Collection and Laboratory Culture of *Ormia ochracea* (Diptera: Tachinidae)[1]

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Abstract The Family Tachinidae is one of the most speciose families in the Order Diptera with approximately 1300 species occurring in North America alone. Research on the species *Ormia ochracea* (Bigot) (Diptera: Tachinidae) has largely focused on the problems incurred by their hosts as a result of parasitism, or on the mechanics of their hearing. Little research effort has been devoted to the behavior or life history of these flies. Part of the reason they have remained lightly researched is the difficultly in maintaining a laboratory culture. Herein, we provide a detailed guide to collecting *O. ochracea* in the field, culturing them in the laboratory, and maintaining stock populations for multiple generations. We also provide data on the effectiveness of capturing *O. ochracea* in wooded versus open field areas, as well as data on the effectiveness of manually parasitizing crickets with *O. ochracea* larvae to propagate stock fly populations in the laboratory. Our results suggest that during field collection, traps should broadcast calls in wooded areas; and that manual parasitization is an effective way of culturing small colonies of *O. ochracea* in the laboratory.

Key Words *Ormia ochracea,* parasite, acoustic trapping, culturing insects

Gravid *Ormia ochracea* (Bigot) (Diptera: Tachinidae) females use the call of their insect hosts to locate and subsequently parasitize them (Sabrosky 1953, Robert et al. 1998, Mason et al. 2001). Once a host has been located, the female deposits her larvae on the host, and the larvae then burrow into the host's body where they develop for 7 d before emerging and killing the host (Cade 1975, Adamo et al. 1995b). Little effort has been devoted to behavioral or life history studies of these *O. ochracea*. One main reason *O. ochracea* is understudied is the difficulty associated with maintaining a colony in the laboratory. This entails capturing wild flies, maintaining both fly and host colonies, and insuring that the colony's females become gravid and that the planidia (1st - instar *O. ochracea* larvae) locate and parasitize a suitable host.

Walker (1989) published an extensive guide on methods of trapping *O. ochracea* in the wild, and Wineriter and Walker (1990) published practical techniques for maintaining a large-scale colony of *O. ochracea* necessary for biological control. Their rearing protocols included detailed drawings and instructions on how to construct rearing enclosures to aid fly propagation in the laboratory. Herein, we provide more details on how to capture *O. ochracea* in the field. We also provide a modified method to culture a small colony of *O. ochracea* in the laboratory. These culture modifications are appropriate for researchers interested in *O. ochracea* for experimentation and

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require a smaller-scale colony than that required when culturing *O. ochracea* as a means of biological control (Wineriter and Walker 1990). We also include data that demonstrate the effectiveness of (1) capturing *O. ochracea* in a wooded area versus an open area, and (2) using manual parasitization methods to propagate *O. ochracea* in the laboratory.

Materials and Methods

Sound trapping *O. ochracea*. When establishing a laboratory colony, if a gravid female fly is not available and the host species is unknown, traps with the calls of local calling insects (often orthopteran and hemipteran species) should be used.

Sound traps consist of a playback device and a catching device (e.g., the component of the trap that physically traps the fly see Walker 1988). The catching device is left to the experimenter's imagination and budget, but should include some variation of a funnel trap, that allows the fly to easily access the acoustic attractant but prevents it from escaping once within the device. The trap we use is modeled after Walker's (1989) slit-trap design. A high quality recording device is necessary to insure that the calls being used in your playback will be recognizable as a host species by a gravid female. We use a Durabrand CD-566 compact disc player (Lennox Electronics Corporation, 35 Brunswick Avenue, Edison, NJ 08817) which is placed underneath the trap and set to continuously broadcast the recorded call of a *G. texensis* male. The call used was recorded from a laboratory-reared male and has a frequency of 4.6 kHz. The call is broadcast through an Amplified Speaker AMX 18 (RadioShack Corporation, Fort Worth, TX) at an intensity of 61 dB from 30cm. Gravid female *O. ochracea* are attracted to the broadcast and enter the trap via the slit, becoming ensnared (see Walker 1989).

Traps should be located in areas with orthopteran activity. If there are no orthoptera in the area, it is unlikely that there are *O. ochracea* nearby. Semiopen habitats (mixture of trees and meadows with ground cover) that are removed from areas with agricultural/pesticide sprays are excellent sites.

Traps should be deployed before sunset as *O. ochracea* are most active at dusk (Wineriter and Walker 1990, Cade et al. 1996). The traps should be left out for several hours. If trapping in an area that is inhabited by imported fire ants, *Solenopsis invicta* (Buren), it is best to check traps early in the morning as worker ants will invade traps and prey upon the inhabitants (SMB, pers. obs.).

Trapping in wooded versus open habitats. In Florida, Walker (1989) found that sound traps placed in wooded areas attracted more flies than traps placed in pastures. To ascertain how universal this elevated capture rate in wooded areas is, we tested whether *O. ochracea* in Texas were also more likely to be attracted to acoustic sound traps in wooded versus traps in more open field locations. In September 2008 we deployed acoustic fly traps in Smithville and Austin. At both sites traps were placed in areas that were designated as 'wooded' or 'field'. An area was designated as being wooded if there was no clearing within sight of the traps (only trees could be seen). The site was designated as being a 'field' if it was located in open clearing and a roadway, parking lot, or street lights could be seen from the trap's location. At each site traps were within 1 km of each other and broadcasted the same recorded male call at an intensity of 61 dB from 30cm. We monitored the number of *O. ochracea* captured over 2 nights (1900h to 2100h; each night in each locality).

Guide to housing *O. ochracea* in the laboratory. Flies can be transported to the laboratory within the traps. If only a few flies are needed or the trap cannot be removed, flies can be transported individually in small (10 mL) vials. Individual flies are collected by gently placing the vial over the specimen and then placing a cap on the vial. A moistened cotton swab should be placed in the vial. This swab should be cut to size so that it fits snugly in the vial and will not move around and damage the fly. The cotton swab should be moist but not wet, as the fly can get stuck to the side of the vial when water droplets form.

Depending on the size of the colony desired, the proposed use of the flies, and the time and resources available to dedicate to the colony, a less sophisticated enclosure than that described by Wineriter and Walker (1990) can be used. We use amphibian terrariums purchased at a local pet store (30.5 × 21 × 21 cm). This housing option is relatively inexpensive (approx. US$10 each) and requires little space. The fly enclosures are placed in an environmental chamber maintained at 26 C on a 14:10 h (L: D) photoperiod. If your environmental chamber allows adjustment of humidity, the relative humidity should be set to more than 55% as fewer than 20% of the flies eclose at humidity levels below 50% (CMV, pers. obs.). If the humidity cannot be programmed, the humidity can be increased by placing a container of water bubbled with an air pump and air stone. This water should be topped daily and changed weekly.

The colony should not be disturbed for maintenance at the beginning of the dark phase of their light cycle as most mating occurs at this time (CMV, pers. obs.). Females may not partake in typical phonotactic behavior if they have not been housed with a light cycle (N. Lee, Dep. Biol. Sci., Univ. Toronto at Scarborough, pers. comm.). A greater proportion of females become gravid as the density of the population increases (Wineriter and Walker 1990, CMV, pers. obs.). Our colonies are kept at a density of about 1 fly for every 38 cm^3.

An excellent food source for *O. ochracea* is Instant Liquid Nectar Concentrate (Yule-Hyde Associates Corporation, 4 Lowry Drive, Brampton, ON) at a concentration of 1 mL feeder solution per 7 mL of water. This sugar-water solution can be supplied to the colony by saturating a long piece of cotton and suspending it from the lid of the colony cage. Feminine hygiene products (tampons) work well for this task as they can absorb a large quantity of the sugar solution and their integrated string can be tied to the cage lid, allowing for easy suspension. The cotton can be refilled by administering the solution through the lid of the terrarium using a pipette. The cotton should be replenished with the sugar-water solution 3 or 4 times a week and should be replaced on alternate weeks. A somewhat less expensive alternative to tampons is dental cotton rolls, for which a means of suspension must be devised (N. Lee, Dep. Biol. Sci., Univ. Toronto at Scarborough, pers. comm.).

Guide to fly propagation through manual parasitization. Though the natural process of host location and subsequent parasitization has been successfully conducted in a laboratory (Wineriter and Walker 1990), it is also possible to manually parasitize crickets. Manual parasitization is a technique where the researcher opens the abdomen of a gravid female fly, removes active planidia, and then places one or more planidia on a host cricket. It is a valuable tool when conducting experiments that require that crickets be parasitized with a specific number of planidia or at a specific time. Furthermore, because crickets increase grooming when they come into contact with planidia (Vincent and Bertram, unpubl. Data), manual parasitizing assures that planidia enter the host. Manual parasitizations also insure that all gravid females are used optimally by preventing females from competing with conspecifics for hosts

(avoidance of superparasitism) and permitting the use of all of the female's planidia (females may not expel all of their planidia during period of exposure to hosts). Avoiding superparasitism is important because the number of *O. ochracea* larvae in a host is negatively correlated with larval success (Adamo et al. 1995b).

It is important that females are carefully selected for manual parasitization. Gravid females should have darkened abdomens (tiny black larvae are visible through cuticle) (Wineriter and Walker 1990). Anecdotal observations suggest that when a gravid female is deprived of a suitable host, her planidia will exit their egg casings and enter her abdomen, eventually killing her (S. Adamo, Dep. Psychology, Dalhousie Univ., pers. comm.). Therefore, once a gravid female has developed a dark abdomen, within a few days, she should be dissected and used for parasitization. Recently deceased gravid females should be immediately dissected, as on 3 out of 7 occasions, we have opened the abdomen of these females to find a few lively, mature planidia. We do not, however, recommend employing this postmortality method to determine whether a female is ready to be used for manual parasitization, because it was only successful 43% of the time and produced far fewer planidia than dissecting the abdomen of a living gravid female *O. ochracea.*

To manually parasitize crickets with *O. ochracea*, the gravid female parasitoid is placed in a small vial (approx. 10 mL). The vial is gently rested in an ice bath for <1 min to cool the female so that she does not fly away during the removal of her abdomen. Females should not be so chilled that they are immobilized following this process. Once the female begins to show signs of cold-induced inebriation, characterized by falling over and the cessation of wing movement, remove her vial from the ice bath and remove her from the vial. Gently grasp the prothorax with a pair of tweezers, placing another pair of tweezers at the proximal end of her abdomen where it meets the prothorax, and gently remove the abdomen. After the abdomen is removed, put the remains of the female in the freezer and place the abdomen on a bed of fresh cotton under a dissecting scope. Gently open the abdomen so that the planidia are visible. Planidia that are 'standing' vertically, with one end on the substrate and the other moving side-to-side in midair in a 'waving' motion, should be used immediately. Throughout the remainder of the parasitization, small sections of the reproductive tract should be removed and teased open to expose fresh 'waving' planidia. Because manual parasitizations can take time, this method of only using small sections of the reproductive tract at a time allows the experimenter to take breaks, as the planidia are not all exposed at once and, therefore, do not wander around the Petri dish and desiccate.

Manually placed planidia often 'wander' on a host cricket and it is unknown whether these wandering planidia successfully parasitize the host. Therefore, it is important to insure that the planidia are placed on the host in a location that optimizes parasite success. The cricket should be held dorsal side up and positioned so that its body is spread over the middle finger lengthwise (head to abdomen). Gently place your thumb on the abdomen and, with your forefinger, depress the pronotum so that the soft tissue between the pronotum and the thorax is exposed. Place the planidia at this location. Waving planidia can be picked up by gently touching them with fine forceps or a dissecting pin to which they will immediately adhere. Once the planidia have attached to the tool, they can be placed on the host. Planidia should be placed specifically on the articular sclerites (area of the wing attachment joint where there is exposed soft tissue; Fig. 1). Planidia will immediately enter the cricket when properly placed. Articular sclerite placement is beneficial because it allows the experimenter to

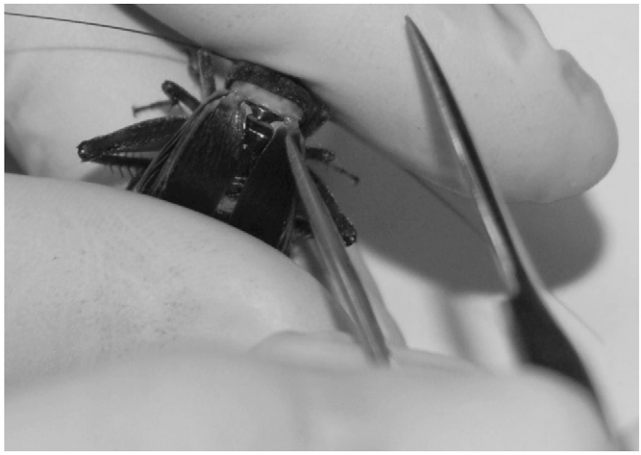

Fig. 1. Articular sclerites. Planidia placed here will immediately enter the host.

visualize the planidia's entry thereby affording more confidence in the success of the parasitization.

Once crickets have been parasitized they should be placed in either individual containers or communal bins and provided with food and water. Housing parasitized crickets communally will result in a slightly smaller fly yield as we have observed pupal cases partially consumed by crickets. Furthermore, when we provided 21 crickets with some laboratory diet, a cricket leg, an *O. ochracea* pupa and an *O. ochracea* larva, 100% (14/14) of the crickets that consumed anything consumed at least some of either *O. ochracea* larva or pupae (3/14 consumed both). Therefore, when the number of crickets parasitized makes it impractical to house them individually, bins must be checked daily for newly-emerged larvae to minimize the number of larvae and pupae consumed by crickets.

Once pupae begin to appear, 7 - 12 d following parasitization, they must be removed and placed in the fly enclosures. It will take 12 d on average for adult flies to eclose from their pupal cases (Wineriter and Walker 1990, CMV, pers. obs.). Therefore, the time elapsed from manual parasitization to fly eclosion is roughly 5 wk (2.5 wk for the adult female to mate and develop mature planidia, 1 wk for the larvae to feed and grow inside the cricket, and 1.5 wk for the development of the pupa in the pupal case).

The efficacy of manual parasitizations. To quantify the effectiveness of manually parasitizing crickets, we parasitized 166 *G. texensis* crickets. *Ormia ochracea* were collected nightly from several locations between Austin and Smithville in the fall of 2007. The *G. texensis* were second generation laboratory reared. We dissected and used the planidia from a total of 7 *O. ochracea* females. Two lively *O. ochracea* planidia were placed on each cricket using the aforementioned manual parasitization techniques. Parasitized crickets were housed individually until larval emergence. We quantified the number of planidia that emerged from each cricket, the number of planidia that successfully pupated, and the number of fly offspring that survived to adulthood from each of the original seven female *O. ochracea*.

Results

Trapping in wooded versus open habitats. Traps placed in 'wooded' areas attracted almost 4X more flies than those placed in fields (Austin: wooded = 124, field = 32; Smithville: wooded = 47, field = 13; Fig. 2).

The efficacy of manual parasitizations. Manual parasitization of crickets with 2 *O. ochracea* larvae, yielded on average, 1.46 ± 0.36 (SE) (n = 352) emerged larvae per cricket. We yielded an average of 30 ± 23.5 (SE.) (n = 7) adult flies per each gravid female used.

Discussion

We found that a greater number of *O. ochracea* can be collected using a sound trap, in wooded areas than in fields. Our findings are in agreement with Walker's (1989) data collected on Floridian *Ormia.*

Manual parasitization is an effective means of maintaining a small *O. ochracea* colony that will have a limited number of gravid females in each generation. An estimated 30% of the females in a generation of a laboratory colony of *O. ochracea* become gravid (Wineriter and Walker 1990, CMV, pers. obs.). Manual parasitizations insure that all gravid females are used optimally by permitting the use of all of a female's planidia (females may not expel all of their planidia during period of exposure to hosts) and preventing females from competing with conspecifics for hosts (avoidance of superparasitism). The number of *O. ochracea* larvae in a host is negatively correlated with larval success (Adamo et al. 1995b), thus, the avoidance of superparasitism is important.

Wineriter and Walker (1990) housed 1 - 25 gravid flies with 200 crickets. We have parasitized no fewer than 50 crickets and often more than 100 crickets, with planidia collected from 1 gravid fly. This implies that the females used in Wineriter and Walker's (1990) study may have been underutilized and that the crickets may have been superparasitized. Furthermore, the authors do not report the fly yield per gravid female,

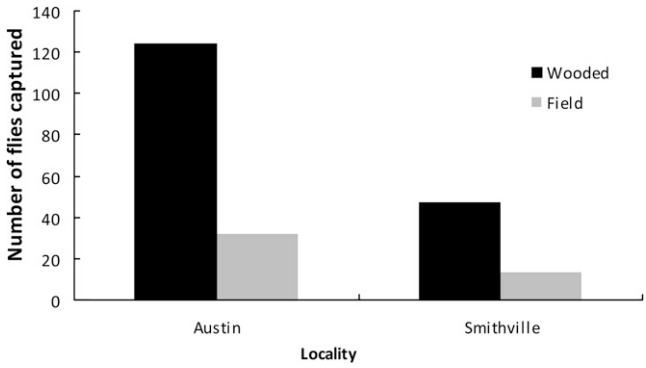

Fig. 2. Traps were set out over two consecutive nights in Austin and Smithville, TX. Each night traps were placed in either a wooded or open area in each locality.

making it difficult to quantify the contribution of individual females to the colony in their study.

The ability to manually parasitize crickets using *O. ochracea* larvae is valuable to any researcher investigating the interaction between *O. ochracea* and its hosts. The number of larvae within a host is known by the experimenter allowing for the entire life history of one *O. ochracea* larvae to be monitored. Furthermore, when conducting behavioral experiments or quantifying the impact of parasitism, it is important to be able to control the number of larvae within a host.

We have successfully maintained 11 generations of *O. ochracea* in a laboratory environment using manual parasitizations. The techniques we present herein should allow any interested researcher to successfully maintain a healthy laboratory population of *O. ochracea*.

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