Ethology Laboratory: Reproductive Behavior of *Mormoniella vitripennis*

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Student demand for courses in ethology has grown markedly in recent years. Stimulated in part by the popular accounts of Robert Ardrey, Desmond Morris, and Konrad Lorenz, public interest has increased and many universities have responded by instituting new courses. However, the conscientious ethology instructor often finds himself in a bind. His subject is best taught through extensive laboratory exposures where the student is taught to observe, appreciate, and quantify in a manner that is probably new to him—but the maintenance, preparation, and performance of good, dependable ethology laboratories is fraught with problems.

Any laboratory work with live animals is difficult, but in ethology labs the risk of failure is particularly great. The animals must be well maintained if they are to "perform" correctly. Nutrition, rearing environment, and genetic background must all be appropriately controlled. Behavior may be disrupted by moving the subjects from rearing place to laboratory or by releasing them into a new and fear-inducing environment for observation. Similarly, jostling, excessive noise, or rough handling by overzealous and impatient students may adversely affect behavior. Finally, after sufficient care is taken in all respects, the animals may still simply "refuse" to behave.

The exercise described here has been used in our ethology laboratory with great success. It demonstrates several important ethologic principles, is easy to set up, is virtually foolproof in its performance, and (most important) provides an impressive demonstration that also stimulates further investigation and the design of simple experiments concerning instinctive behavior. The materials required are inexpensive, occupy very little space, and should be readily available to most high school and college biology departments.

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Life Cycle

Mormoniella (=Nasonia) vitripennis, a member of the order Hymenoptera, is a small wasp that parasitizes various fly pupae. For a depiction of the life cycle see fig. 1. The adult female lays her eggs on a fly pupa developing inside a puparium (*3*, in the figure). At 25 C larvae hatch approximately two days after the eggs are laid. For six to seven days the wasp larvae feed on the tissues of the fly pupa (*5*). The wasp larvae pupate inside the host puparium (*7* and *8*) and emerge as adults five to six days later. The adult wasps (*1* and *2*) range in size from 0.6 mm to 3.5 mm (Whiting, 1967); males generally are smaller than the females. Normally the males are haploid, having developed from unfertilized eggs, and the females are diploid, having developed from fertilized eggs. A mated female produces a majority of female offspring; a virgin female produces only male offspring. Whiting (1967) discusses the biology of *Mormoniella* in detail, and teachers considering the use of *Mormoniella* should refer to this fine paper.

Maintenance

Several weeks before using *Mormoniella* for this ethology laboratory, a starter culture of the parasitic wasp and a supply of the host, *Sarcophaga bullata*, should be obtained. Small starter cultures of *Mor-*

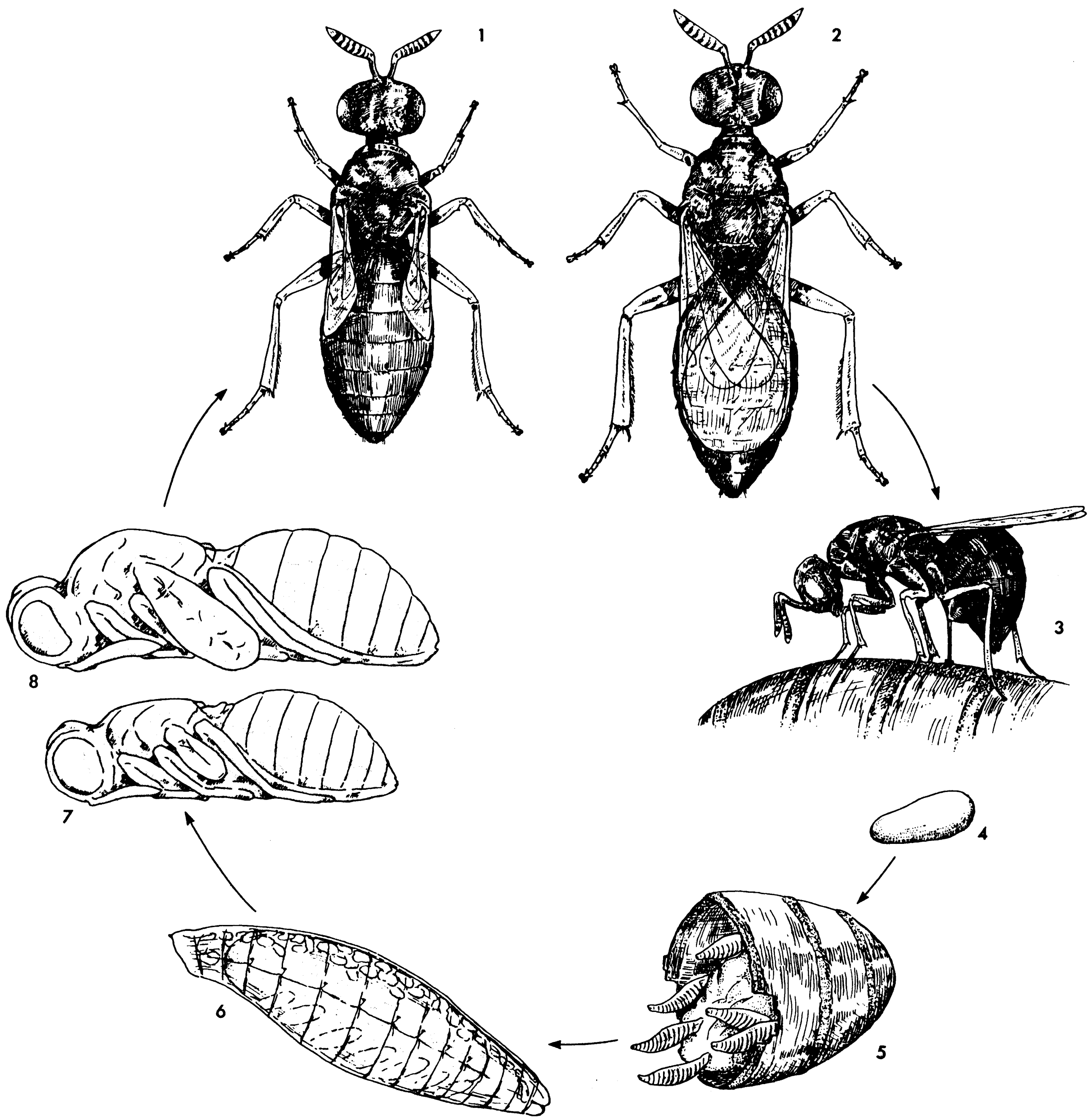

Fig. 1. Life cycle of *Mormoniella vitripennis*. *1*, adult male; *2*, adult female; *3*, female drilling through puparium; *4*, egg; *5*, developing larvae in *Sarcophaga* puparium; *6*, larva; *7*, male pupa; *8*, female pupa. (Courtesy of Carolina Biological Supply Co.)

moniella for teachers and a helpful paper, "The Biology and Culture of *Mormoniella*," by George B. Saul are available from the NSF-supported *Mormoniella* Stock Center at Middlebury College, Middlebury, Vt. 05753. Larger quantities of *Mormoniella* can be ordered from biology supply houses. These houses also stock *Sarcophaga*, which should be purchased as young pupae and refrigerated (at about 4 C) upon arrival. The fly pupae can be stored in the refrigerator for several months and then removed to serve as hosts whenever needed.

The *Mormoniella* will probably arrive as pupae. If they have not been removed from the puparium of the host before shipment, crack open the puparium and pour the wasp pupae onto a card. The young pupae will be light yellow in color, but over a period of a few days they will gradually turn darker, starting at the head and thorax. Choose individuals of the same color pattern to start a new culture. Using a dissecting microscope and a fine brush to move the pupae gently, sex the wasps according to the relative sizes of wing pads (see fig. 1, *7* and *8*). Place five to

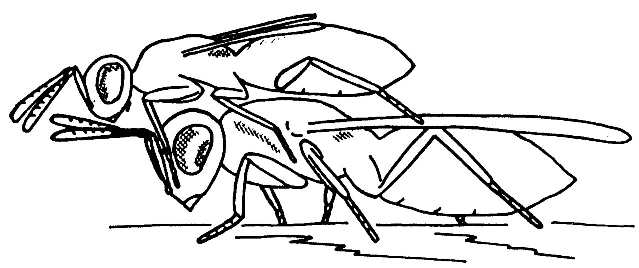

Fig. 2. Courtship position, male above female. (From Barrass, 1960a.)

10 males and five to 10 females in a small vial, such as a 5.3-g (three-dram) shell vial. Securely plug the vial with cotton and keep it at room temperature (22 to 25 C). After emergence of the adults and reproduction by them, the majority of offspring in this vial will usually be females. When a large number of males is needed, place several female pupae in a vial without males, so that only unfertilized eggs will be laid after emergence of the adults. Store extra pupae in the refrigerator. Young (yellow) pupae can be stored for up to one month; older (darkened) pupae can be stored for several days. Such storage provides insurance in case the first culture does not reproduce.

Approximately 24 to 48 hours after adult wasps have emerged, place six to eight hosts (fly puparia with fly pupae inside) in the vial. During the next few days the female wasps will feed on fluid, which they obtain by puncturing the puparia, and will lay eggs inside the puparia. The males die a few days after emergence. The rate of development of *Mormoniella* depends on the temperature at which the wasps are kept. If the cultures are left at a temperature in the range of 22 to 25 C, crack the puparia open to check for the presence of wasp pupae eight to 10 days after the hosts have been added. When young (yellow) wasp pupae fall out of the puparia they can be stored in the refrigerator. Thus it is possible to raise many wasps a few weeks ahead of time and temporarily store the young pupae for up to one month in the refrigerator. Six to eight days before adults are needed for the ethology laboratory, remove the pupae from the refrigerator, separate the sexes, and keep the pupae at room temperature until the adults emerge. (Occasionally some larvae will go into diapause and not pupate at room temperature. These larvae can be refrigerated for several months. When returned to room temperature, they will then pupate and continue development.)

Because the length of the life cycle of *Mormoniella* depends on temperature and adults are needed for this ethology laboratory, we recommend that the teacher obtain the culture several weeks before the laboratory session, so as to have time to become familiar with the life cycle and handling of these wasps and to raise the required number of them.

Reproductive Behavior

Reproduction in *Mormoniella* involves distinct courtship, copulation, and (usually) postcopulatory courtship. Males respond, apparently visually, to the presence of a female within about 4 to 7 mm. They generally approach immediately and begin courtship, during which the male mounts the female with his forefeet on the female's compound eyes or between them and his head above and forward of the female's antennae (fig. 2). The male then engages in head movements. First, he lowers his head, bringing the mouthparts into contact with the female's antennae. The mouth is then moved backward along the antennae, forward almost to the tips, and backward again (fig. 3). The mouthparts are generally protracted during the forward movement and retracted during the backward movement. The complete act takes about one second and may be repeated several times, usually separated by intervals of two to five seconds. During these movements, the male also strokes the female's antennae with his own, raising his flagella between head movements. Throughout courtship, the male also flutters his wings and gently raises and lowers his head and thorax.

The female indicates willingness to copulate by several postural changes. The antennae are lowered (fig. 4) and the tip of the abdomen is raised while the abdominal sternites are held perpendicular to the body, exposing the genital opening. The male responds immediately by backing into the copulatory posture (fig. 5). Copulation takes three to 15 seconds. It is often followed by a postcopulatory courtship, which is generally indistinguishable from precopulatory courtship except that it only rarely

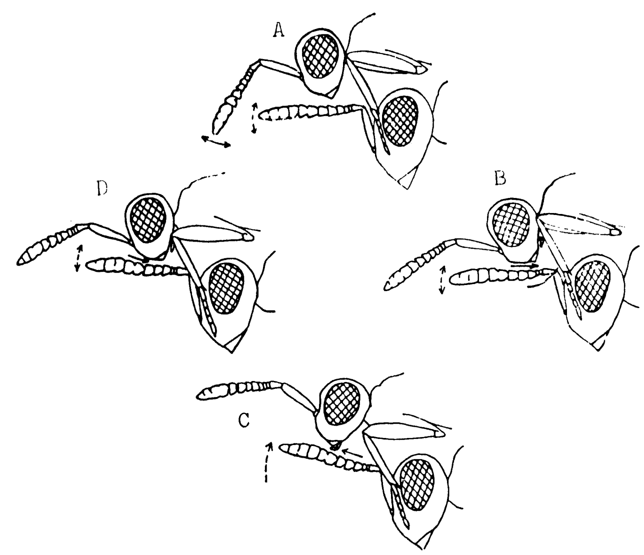

Fig. 3. Head movements of a courting male. Note retraction of mouthparts during posterior movements *B* and *D* and extension during anterior movement *C*. (From Barrass, 1960a.)

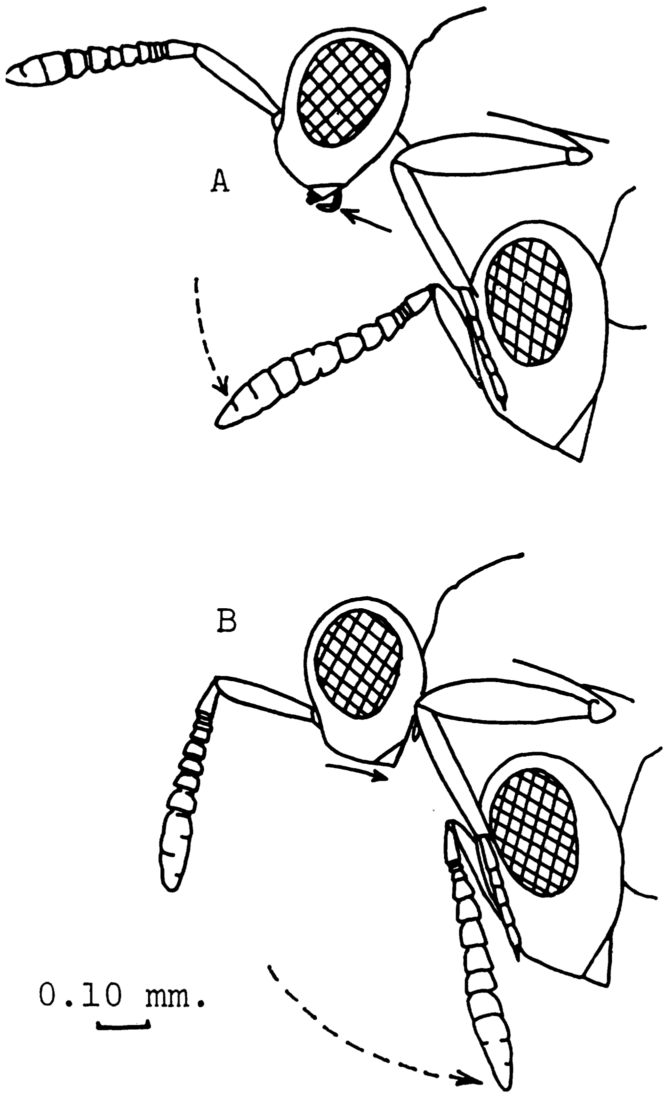

Fig. 4. Lowering of female's antennae in response of courting male. (From Barrass, 1960a.)

results in a repetition of the female's "acquiesence" behavior and, thus, a second copulation. Technical aspects of these behavior patterns are reviewed by Barrass (1960a, 1960b, 1961).

Upon encountering a fly puparium, a *Mormoniella* female touches it with her antennae. She then climbs onto it and "drums" vertically upon it with her antennae. Following this, she taps rapidly with the tip of her abdomen and finally pierces the puparium with her ovipositor, which is located about one-third body length anterior of the abdominal tip. Drilling is generally accomplished with the ovipositor elevated and the abdominal tip pointed down (fig. 1, 3); oviposition itself usually involves straightening the abdomen and applying the region of the ovipositor directly to the puparium, thus providing maximum reach. Details of host-selection and oviposition are reviewed by Edwards (1954) and Wylie (1958).

Suggestions for Study

Observations of the complete sequence of reproductive behavior can be made in any petri dish with a close-fitting cover to prevent escapes. Although a binocular dissecting microscope is ideal for this study, any standard magnifier, tripod-mounted or even hand-held, is adequate. In these observations adult males and females can readily be distinguished by the length of the wings: the males have much shorter wings (fig. 1, *1*) than do the females (fig. 1, *2*). To insure against unobserved copulation, males and females should have been segregated in the pupal stage before they emerged as adults.

Each student can be provided with a vial containing at least six one-to-two-day-old adult females, a vial containing at least six one-to-two-day-old males, and a petri dish. In addition, stock vials of a few hundred separated males and females can be available for extra experiments suggested by the students. For observations on oviposition a vial containing several adult males and females, which have had at least one day to mate, and a petri dish containing several hosts can be provided for each student.

Mormoniella adults may be handled approximately like *Drosophila*—tapped to the bottom of a vial and then shaken out as desired. Because the females can fly but the males are flightless, it is best to introduce a male into the petri dish first. Age of the subjects does not appear to be critical: the adults may be newly emerged or up to several days old. Within a few minutes (sometimes seconds) after introduction of both sexes, courtship, copulation, and postcopulatory courtship generally will be observed. Occasionally a second copulation will follow. Because the subjects are readily available in large numbers for repeated observations and all relevant behavior is predictable, the laboratory can be interrupted periodically with discussion neatly sandwiched between observations. Depending on the questions raised during discussion, the students can be primed to

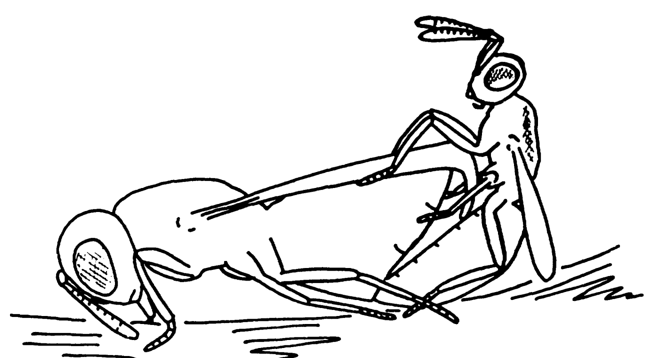

Fig. 5. Copulatory posture, male behind female. Note the female's lowered antennae and elevated abdomen and the contact made at the female's genital pore. (From Barrass, 1960a.)

concentrate on particular aspects of the behavior during the next observation period.

In one successful procedure the students first observe gross aspects of courtship and copulation, then concentrate of finer details. Exactly what is the male doing? How does the female indicate receptivity? In the event of postcopulatory courtship that is not followed by a second copulation, what has the female done differently (that is, how has she indicated unreceptivity)? This demonstration lends itself to the construction of an ethologic "reaction chain", indicating fixed stimulus–response interactions, with successive male and female actions. Furthermore, if stopwatches are available these acts lend themselves to ready quantification. Thus, latency between introduction of both sexes and initiation of courtship may be determined, along with duration of courtship, of copulation, and of postcopulatory courtship.

This study also raises many basic questions, which can be readily investigated. Will a female, having copulated with one male, mate with another? Will she mate with the same male again? (She usually will not.) Will males mate successfully with many different females? (They will.) Is there any change in latency of response, duration of acts, or frequency of head movements with successive matings? Does age of the male and the female influence any of these aspects of reproductive behavior?

A simple manipulation will reveal the important role of specific stimulus-input in maintaining the reaction chains and coordinating male–female behavior. The antennae are clearly important as sense receptors and can readily be removed with no serious bodily damage by simply enmeshing the animal in cotton and pulling the antennae with fine tweezers. Intact males approach females and generally make initial contact with their forelegs and antennae before commencing courtship. Antennaeless males will still approach—apparently they are visually guided—but will generally fail to mount, having not received the proper tactile stimulation. An intact male will court an antennaeless female, and after appearing to "search" with his head and antennae for her missing structures he often will begin characteristic head movements in the region where her antennae would normally occur—which is striking evidence of the stereotypy of this behavior.

Antennae may of course be removed from both virgin and mated females. An intact mated female will normally reject a courting male and signal this in part by keeping her antennae in the upright position. Following amputation of the antennae she is unable to signal her rejection, and the male may then attempt copulation with an unreceptive female, which will often respond by walking away, dragging the rejected male behind her. This striking lack of coordination is never observed when communicative structures are intact. When antennae are amputated from virgin females the results are equally extraordinary: intact virgins will invariably acquiesce to a courting male, but amputees usually do not. Failing to receive the "no" signal, the persistent male will attempt copulation, but the female will respond like a mated female and generally walk away. Thus, during the head movements of normal courtship the female apparently receives through her antennae the stimulation that induces sexual receptivity. In the absence of such stimulation, even a virgin remains unreceptive.

Finally, the cycle can be completed by introducing females into petri dishes containing puparia of *Musca, Sarcophaga,* or *Calliphora.* Drilling and oviposition behavior (described above) can then be observed, although with less rigid predictability than with mating behavior. Again, however, this sequence provides much behavior that can profitably be counted and measured, and it suggests numerous questions which can be answered with simple experiments. For example, oviposition will usually not follow if the female has located the emergence hole of a fly or parasite. Thus, by inserting a needle, such a hole can be mimicked, resulting in rejection.

These preliminary suggestions are only a beginning to a potentially rewarding student inquiry. The wasp *Mormoniella* deserves a place in the ethology laboratory: it contributes to the students' education and to the instructor's peace of mind—with the comforting assurance that this behavior lab will really work.

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Groundwater Ecology Conference

The Underwater Research Institute will sponsor a conference on groundwater ecology at St. Louis University on April 29-30. Current concepts of aquatic troglodytic organisms will be examined. For details inquire of William Cate, director of the institute, 3411 Hampton Ave., Suite 202, St. Louis, Mo. 63139.