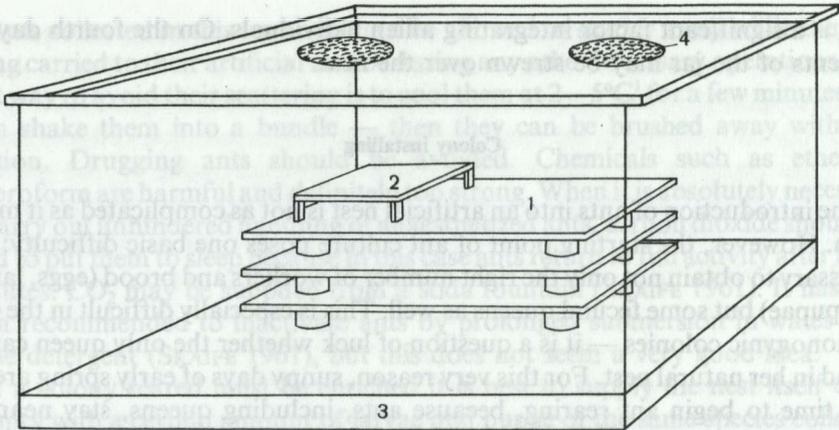


Fig. 22. Copulatorium for ants of the *Formica rufa* group: 1 — podiums of peat slabs; 2 — bait; 3 — gypsum base; 4 — ventilation holes (after GÖSSWALD 1978).

phototaxis — uninseminated ones — positive). At the darker side of the vessel there are blackened glass flask (Fig. 23) and inseminated queens gather there. From these they are carried to open shallow dishes and in groups (100 individuals in each) are placed in glass transport tubes with a layer of gypsum and perforated cork. In order to make it easier for young queens to adopt in a selected colony they should be kept for a few days with a group of workers from there. To do this, 200 queens are put into a glass jar with its bottom poured over with gypsum, and about 1000 workers are added with their natural nest material. The closed jar is then planted into the ground in the nearest vicinity of the nest to be enriched. On the second or third day some more (a few thousand) workers should be added to the jar. Throughout this time the ants are given a solution of honey — trophal-

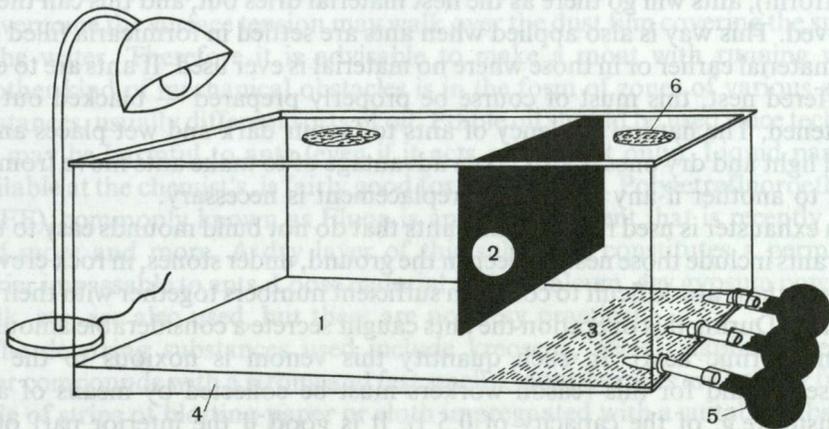


Fig. 23. Device for separating inseminated queens: 1 — lit part; 2 — non-transparent semi-partition; 3 — darkened part; 4 — gypsum base; 5 — darkened flasks where inseminated queens gather; 6 — ventilation holes (after GÖSSWALD 1978).

laxis is a significant factor integrating alien individuals. On the fourth day the contents of the jar may be strewn over the nest.

#### Colony installing

The introduction of ants into an artificial nest is not as complicated as it might seem. However, the starting point of ant culture poses one basic difficulty: it is necessary to obtain not only the right number of workers and brood (eggs, larvae and pupae) but some fecund queens as well. This is especially difficult in the case of monogynic colonies — it is a question of luck whether the only queen can be found in her natural nest. For this very reason, sunny days of early spring are the best time to begin ant rearing, because ants, including queens, stay near the surface of their nest. It is of course possible to catch young inseminated queens that abound on the ground during the period of nuptial flights, and their eggs can be the basis of ant culture. This, however, is a long process, and rarely crowned with success. Moreover, in the case of species that found colonies in the dependent way (by taking advantage of colonies of other species) it is necessary to have an already existing culture of a colony of the host species, and this greatly complicates matters.

Ants nesting in mounds or touchwood are collected with a scoop, spoon or by hand together with their nest material into a properly protected vessel or bag of thick cloth. The more larvae and pupae are collected the better, because their presence has a positive influence on the process of adaptation of ants in their new nest. If ants are to be reared in a formicarium with natural nest material, a whole nest or a part of it is put directly into the formicarium. If any material is left over it should be strewn near the formicarium, for instance in the future arena (but first it must be made sure that the ants will have no possibility to scatter). Given a means of getting to the nest container (through a hole, tube or along a platform), ants will go there as the nest material dries out, and this can then be removed. This way is also applied when ants are settled in formicaria filled with nest material earlier or in those where no material is ever used. If ants are to enter an offered nest, this must of course be properly prepared — blacked out and moistened. The natural tendency of ants to favour dark and wet places and to avoid light and dry ones is also taken advantage of to make ants move from one place to another if any cleaning or replacement is necessary.

An exhauster is used for collecting ants that do not build mounds easy to take. Such ants include those nesting deep in the ground, under stones, in rock crevices, etc., and these are difficult to collect in sufficient numbers together with their nest material. During this operation the ants caught secrete a considerable amount of venom (formic acid). In such quantity this venom is noxious to the ants themselves and for this reason workers must be collected by means of a big exhauster (e.g. of the capacity of 0.5 l). It is good if the interior part of the exhauster is covered with a thin layer of lime which neutralizes the acid and the suction tube has a filter (of charcoal or at least cotton wool). Queen, larvae and pupae should be collected into a separate, smaller exhauster (SKAIFE 1961).

Ants collected in this way must not be allowed to scatter away while they are being carried to their artificial nest or during any other necessary operations. The best way to avoid their scattering is to cool them at 2—5°C<sup>1</sup> for a few minutes and then shake them into a bundle — then they can be brushed away with one motion. Drugging ants should be avoided. Chemicals such as ether or chloroform are harmful and definitely too strong. When it is absolutely necessary to carry out unhindered handling of anaesthetized ants, carbon dioxide should be used to put them to sleep because in this case ants return to full activity after a few minutes. CO<sub>2</sub> may be supplied from a soda fountain (SKAIFE 1961). It has also been recommended to inactivate ants by prolonged submersion in wates with some detergent (SKAIFE 1961), but this does not seem a very good idea.

If a colony reared must be enriched it is best to supply the nest itself or its vicinity with a certain amount of larvae and pupae of the same species collected from any natural nest — but it is better if they are form their maternal colony or related to it. Even though some part of the foreign brood may be utilized as food, most of it is generally adopted (and much more easily than alien adult workers). Introducing new queens is far more difficult because a lot depends on the social structure of he colony reared and on the origin of queens introduced into it (SKIBIŃSKA 1982).

#### Protection of a culture

Every culture, even if it is in an apparently tightly-closed vessel, must have some protection against the scattering of ants. Otherwise, the whole swarm may move out unnoticed. In the case of synanthropic species (e.g. Pharaoh ants) this would mean not only a loss of the culture but also a sanitary-epidemiological threat.

Mechanical obstacles or substances with a deterring smell are used as barriers for ants. The former are usually moats with water small ants, however, too light to overcome the surface tension may walk over the dust film covering the surface of the water. Therefore it is advisable to make a moat with running water. Another kind of mechanical obstacles is in the form of zones of various greasy substances, usually different sorts of oil. Edible oil should be used since technical one may be harmful to ants (even if it acts on contact only). Liquid paraffin, available at the chemist's, is fairly good for this purpose. Polytetrafluoroethylene (PTFE), commonly known as Fluon is an effective agent that is recently being used more and more. A dry layer of this substance constitutes a permanent barrier impassable to ants. Loose material such as talcum, dry gypsum powdered chalk, etc. are also used, but these are not very practical.

The deterring substances used include kreosene, petrol, naphthalene, and other compounds with a strong and fast smell. Odour barriers are usually frames made of strips of blotting-paper or cloth impregnated with a suitable substance

<sup>1</sup> Forbidden in the case of tropical species.

and dried. The intensity of the odour must be renewed from time to time. Insecticides, for instance DDT, are also used (SKAIFE 1961), but it seems a misconception if strong insecticides are consciously applied in ant rearing.

No way, either used in the past or at present, of protecting a culture (SKAIFE 1961) has been entirely successful. It is therefore advisable to use multiple protective belts. They should surround not only the nest itself, or this and the foraging area, but also the whole area of any manipulations, where ants may be carried unconsciously.

### Food

Food should be accessible to ants all the time and — if it is not given as a blend prepared after a special formula — it must be as varied as possible so that the ants are provided with all the indispensable components, they normally find in their natural environment.

Energetic carbohydrate food is generally supplied as water solutions of sugar (sucrose) or honey. Opinions differ as to what is the optimum concentration of these solutions. In any case, sugar syrups with concentration exceeding 50% are harmful to ants. It seems that the most appropriate should be from 10 to 15%<sup>1</sup>. While diluting boiling water should be added and syrup should be prepared only for current needs because it ferments very quickly. Ants can even be supplied with honeydew, their natural carbohydrate food, if aphids are reared at the same time. The common pea aphid (*Acyrtosiphon pisum* HARRIS) is excellent for this purpose and it can easily be reared on shoots of plants of the family *Papilionaceae* in pots arranged within the foraging area of the ants. Other aphids can also be reared, for instance those living on underground rhizomes of grasses. The grass is planted in pots, and parts of its rhizomes with aphids grown along glass tubes fixed in the foraging area (BRIAN 1977).

Protein food is indispensable to ants, especially at the time when queens reproduce and larvae develop. Various small arthropods, mainly insects, usually constitute the source of this food. These should be served freshly killed or alive but slightly crushed. It is advisable to have permanent cultures of insects that reproduce easily and quickly, for instance fruit-flies, house-flies or meal worms. Larvae and pupae of other ant species are a valuable food which is easy to handle. Ants can also be fed on tiny pieces of raw or boiled meat (especially liver), the yolk of boiled eggs, cottage cheese, milk.

Certain species, due to their biology, require specific food. For instance, harvester ants (e.g. the genera *Pogonomyrmex* MAYR and *Veromessor* FOR.) must feed on plant protein contained in seeds of certain plants. Ants feeding on fungi (the genus *Atta* FOR.) from their own cultures must be provided with the

<sup>1</sup> The concentration of sugars in honeydew — the natural carbohydrate food of ants — is 1–2% (ETTERSHANK 1967).

appropriate parts of plants which can be used to make compost for the cultures of these fungi.

The food supplied should be varied thus providing all the nutrients indispensable to the proper functioning of an organism. The diet must be supplemented by fruits, preferably ripe and sweet, as a source of vitamins and other microelements. Under our conditions, apples, pears, plums, cherries and tomatoes can be given (SKIBIŃSKA 1982).

Looking after ant cultures is less troublesome if the basic food is in the form of multicomponent mixtures — from the simplest such as solutions of sugar or honey in milk (KONDOH, MATSUMOTO 1977) to very complex ones. For instance, what is recommended by BHATKAR and WHITCOMB (1970) as very useful for many ant species is a protein-carbohydrate-vitamin-mineral mixture solidified with agar, and its composition is as follows: one raw hen's egg, 62 ml of honey, one vitamin-mineral capsule (made by Mc Kesson Bexel)<sup>1</sup>, 5 g of agar, 500 ml of water. All the components are mixed in a centrifuge with half the amount of water and added to a solution of agar previously prepared hot and cooled in the rest of the water. This nourishment is cut into the right helpings and stored in a refrigerator. Protein baby food is another ready-made product used (SORENSEN, MIRENDA, VINSON 1981).

Mixtures of standard value and with a precisely measured composition are used in some physiological studies when it is necessary to determine precisely the quantity and quality of the food taken by the ants. These may be exemplified by a blend recommended by ETTERSHANK (1967) and containing amino acids, vitamins, sugars and salts — this perfectly meets the food requirements of ants. The composition is as follows:

Alfa amino acids and amides (in  $\mu\text{g}$ ):

Alanine	— 100	Leucine	— 40
Arginine	— 270	Lysine	— 120
Asparagine	— 550	Methionine	— 10
Aspartic acid	— 140	Phenoloalanine	— 10
Cysteine	— 10	Proline	— 40
Glutamine	— 150	Serine	— 80
Glutamic acid	— 140	Threonine	— 140
Glycine	— 40	Tryptophan	— 40
Histidine	— 40	Tyrosine	— 40
Homoserine	— 70	Valine	— 40
Isoleucine	— 30		

<sup>1</sup> This may be substituted with a Vitalar tablet (SKIBIŃSKA 1982).

Vitamins (in  $\mu\text{g}$ ):

One capsule of Vitamin B Complex<sup>1</sup> of the following composition:

Thiamine	—	2	Choline	—	20
Riboflavin	—	2	Inositol	—	10
Niacinamide	—	10	Vitamin B <sub>12</sub>	—	0.001
Calcium Pantothenate	—	3	Insoluble Liver Fraction	—	414
Pyridoxine	—	0.2			
and:					
Folic acid	—	0.5	Ascorbic acid	—	100
Biotin	—	0.1			

## Sugars (in g):

Sucrose	—	15	Fructose	—	2	Glucose	—	2
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Salts (in  $\mu\text{g}$ ):

K <sub>2</sub> HPO <sub>4</sub>	—	500	MgCl <sub>2</sub> · 6H <sub>2</sub> O	—	200
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Cholesterol — 15  $\mu\text{g}$

Water — to make 100 ml

The general principle in feeding ants is that they must be given only fresh food. The portions of food should be in doses that can be assimilated within one day. All left-overs must be removed to prevent fermentation and moulding. That is why food should be served on glass or ceramic tiles, pieces of parchment, trays made of aluminium foil etc. which simplify the removal of the left-overs. Small test-tubes placed on a special stand may also be used (Fig. 24). Since ants frequently drown in open containers, liquid food must be served in such a way that will enable them to lick it. This is made possible thanks to glass tubes with capillary endings within the ants' reach. Every day, these tubes must be replaced by clean ones.

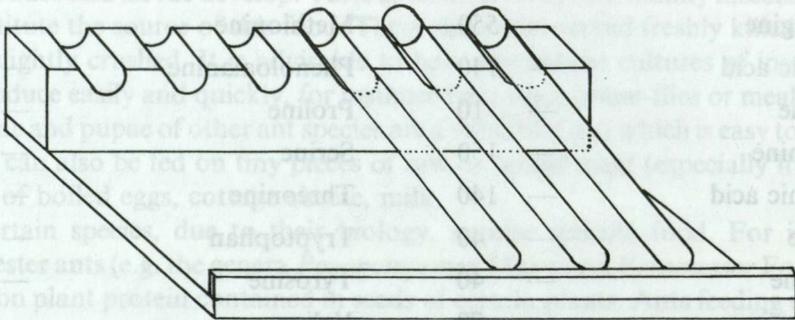


Fig. 24. Bait-stand for test-tubes with food (after SKAIFE 1961, modified).

<sup>1</sup> Producer: Lederle Laboratories Division, Cyonamid of Great Britain Ltd.

Drinking water (tap water must be boiled first) can be supplied in the same way. But it is simpler to put, on the bottom of the rearing vessel or in the arena, a test-tube stopped with a loose swab of cotton wool (nothing synthetic should be used) in such a way that the cotton wool is always wet, but the water never pours out. The water must be fresh every day.

#### Wintering

Ants which hibernate (probably all European species, among others; BRIAN 1977) spend winter in their formicarium removed to a dry room, where the temperature is a few degrees above 0°C. For the winter, ants are generally kept in the same nests in which they have spent their active period, but it is better to prepare special places for ant species that, in nature, have separate winter nests. A description of a winter formicarium for *Formica polyctena* FOERST. ants (this may also pertain to other ants of the *Formica rufa* group) is given by CHAUVIN (1972). The nest he used was in the form of a shallow wooden box of 54 x 33 x 1 cm with an opaque bottom and a transparent top covered with a non-transparent lid. The inside was sparsely strewn over with a lot of wooden chips — the free spaces between them imitated nest chambers. The winter and summer nests were connected by means of a rubber tube (Fig. 1). When autumn was approaching the ants changed nests spontaneously (usually in October). That was the time to disconnect the winter nest, close it and take to a cool room.

In the fairly rich myrmecological literature (even in European publications) on laboratory cultures there is very little about the need of hibernation in ants (FABER 1966, CHAUVIN 1972). It may therefore be assumed that most ant-keepers prolong the ants' state of activity for the whole year. This is clearly indicated by recommendations about winter rearing of different insects as food for ants at that time (SZCZERBAKOW 1957, BRIAN 1977), and by description of devices which heat nests in winter (SKAIFE 1961). No doubt, this is possible but if the colonies reared are to develop normally they should be given conditions as close to the natural ones as possible. In cultures meant for many years this is simply indispensable.

#### CONCLUSION

The methods of ant culture or, to be more precise, the models of devices used in ant rearing presented here do not exhaust all the possibilities. Scattered all over the rich myrmecological literature are a lot of descriptions of formicaria used by particular researchers for various purposes. However, with the general principles observed it is possible (and necessary) to introduce and try out newer and better solutions. A lot depends on the ingenuity of the experimenter. Nevertheless, it must be stressed once again that ants are difficult to rear and — unlike many other animals, including some insects — they are unsuitable for so called "keeping for pleasure".

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# Laboratory methods for rearing ants (*Hymenoptera, Formicoidea*)

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