

## Rearing Blister Beetles (Coleoptera, Meloidae)

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### Introduction

The receipt, recently, of several requests for information and assistance in rearing blister beetles (Meloidae) has prompted me to prepare the following account of the rearing method used in my laboratory. In order to make the account as useful as possible to new students, I have included a considerable amount of information on meloid bionomics. Larval phases are designated as triungulin (T), first grub (FG), coarctate (C), and second grub (SG). Where necessary, instar is indicated by a numerical subscript. The pupa and adult are symbolized by P and A, respectively. I assume that the reader has some knowledge of the taxonomy of the Meloidae.

The rearing method described was developed over a period of many years as an extension and refinement of procedures used initially by W. R. Horsfall in rearings of species of *Epicauta* in Arkansas, U.S.A. It has been used in my laboratory with great success in complete and, often, extensive rearings of nearly 50 species of *Epicauta* (including species of the nominate subgenus and *Macrobasis* from a great variety of habitats in North and South America) as well as one or more species of the genera *Linsleya*, *Lytta*, *Meloe*, *Pyrota*, and *Pseudomeloe*. In addition, it has been used, with modification, by Erickson and Werner in partial or complete rearings of one or more species of *Lytta*, *Nemognatha*, *Pseudozonitis*, *Tegrodera*, and *Zonitis*.

The method is appropriate for the researcher interested in larval behavior, ontogenetic patterns (including number and duration of instars), and specimens of immature stages. It is equally suitable for initial, exploratory rearings, where day-to-day observation of progress and possible adjustment of conditions are required, and for experimental, comparative studies involving modification of temperature, humidity, and food material. While designed for a complete rearing, from adult to adult, it may be adopted in part for obtaining T larvae, the anatomy of which is of primary importance in the taxonomy of the Meloidae. It may also be adopted in part for rearing to the adult stage partially developed larvae encountered in the field. If a rearing is planned

solely to obtain adults or to investigate the seasonal distribution of life stages, various modifications of the method may be made, as discussed in the last section of this paper.

### Maintaining Adults

If one has no laboratory colony of meloids, it is necessary to obtain eggs either in the field or from captured adults. Except in a few species that do not feed in the adult stage, female Nemognathinae oviposit on their food plants. Egg masses are commonly placed on the underside of flower buds or flowerheads but sometimes on leaf surfaces or stems. Frequently it is possible to find egg masses or triungulin larvae on food plants in nature, and where adults of one species occur by themselves it is reasonable to assume (although not guaranteed) that the eggs or larvae belong to that species. Finding eggs masses in the field and associating them with adults is much more difficult in the case of the Meloinae, since in this subfamily the female may oviposit in a cavity excavated in the soil, beneath a stone, or at the base of a plant. One can sometimes obtain egg masses by sifting soil or, with luck, by finding females in the act of oviposition. But if the adult population is so large at a locale that there is a reasonable chance of doing either, there should be no difficulty at all in collecting a sample of adults large enough to virtually guarantee oviposition in captivity. Fortunately, captive adults of both Meloinae and Nemognathinae will oviposit readily if maintained under proper conditions.

**Cages.** Adults may be transported from the field and kept in a screened cage or in a plastic box provided with a screened port. I regularly use a transparent plastic box about the size of a shoe box; its port, about 7 cm in diameter, is cut in the lid. As flooring I use blotting paper, sheets of which are cut to the proper size ahead of time. Because females may oviposit on the floor or egg masses laid in plant material may fall to the floor, it is advantageous to change the flooring material daily, in order to lessen the probability that eggs will be contaminated with feces on the floor.

**Number of Adults per Cage.** No more than 20 adults of a small species (length 1 cm or less) and no more than 4-6 adults of a large species (length 2.5 cm or more) should be kept in a cage of the type described above. Excessive crowding may decrease longevity, due to hyperactivity and

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to injuries resulting from fights. More important, it will almost certainly reduce the frequency of oviposition; in fact, in the worst case it will inhibit it completely.

It is neither necessary nor particularly desirable to maintain a balanced sex ratio in a caged population. Indeed, if a population is to be kept for only a few days, males need not be included, since females collected in the field will almost certainly have mated before capture. In any event, because males are capable of mating repeatedly during adult life, an excess of females in a captive population is not likely to reduce individual fecundity.

For studies of behavior and other aspects of biology it is sometimes useful to cage adults individually or in pairs. For this purpose a plastic cage about half the size of the one described above is suitable.

**Physical Conditions.** If environmental control is available, use a constant temperature between 25 and 30°C, a relative humidity of 40-60%, and a daily photocycle similar to the natural one. At the very least, protect cages from direct sunlight and rainfall.

Meloid adults, even those inhabiting deserts, are capable of obtaining all the water they need from their food. Therefore, if fresh food is given in abundance, a separate source of water is not necessary. If food material tends to dry out rapidly and cannot be replaced more than once a day, the screened port of the cage may be covered with paper, to reduce evaporation. Do not close the port with plastic or other material that is impermeable to water, since the cage is then likely to become excessively humid and the food will mold.

**Adult Food.** Some meloid adults eat only floral parts (mainly flowers but sometimes fruits as well); others eat only leaves; still others eat both floral parts and leaves. If the natural food plants are known and obtainable, cuttings of these should be offered to captive beetles. Otherwise, experiment with a variety of potential food plants. Solanaceae, Amaranthaceae, and Leguminosae, particularly soft-leaved species, are good bets for leaf and flower/leaf feeders. Flower feeders tend to be more host specific than leaf feeders, but by preparing test bouquets containing flowers of a wide variety of plants, with emphasis on Compositae and Leguminosae, there is a good chance of discovering an acceptable food item. For emergency maintenance of leaf and petal feeders over a period of 1-3 days, commercial lettuce leaves may be used. According to Leppla,

Standifer, and Erickson (1974), a 5-10% solution of sucrose in water to which 1 g of honey per 100 ml has been added is an adequate diet for *Nemognatha* adults, but I believe that it would be much improved by a source of protein, such as pollen. For any meloid, if no food is available, at least provide a dish of water containing a cotton or paper wick.

Gather cuttings of plant material in large plastic bags and hold them in a refrigerator at about 10°C. Many flowers can be kept fresh over a period of several days by arranging them as bouquets in jars of water (containing sugar) and refrigerating them. In most cases cuttings may be given as food simply by arranging them loosely on the floor of the cage, without a container. Experience will dictate how much material is to be used. An excessive amount will only make examination for egg masses more difficult.

In order to keep cuttings of delicate flowers fresh enough for sustained use by the adult beetles during the day, it may be necessary to offer them in a bouquet, with the stems in water. This will, of course, require the use of a relatively large cage. In preparing a bouquet, pack cotton around the stems at the rim of the container. Females are likely to oviposit between stems of bouquets, and there is a good chance of egg masses falling in the water if the packing material is omitted.

If possible, remove all old food from cages daily and replace it with fresh material. Daily replenishment of food is especially important for meloids feeding on flowers or thin leaves and critically so for those feeding on nectar.

It is efficient first to prepare the required number of new cages daily, complete with appropriate food material, and then to transfer adults from the old cages to the new ones. After the transfers have been made, each of the old cages can be examined at leisure for egg masses. Adults should be handled with the fingers or, carefully, with coarse forceps. Keeping a count of the number of adults in a cage will save time and prevent loss of adults by oversight.

#### Oviposition

Females in good health will live several weeks to several months and will produce egg masses periodically (at intervals of 1-2 weeks for most Meloinae). The number of eggs per mass is quite constant within females but varies with body size among females. Further, it varies inversely with egg size among species. In *Epicautina*, *Mylabrina*, and many *Nemognathinae* the number is on the order of 50 to a few hundred; in some *Meloini* and

Lyttini it may be as high as three or four thousand.

**Meloidae.** To simulate, roughly, the natural conditions of oviposition for females of this subfamily, I commonly place in each cage a plastic dish measuring 20 mm in diameter and 15 mm in height and filled with lightly packed, moist silica sand. (The sand is prepared by adding water to dry sand in the amount of 10% of its volume. It may be prepared in bulk and stored in a closed container.) After a dish has been filled I punch 2-3 holes in the sand with a sharpened pencil, on the assumption that females in nature are stimulated to dig by finding an opening in the soil. I have no real evidence, however, that this is so. It is often possible to tell from the surface of the sand whether a female has dug and filled an oviposition cavity. But this is not always true, and it is therefore necessary to examine each dish carefully. This is done by dumping the sand as a loaf onto a piece of paper and carefully breaking the loaf apart. Egg masses, yellow, cream, or white in color, are relatively large and are easily recognized without magnification.

The attractiveness of sand-filled dishes to ovipositing females varies greatly with species of Meloidae. Some seldom oviposit outside the dishes when they are available; others almost never use them. In any event, there is no evidence that lack of sand or soil inhibits oviposition. As a matter of fact, when working in the field I do not provide adults with anything more than food. Yet, even if I collect as few as two or three females, I usually obtain at least one oviposition. The main value of the dishes is that egg masses laid in them are easily recovered intact and without contamination by feces and plant material.

Females that do not oviposit in sand dishes commonly do so on the food material or on or beneath the paper floor covering. In many cases it appears that females select tight places, as between leaves or stems or a leaf and the floor. Shaking the food material as it is removed and a cursory examination of the cage floor are generally sufficient to disclose egg masses laid in the cage.

**Nemognathinae.** For adults of this subfamily it is doubly important to keep the food fresh, since there is little question but that withered plants are unattractive to ovipositing females. Old food, particularly the undersides of buds, flowers, and flowerheads, should be examined carefully for egg masses before being discarded. It is also a good idea to examine the sides of the cage and both sides of the flooring material, since some

Nemognathinae, especially *Tetraonyx*, will oviposit on these surfaces.

**Recovering Egg Masses.** An intact egg mass may be lifted carefully with forceps and dropped into a clean glass (not plastic) vial (I use a 3-dram lip vial) which is then stoppered with a cotton ball. Feces and other extraneous material should be removed with fine forceps before the mass is placed in its vial. It is not necessary to remove sand grains. Egg masses laid on the floor and heavily trampled are seldom worth saving. Similarly, unless one is interested in counting the number of eggs in a mass, it is not worthwhile gathering up single eggs or small clumps that have become detached from the mass. If single eggs or small clumps must be handled, use a small brush rather than forceps.

In some Nemognathinae the eggs are so sticky that they are difficult to remove from the substrate as an intact mass. In this case, if the substrate is plant tissue or the paper floor covering, simply cut out the section holding the mass and transfer it with the mass to a vial.

**Labeling Egg Masses.** Attach to the outside of each egg vial a label including data such as: name of species, note number of adult population, date of oviposition, number of eggs discarded or not recovered, site of oviposition, and color of eggs (if of interest). Space should be left for later recording the hatching date and number of larvae removed for rearing or other purposes. It is useful also to assign a serial number to each egg mass as it is recovered.

#### Incubation of Eggs

Incubate egg masses at 25-30°C, 100% relative humidity, in darkness. In this temperature range eggs that are not in diapause will hatch within 10-14 days. In some species hatching is nearly simultaneous in a mass; in others it occurs over a period of several days.

Exact temperature level is not important. I usually use 27°C, but I have had success with temperatures as high as 35°C. A nearly constant temperature is not only adequate for incubation but is desirable because it minimizes condensation of moisture within the egg vial. In any event, avoid wide swings in temperature.

Humidity level, on the other hand, is critical. In particular, 100% R.H. is mandatory. In order to maintain the proper humidity level, place vials in a covered glass container provided with a porous floor over standing water. For incubating eggs as well as for rearing larvae, I use a large glass vacuum desiccator jar (21 cm ID) that has an internal platform consisting of a perforated por-

celain plate. The insect materials are set on the plate over 500 ml of water in the bottom of the jar. The heavy glass lid of the jar fits tightly by means of contact of flat, ground surfaces. The only opening is a hole about 7 mm in diameter in the turret at the top of the lid. This provides ample circulation of air, even when the jar is virtually packed with egg vials.

Alternatively, on extended field trips I have had excellent results simply by keeping egg vials in a plastic box lined with several layers of moistened blotter paper. If condensation becomes a problem, as it is apt to periodically, I leave the lid off the box for a day.

Unfertilized eggs soon become clouded. Damaged eggs will likely develop fungal growth. Intact eggs will not be injured by fungus, but triungulins eclosing from a heavily molded egg mass will become covered with spores and die rapidly. Therefore, if a particularly valuable egg mass molds heavily, separate the healthy eggs from the rest and place them in a fresh vial.

Dark eye spots become visible relatively early in development. Mandibles (darkened), legs, and body segments are clearly visible a day or two before hatching occurs. Triungulins are largely colorless when newly emerged but darken within a day or two.

#### Maintaining Unfed Triungulin Larvae

Having tried several ways of maintaining T larvae before use in a rearing, I have come to the conclusion that they will live longest without food if they are simply allowed to remain in their original vials under the conditions of incubation.

Newly emerged T larvae are strongly gregarious and tend to be quiescent. If placed in a Petri dish, for example, they will soon gather in clusters. After a few days, however, they become quite active and, given the opportunity, will disperse rapidly. Most epicautine and, presumably, mylabrine larvae and some of the larger lyttine larvae cannot climb on a vertical glass surface, and if these are kept in their incubation vial they are forced to remain in a dense group. Apparently because close contact with other larvae inhibits movement, triungulins in this situation commonly survive for one to several weeks without food or water. Larvae of Nemognathinae, Meloini, and many Lyttini, which are capable of climbing on a vertical glass surface, tend to exhaust themselves by continual wandering and usually live only a few days if not given food. (I have not tried confining larvae of this type to containers smaller than incubation vials.)

For rearing, select triungulins 2-3 days old, if

possible. But if they are still active when placed in a Petri dish and have suffered little mortality, older triungulins are satisfactory. For experimental, comparative purposes, of course, it is important that there be little or no mortality among larvae in a vial before selection of individuals for rearing. There is no evidence that initial age of larvae affects later development.

#### Postembryonic Development

The procedure described here is one that I use when no special experimental conditions are imposed. Numerous variations have been tried, with greater or lesser success. Although many elements of the procedure have no hard experimental evidence to justify their inclusion, most have been incorporated on the basis of extensive experience. I have tried to call attention to those elements that seem especially important.

The procedure is designed for rearing larvae individually and is therefore work-intensive. As far as I know, there has been no successful attempt at mass rearing. The principal stumbling block here is the fact that the larvae, once they have begun to feed, are cannibalistic. At the same time, feeding larvae are quite sedentary. Horsfall reported sometimes finding two *Epicauta* larvae feeding at different ends of a single *Melanoplus* egg pod. I have reared pairs of larvae of *Pseudomeloe* (a South American genus preying on bee cells) together in small dishes. Thus it might be possible, by providing ample food and space or perhaps by partially compartmentalizing the environment, to develop some sort of mass rearing technique.

**Physical Conditions.** Except when, with temperate-zone species, attempting to break larval diapause, rear at a moderate temperature. I have commonly used 27°C constant. Development is possible at a temperature as low as 20°C but is extremely slow. Heavy mortality is the rule at temperatures above 35°C. Experimental work in my laboratory with the North American *Epicauta segmenta* (Say) indicates that mortality may be minimized by using a daily temperature cycle. In extensive rearings of species of the Vittata Group of *Epicauta* (Selander and Adams, unpublished), daily temperature cycles with an amplitude of 7°C were used. Although the incubators had only two thermostats, the effect of switching thermostats was buffered within the desiccator jars containing larvae. As a result, the larvae were exposed daily to a very gradual cycle of temperature change closely approaching the temperature regime they would encounter under natural conditions in the soil.

I have almost invariably kept larvae in the

dark, if for no reason other than the fact that this is the normal condition in nature. However, I have occasionally reared them simply by keeping a rearing jar on a laboratory table, with exposure to artificial light for much of the day.

As in the case of incubating eggs, it is vital to maintain relative humidity at 100%, which is best done by placing tubes and vials containing larvae in a jar of the type described earlier (desiccator with water in the bottom). In experiments with *Epicauta segmenta*, Selander and Weddle (unpublished) found that larvae exposed to a relative humidity of 75% or less invariably stopped feeding and failed to develop further.

In early rearings of meloids I routinely autoclaved all glassware, including vials and tubes, storage dishes for grasshopper eggs, etc., as well as all sand and soil. Extensive experience over the past few years indicates, however, that thorough washing (in detergent) and rinsing is sufficient for glassware. Still, to avoid the possibility of introducing predators and disease organisms, I recommend autoclaving or baking sand and soil before use.

#### Larval Food of Meloidae

Certain groups or species of Meloidae differ markedly in the nature of their larval food. Except for members of the lyttine subtribes Epicautina and Mylabrina, most Meloidae attack, as larvae, the provisioned cells of wild bees, where they feed on pollen and honey as well as, in many cases, the immatures of the bees. A notable exception is the African *Lytta enona* (Péringuey), nominally a member of the Lyttina, the larva of which feeds on larval Psychidae (Lepidoptera) provisioned by *Parachilus* wasps (Eumenidae) (Gess and Gess 1976). Triungulin larvae of all Meloini (*Meloe*, *Spastonyx*, and relatives) and Nemognathinae (*Apalus*, *Nemognatha*, *Zonitis*, *Zonitoscema*, and others) are phoretic, attaching to adult bees from flowers or, in some degenerate forms (*Hornia*, *Tricrania*, and others), from the ground at or near a bee nesting site. All other triungulins, so far as known, reach their feeding sites by active searching.

As a rule, larvae of Epicautina (*Epicauta* and relatives) and Mylabrina (*Mylabris* and relatives) feed on the eggs of grasshoppers. But the larva of the African mylabrine *Ceroctis groendali* (Billberg) attacks cells of masarid wasps of the genus *Ceramius*, which, like bees, provision pollen (Gess and Gess 1980), and there is evidence that the North American *Epicauta atrata* (Fabricius) feeds on the egg masses of Meloidae, specifically those of *Epicauta* (Selander 1981, 1982).

While there has been sufficient study to indicate the general features of adult feeding and reproductive behavior and of larval food types and patterns of development in the subfamilies Meloinae and Nemognathinae, there is little information, aside from scattered food plant records of adults, on the bionomics of the primitive subfamily Eleticinae (including *Ceriselma*, *Eletica*, and *Iselma* in Africa, and several genera, principally, *Spastica*, in South America).

The great range of intraspecific variation in adult body size commonly seen in natural populations of Meloidae is indicative of the marked capacity of developing larvae to adapt their ultimate nutritional requirements to the amount of available food. On the other hand, variation in adult body size within species seems to have little if any genetic component. Thus when individuals of a species are given equal amounts of food, final body size is remarkably uniform.

#### Obtaining and Preparing Larval Food

I consider here grasshopper eggs, provisions of bee cells, and the egg masses of meloids themselves as larval food.

**Grasshopper Eggs as Larval Food.** Grasshoppers whose eggs are recorded as prey of meloid larvae belong to the families Acrididae or (one case) Pyrgomorphidae.

When grasshoppers are abundant, sifting of soil in areas known to be favored by ovipositing females will provide a supply of egg pods. This was the procedure used by Horsfall in his studies of *Epicauta*.

Although grasshoppers may be reared and maintained as a colony, doing so requires a great deal of work. I have found it much more efficient to collect adult grasshoppers live (mainly of *Melanoplus* species) and hold them for several months in captivity. Whether rearing grasshoppers or merely maintaining an adult population, one should consult the work of Hunter-Jones (1961) for details of housing and feeding.

I keep adult populations in large screened cages with coarsely screened floors (to facilitate cleaning) in a dry environment. Naked incandescent light bulbs are placed next to the cages (and are much used by the grasshoppers for "sunning"). A 18L:6D or 12L:12D photocycle or one approximating the natural one may be used. Temperature should be fairly high (30-35°C during the light phase). As fresh food I provide cuttings of plants such as corn, Sudan grass, ragweed, and soybean. In addition, a dry mixture of alfalfa meal, powdered milk, and yeast is always available, as is a supply of water. I have obtained good yields

of egg pods (a mean of four pods per female) by providing adults with bread pans containing moistened silica sand (10% water by volume). Egg pods are easily recovered by straining the sand.

In my rearings I have generally used eggs of *Melanoplus differentialis* (Thomas). This is a good-sized species producing pods 2.5-5 cm long containing 100-150 eggs. Occasionally I have used eggs of a smaller species, *M. femurrubrum* (DeGeer), with shorter, more slender pods containing 25-50 eggs. The species of grasshopper used as a source of eggs is, in all probability, not important. My work and a review of the literature fails to reveal any evidence of food specificity or preference among larvae of *Epicauta* or *Mylabris*. Undoubtedly, various degrees are expressed in nature, but these would not seem to depend on chemical or other feeding cues once larvae reach the eggs or, for that matter, on differences in nutritional adequacy of eggs of different grasshoppers.

The exact procedure for maintaining a supply of grasshopper eggs will depend on the life history of the species involved. In both species of *Melanoplus* that I have used there is an embryonic diapause, and eggs in this state may be kept for 1-2 years under refrigeration (5-10°C, 100% R.H.) with relatively little mortality. Egg pods are left intact until shortly before eggs are needed.

It is feasible to rear larvae by simply confining them individually with an egg pod. However, to make the most economical use of a supply of grasshopper eggs and, in any event, to facilitate observation of developing larvae, it is preferable to remove the eggs from the pods and feed them to the meloid larvae in loose form. To separate the eggs, break the pods apart in a coarse sieve (opening about 2.4 mm) that allows the eggs to pass through but retains coarser pieces of egg pod material. Then shake the eggs in a finer sieve (opening about 0.8 mm) that retains the eggs but permits sand and other small particles to pass through. Once this has been done, quite a bit of froth material will remain with the eggs. This can be largely removed by winnowing in a light stream of air (most simply by blowing on a dish of eggs while shaking them about). Store eggs in a dish at 5-10°C and 100% R.H. before use. If eggs are in short supply, give larvae only a few to begin with or, if a full supply is given initially, recover uneaten eggs from containers in which larvae fail to feed or are killed for study.

The number of eggs required by a larva depends, of course, on the relative sizes of the eggs

and the larva. The larva of *Epicauta segmenta*, a fairly large species (2 cm or more in length and heavy-bodied as an adult), will eat about 60 eggs of *Melanoplus differentialis*. In any case, most of the eggs are consumed in the last FG instar (generally FG<sub>5</sub>). When eggs are in good supply I commonly give the larva of a "moderate-sized" species of *Epicauta* a scoop of about 20 eggs initially and add about 40 more when the larva reaches FG<sub>5</sub>. For one of the smaller species I would reduce the number by half.

**Pollen as Larval Food.** If the cells of the bee serving as the natural prey of the meloid species are available, one would, of course, choose to use them. Short of that, considerable success has been achieved by substituting pollen (with or without honey) from cells of the domestic bee *Apis mellifera* Linnaeus and, on occasion, larvae and pupae of this species. I have also had success with pollen removed from worker bees by a pollen trap.

Pollen is prepared by moistening it with water and mixing it into a paste. Addition of a small amount of honey improves the texture of the pollen mass but is not necessary. The consistency of the pollen paste is especially important in the early instars (T<sub>1</sub> and FG<sub>2</sub>). If the paste is too dry the larva will not be attracted to it or able to use it. On the other hand, if it is too moist, a larva not specifically adapted for feeding on a liquid medium is apt to become mired in it and drown. As the larva grows, its own feces will make any paste watery, but there is little likelihood that a large larva will drown. Determining the proper consistency requires trial and error. For larvae of many species it is appropriate to add just enough water so that the pollen can be easily shaped into a solid ball that is moist on the surface. But some larvae, such as those of two species of *Pseudomeloe* reared recently in my laboratory and those of a few *Nemognathinae* attacking cells of colletid bees, are adapted for floating in liquid provisions and require a fairly "soupy" mixture of pollen and water.

In early instars the accumulation of pools of liquid and the development of mold on the pollen paste are likely to be serious problems. Using a fairly dry, well mixed pollen paste, together with a mold inhibitor (0.5 mg methyl para-hydroxybenzoate per 1 g of diet), Leppla, Standifer, and Erickson (1974) were able to rear species of *Nemognatha* by providing the entire food supply at the beginning of the rearing. When using a moist paste, it is not unlikely that some or all of the larvae will have to be transferred to new tubes during the first three instars. Mold does not

seem to harm larvae directly. Rather, it inhibits feeding and, in the worst case, may actually prevent them from reaching their food. By the time larvae reach FG<sub>4</sub>, mold ceases to be a problem, presumably because of inhibitors produced by the larvae.

Because of the potential problem with mold, food is best added in small increments, on a daily basis. When using glass tubes, smear the paste on the tube wall, making sure that it does not contact either of the cotton plugs. Otherwise the cotton will soon absorb much of the moisture in the paste. If a very moist paste is being used it will run down the side of the tube to the cotton. To avoid this in my rearings of *Pseudomeloe*, where the paste was nearly liquid, I replaced the glass tubes with small glass vials and, later, small Syracuse dishes.

Although it is probable that the first food of a meloid T larva attacking a bee cell is usually the egg of the host bee, it has been the experience of several workers, including myself, that this is not a necessary part of the diet. Similarly, while FG larvae, especially in instars FG<sub>4</sub> and FG<sub>5</sub>, are capable of killing and eating bee larvae and pupae and in many cases undoubtedly do so in nature, these do not seem to be necessary for complete and apparently normal development. If bee larvae or pupae are offered, uneaten portions must be removed promptly since they will rot quickly.

**Meloid Eggs as Larval Food.** If T larvae whose natural food is unknown will eat neither grasshopper eggs nor pollen. I would suggest fresh meloid egg masses as the first alternative diet to be tested. These can be offered intact or broken into small clumps.

#### **Feeding Period of Development**

This period includes the T phase (first instar) and 4 or 5 instars in the FG phase, the last feeding instar being either FG<sub>5</sub> or (much less commonly) FG<sub>6</sub>.

**Larval Containers.** Glass tubes 8 ID x 50 mm plugged at each end with cotton are ideal for larvae of most species in the T and FG phases. These tubes are large enough for the entire food supply, if it is desired to provide it at the outset. For very large species a 10-12 ID x 70 mm tube may be used, either from the beginning or when the larva reaches FG<sub>5</sub>. The bottom cotton plug may be inset about 1 cm. Tubes should be labeled externally with rearing and larval number.

It is convenient to place tubes upright when stocking them with food and larvae. Add the food to the tubes first. Then dump the T larvae from their vial into a Petri dish and transfer them

individually to the tubes with a fine brush. Because of normal static charge on a brush, a larva will tend to adhere to it when touched. If the brush is then drawn lightly over the rim of an open feeding tube, the larva will drop off and fall to the bottom. A little practice will permit one to transfer several hundred larvae in an hour or less. I would not advise trying to pick up larvae with forceps. After larvae are in their tubes, the latter receive their top plug of cotton. In early rearings of *Epicauta* species I pushed down the top plug so that it touched the grasshopper eggs, with the idea of simulating the packed condition of eggs in a pod. Experience has shown that this is not necessary.

If additional T larvae will be selected subsequently from an egg mass, return the remaining larvae from the Petri dish to their vial. As indicated above, allowing larvae to remain in a Petri dish for more than a day or two will, in all likelihood, reduce their longevity significantly.

Once stocked with food and larvae, the tubes are transferred to a rearing jar of the type described earlier and placed in a temperature control chamber or other holding facility. I prefer to keep the tubes upright. If the rearing is small, a plastic or wood plate may be perforated with holes of appropriate size to receive individual tubes. Otherwise, tubes may be assembled in lots of convenient size and held in boxes of plastic or other material (preferably with porous bottoms). The day on which larvae first receive food is designated as day zero of the rearing.

As an alternative to glass tubes, I have experimented with glass vials of various sizes. Vials are more convenient to use because they require only a single cotton plug. However, the disadvantages outweigh this advantage. Larvae are more difficult to remove from vials (if this is necessary) than from tubes. Unless vials are inverted, which makes for awkward handling, there is the possibility of accumulation of condensed moisture. Finally, vials are harder to clean.

When larvae reach the FG phase they may be transferred to small dishes (such as miniature Syracuse dishes) arranged, conveniently, in Petri dishes. This procedure is particularly useful for close observation and photography of behavior but is not to be recommended generally, since it vastly increases utilization of space. Because FG larvae are quite sedentary in the presence of adequate food, it is not necessary to cover the individual small dishes. T larvae, however, are apt to escape from open dishes.

**Feeding Behavior.** T larvae may be expected

to begin feeding in 1-5 days. General deviation from this rule may indicate that the larvae are in diapause (unlikely) or that the food or physical conditions are not appropriate. In the temperature range of 25-30°C larvae will reach instar FG<sub>5</sub> in 10-20 days. With an additional period of, say, 5 days feeding in FG<sub>5</sub>, they will be ready for transfer to soil in a total of 15-25 days from the start of the rearing.

Larvae feeding on pollen may do so from the side of the pollen mass or may mount the mass and, if it is liquid enough, actually float in it.

T larvae of *Epicauta* generally open a hole in the side of a grasshopper egg from which they extract fluid. They often may be observed feeding with the head or anterior part of the body inserted in an egg, and occasionally they will crawl entirely inside. One or two eggs are sufficient for the first instar. FG larvae, particularly in later instars, chew large holes in eggs and consume the entire contents. In this phase they are capable of utilizing quite mature grasshopper embryos and will even eat newly hatched grasshoppers. As indicated earlier, much the greater part of the food used by any meloid larva is taken in the last instar of the FG phase.

If, as recorded in some species of *Epicauta*, there is to be an extra instar in the FG phase (FG<sub>6</sub>), the FG<sub>5</sub> larva will undergo ecdysis within perhaps 2-3 days, before it has become greatly engorged.

**Counting and Timing Instars.** Although there are reports in the literature to the contrary, meloid larvae do not consume their cast skins, except perhaps accidentally. Consequently, if skins are removed from tubes as they are observed, the presence of a skin will indicate that a larva has undergone ecdysis since the last observation. Skins, especially the head capsule, are generally easily seen. Frequently they will be found in the immediate vicinity of the larvae.

If larvae are checked daily, the observer will soon learn to recognize by inspection whether a larva has ecdysed since the last examination. In a recently ecdysed larva the body cuticle has a dull, almost velvety appearance, the body is deeply set with folds and wrinkles, and the head capsule appears disproportionately large. Moreover, in a recently ecdysed larva the head capsule is paler than usual, although the difference may be subtle. As the instar progresses the body cuticle becomes smoother and shinier, the body more plump, and the head capsule relatively small.

It is possible to specify the instar of an FG larva at sight, without reference to state of the

larva on the previous day. But this requires a great deal of experience. In particular, visual distinction between FG<sub>3</sub> and FG<sub>4</sub> larva may be difficult. (Reports in the literature of only three instars in the FG phase, with FG<sub>4</sub> as the last feeding instar, are attributable largely, I believe, to failure to note the ecdysis between the third and fourth instars.)

Probably the best procedure initially is to remove cast skins as they are found. Later, with more experience, a combination of ability to spot newly ecdysed larvae and to specify instar with some confidence at sight will eliminate the need to recover cast skins.

Except at an unusually high temperature, and then only early in development, a larva is not capable of completing an instar in less than a day. Therefore, as far as counting instars is concerned, there is no need to examine larvae more than once daily, and this has been my usual procedure. For measuring instar length, however, daily examination results in a relatively crude scale of measurement, especially in the early instars. This will not bias estimates of mean duration of instars, provided that the larvae are examined at the same time each day. But it will, of course, tend to inflate estimates of variance. On the other hand, since I am convinced that even daily handling of tubes disturbs larvae enough to prolong developmental time significantly, I am reluctant to examine them more than once a day. A further consideration is that in experimental studies involving temperatures appreciably different from the temperature of the room in which the examination takes place, larvae should be examined as infrequently and as quickly as possible, within the limits of the experimental objectives, in order to minimize deviation from treatment temperatures.

**The End of Feeding.** In many Nemogetininae and some Meloinae the larva reaches the adult stage in the bee cell in which it began its development. Consequently, in rearings of species of this type the fully fed larva may be allowed to remain in its feeding tube or vial. In rearings of most Meloinae, however, the FG<sub>5</sub> (or FG<sub>6</sub>) larva should be given access to moist soil when feeding is completed. In experiments with *Epicauta segmenta* I have found that replete FG<sub>5</sub> larvae forced to remain in their glass feeding tubes will, with rare exception, eventually become comatose and die, without further development. Numerous observations in the course of rearings of other species and genera indicate that this is generally true (but see below). Excavation in the soil of a chamber in which ecdysis will occur involves elaborate larval



behavior as well as production of an oral fluid that is mixed with the soil lining the chamber. Experiments with *E. segmenta* suggest that access to water in soil, rather than the opportunity to tunnel and excavate in it, is the key element necessary for continued larval development.

It must be noted that in contrast to my experience, Frickson and Werner (1974b) were able to rear *Lytta magister* Horn, *L. mutilata* (Horn), and *Tegrodera erosa* LeConte (only the first species in appreciable numbers) without providing soil for FG<sub>5</sub> larvae. They reported that the larvae worked the cotton from their tubes into a "cell," incorporating oral fluid. I have not observed this behavior in my rearings, perhaps because I tamp the bottom cotton tightly into the tube.

As "soil" I use a mixture of 1 part fine loam soil and 2 parts of silica sand moistened with water (10% of total sand/soil volume). Add the water to the (dry) sand and mix by shaking in a container. Then add the (dry) soil and again mix by shaking. The mixture (hereafter referred to simply as soil) can be stored for 2-3 weeks in a glass or plastic container. It is better to mix a fresh batch of soil than to moisten an old batch that has dried out.

Soil is packed lightly into a glass vial (or a glass tube stoppered with cotton), leaving sufficient room for the larva to be added at the top. Punching a hole in the soil with a pencil will help the larva to begin digging. After dropping the larva in a soil vial, stopper it with cotton. If the larva does not dig into the soil within a day, try a slightly moister mixture. This is indicated also if the soil tends to collapse on the larva as it excavates its chamber. I use 3-dram vials for soil, substituting smaller vials if the species being reared is unusually small. The idea is to use a tube that is wide enough for the larva to excavate its chamber comfortably but at the same time narrow enough that the chamber will be visible from the outside. In this way it is possible to observe the behavior of the larva and to record accurately its ecdysis to the next instar.

Either of two procedures may be adopted for giving the last-instar FG larva access to soil. The one that I now recommend for rearings of *Epicauta* is to transfer the larva, together with its food, to a soil vial as soon as the larva reaches FG<sub>5</sub>. This procedure may also be used with Meloidae feeding on relatively dry pollen masses, but severe problems with mold and absorption by the soil make it unsuitable if the pollen material has a high moisture content. The other procedure is to let the FG larva remain in its feeding tube

until it is judged, on some criteria, to have completed feeding. The problem here is that even after it has stopped feeding, a larva may continue to chew on its food as part of its attempt to escape from the tube. Smearing of food material about the tube, especially on the sides, is a good indication that feeding has stopped. Other indications are restlessness and chewing at the cotton plug. Finally, with some experience one can tell that a larva is replete by inspection. In any case, five days should be sufficient for feeding in the last FG instar, and no great harm will be done by arbitrarily adopting this length of time as the feeding period.

#### Postfeeding Period of Development

The general pattern in Meloidae is for the C (coarctate) phase to follow the FG phase. In many species of *Epicauta* an alternative, abbreviated pattern is often expressed in which the FG phase gives rise immediately to the pupal stage.

**The Abbreviated Pattern (T-FG-P-A).** When this pattern occurs one can expect the pupal period to last on the order of 10 days. It is best to allow the pupa to remain in the soil chamber excavated by the FG larva. Once the adult stage is reached the soil vial can be unstoppered and placed in a small cage. Unless there is diapause in the adult stage (not common), the newly formed adult will become active and emerge from the tube in a matter of days. If the soil is strongly compacted or has dried out appreciably, the adult may require assistance in emerging from the vial.

**The Longer Pattern (T-FG-C-SG-P-A).** As a general rule the C phase will be characterized by diapause. When this occurs C larvae may be allowed to remain in their soil chambers or may be removed from the soil and placed in individual cotton-stoppered tubes. The latter procedure will facilitate observation and will reduce the amount of space required for storage of the larvae.

Once diapause is broken the C larva will generally give rise to a second grub larva (SG phase). The SG larva does not feed. Further, in many cases it is capable of developing to the pupal stage without access to moist soil. But since there are exceptions, it is wise to provide SG larvae with soil unless it has been shown that soil is not required in the species under study. In this connection it is important to note that if a C larva is allowed to remain in its soil chamber, the soil will probably be too dry for use by the SG larva by the time it forms, and the SG larva will have to be transferred to a fresh soil vial.

Normally the SG larva will excavate a chamber like that of the last-instar FG larva and

in a few days will enter the pupal stage. Again, the pupa is best left in its chamber for development to the adult stage. Rarely, the SG phase will give rise to a second C phase, which may or may not be characterized by diapause. Repeated cycling between SG and C phases has been recorded.

In the Nemognathinae the C larva is encapsulated by the FG<sub>5</sub> skin and the SG larva, pupa, and adult form within the C skin. In the Meloinae the C larva is free. In this subfamily the SG larva and succeeding stages are generally free also, but in *Meloe* and some other genera they remain within the ruptured skin of the C larva.

**Diapause in the C Larva.** This subject is an extremely complicated one which has been hardly investigated experimentally and then only in temperate-zone forms. The following discussion is based on a survey of published literature, much of it anecdotal, and unpublished studies in my laboratory.

In most studies of temperate-zone species of Meloidae the C larva enters a "strong" diapause state in which it passes the winter and which is broken only after the larva has experienced an extended cold period. I suggest chilling C larvae of species from northern temperate regions for three months at 5°C and those from southern temperate regions for the same period at 15°C. After chilling, C larvae are returned to the temperature regime used in the feeding period of development. A latency period of several weeks to several months may be expected before the next ecdysis occurs. Some authors (e.g., Milliken) have suggested that wetting of C larvae previously exposed to dry conditions will terminate diapause, but the evidence for this is, in my opinion, questionable.

In the temperate-zone *Epicauta segmenta*, at least, there is an additional "weak" diapause state that occurs when larvae are reared under a cyclic temperature regime (daily amplitude of 10-20°C) and that increases in incidence directly with the maximum temperature of the cycle (Selander, unpublished). This diapause persists only so long as the larvae remain at the cycle under which they reached the coarctate phase. Diapause is broken within a few days after either the amplitude or mean temperature of the cycle is reduced a few degrees. No temperature treatment even approaching chilling is required. Like the pattern T-FG-C-P-A, the "weak" diapause state would appear to be an adaptation for the production of multiple generations in a single season.

The only extensive data on C diapause in a

strictly tropical species of Meloidae is derived from a rearing of *Pseudomeloe collegialis* (Audoin) that I recently completed using larvae derived from adults collected in Ecuador. In this rearing I took more than 100 larvae to the C phase of development (T-FG-C) at 25°C constant and 100% R.H. and then allowed them to remain under these conditions (no chilling or drying). All larvae broke diapause spontaneously in fairly synchronous fashion after a period of about five months.

Since so little is known about diapause termination in C larvae of tropical species, one should be prepared to experiment extensively with environmental variables. Some diapausing larvae should remain at the temperature at which they formed, under fairly high humidity; others should be subjected to combinations of somewhat lower temperatures and various humidity levels. In a region characterized by a marked difference between wet and dry seasons, exposing C larvae to very dry conditions first and then to humid ones (including periods of contact with liquid water) would be a worthwhile experimental procedure.

**Behavior of New Adults.** It is normal for a new adult to rest within its chamber for one to several days. Even after it has emerged it may not become fully active for a few days more. The length of the "resting" period varies considerably among species. Soon after the adult becomes active it will discharge the meconium and begin to feed. Sexual behavior generally develops last. Adults that do not attempt to emerge from their tubes or that, having done so, walk or feed only sporadically are probably in diapause.

In some species (e.g., those of the Vittata Group of *Epicauta*) the female initiates the egg production cycle before mating occurs. In others (e.g., those of the Albida Group of *Epicauta*) one or two matings are required as a stimulus.

#### More Natural Rearing Conditions

If one is not interested in the FG instars, the rearing method can be greatly simplified for larvae that eat grasshopper or meloid eggs. Merely add a T larva and its complete food supply to a vial partially filled with moist soil mixture and then allow two or three weeks to pass before examining the vials. For reasons already given, this procedure may not be appropriate for larvae feeding on pollen.

To obtain reliable information on the natural incidence of the various ontogenetic patterns and the seasonal timing of the life history, it is, of course, necessary to rear under more natural conditions than described above and, ideally, to supplement rearings with survey data from the field.

In some of Horsfall's rearings of species of *Epicauta*, diapausing C larvae in stoppered glass tubes or ceramic thimbles were buried in the ground over winter and then recovered in the spring for observation of further development. Some rearings of larvae to the C phase were accomplished by placing larvae and supplies of grasshopper eggs in individual ceramic thimbles which were then inverted in a larger container (crock) of moist soil placed outdoors. In addition to using temperature control chambers, Horsfall built an outdoor rearing chamber by suspending a 2-gallon ceramic crock inside a 3-gallon crock buried to its rim in the ground. Vials and tubes containing larvae were placed in the inner crock. Both crocks were closed with Cellotex disks, and the whole was protected from rain by an inverted saucer. According to Horsfall, temperatures in such chambers were very similar most of the year to those recorded at the 7.5 cm level in soil.

If an outdoor chamber similar to Horsfall's is constructed, a screened bottom on the outer container might be preferable to a solid one, so as not to isolate larvae from changes in moisture level in the ground. However, in some areas the likelihood of predation by ants and other arthropods may mitigate against this. Reference to published works dealing with the rearing of other soil-inhabiting insects should provide an abundance of ideas for rearing meloids under more or less natural conditions.

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