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Authors: Thomson, Ian R., Vincent, Crystal M., and Bertram, Susan M.

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SUCCESS OF THE PARASITOID FLY ORMIA OCHRACEA
(DIPTERA: TACHINIDAE) ON NATURAL AND UNNATURAL CRICKET HOSTS

IAN R. THOMSON1, CRYSTAL M. VINCENT1,2 AND SUSAN M. BERTRAM1
1Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6
2Current Address: Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks Street, Toronto, Ontario, Canada M5S 3B2

ABSTRACT

The parasitoid fly Ormia ochracea acoustically stalks signaling male field crickets and lays live larvae on and around them. Ormia ochracea have been observed to acoustically stalk various cricket species. In Texas the natural host of this fly is Gryllus texensis (Orthoptera: Gryllidae). Two larger potential gryllid hosts, Gryllus assimilis and the undescribed G. sp. #45, have been found in the same mating territories as G. texensis and therefore have the potential to be parasitized as well. It is unknown, however, whether O. ochracea can successfully survive in G. assimilis and G. sp. #45 hosts. We manually parasitized G. texensis, G. assimilis, and G. sp. #45 to compare parasitoid survival rates at different stages of parasitoid development. Ormia ochracea had the highest survival rate in males and females of its natural host, G. texensis, survived at about half the rate in female G. sp. #45, and survived at about one quarter the rate in male G. assimilis. No parasitoid flies survived through eclosion in male G. sp. #45 or in female G. assimilis. Our results indicate that larvae from this population of O. ochracea show highest survival in the natural host and show limited survival in other potential host species.

Key Words: parasite; specificity; host switching; Gryllus

RESUMEN

La mosca parasitoide mosca, Ormia ochracea, acústicamente acecha los machos de grillos de campo por sus señales y pone larvas vivas en y alrededor de ellos. Se ha observado que Ormia ochracea acústicamente acecha una variedad de especies de grillos. En Texas, el hospedero natural de esta mosca es Gryllus texensis. Se han encontrado dos especies mas grandes de Gryllidae que son hospederos potenciales, G. assimilis y G. sp. # 45 (una especie no descrita), en el mismo territorio de apareamiento que G. texensis, y por lo tanto tienen el potencial de ser parasitados también. Se desconoce, sin embargo, si O. ochracea puede sobrevivir con éxito en los hospederos G. assimilis y G. sp. # 45. Parasitamos manualmente individuos de G. texensis, G. assimilis y G. sp. # 45 para comparar la tasa de supervivencia del parasitoide en diferentes etapas de desarrollo del parasitoide. Ormia ochracea tuvo la mayor tasa de supervivencia en machos y hembras de su hospedero natural, G. texensis, sobrevivió como la mitad de esta tasa en hembras en G. sp. # 45, y sobrevivió como una cuarta parte de esta tasa en machos de G. assimilis. No moscas parasitoides sobrevivieron hasta la eclosión en los machos de G. sp. # 45 o en las hembras de G. assimilis. Nuestros resultados indican que las larvas de esta población de O. ochracea muestran una mayor supervivencia en el hospedero natural y muestran supervivencia limitada en otras especies de hospederos potenciales.

Ormia ochracea (Bigot 1889) (Diptera: Tachinidae) is a tachinid parasitoid that exploits multiple host species throughout its geographical range (Gray et al. 2007). Ormia ochracea females are known to parasitize multiple species of field cricket (Orthoptera: Gryllidae) such as Gryllus rubens (Scudder 1902) and G. firmus (Scudder 1902) in Florida (Walker 1986; Walker & Wineritter 1991), G. texensis (Cade & Otte 2000) in Texas (Cade 1975), G. integer (Scudder 1901) and G. lineaticeps (Stål 1858) in California (Cade 1975, Wagner 1996), and Teleogryllus oceanicus (Le Guillou 1841) on some of the Hawaiian islands (Zuk et al. 1993).

Gravid female O. ochracea acoustically locate singing male field crickets and subsequently parasitize them (Cade 1975). Once a cricket host is located, the female will oviposit several first instar larvae (planidia) on and around the host (Cade 1975). Planidia will wave their anterior ends in the air and cling to passing crickets resulting in parasitization of both males and females (Adamo et al 1995b). Once inside the host body, planidia lodge themselves into a muscle
capsule (usually the flight muscles) in the thorax of the cricket. Planidia will then migrate to the abdomen; here they grow and feed on the cricket’s fat body and abdominal and thoracic muscles (Adamo et al. 1995a). Parasitized crickets usually initiate an encapsulation response to kill the planidia (Vinson 1990). However, the hosts’ encapsulation response is co-opted by the parasitoid larvae and used to construct tracheae that connect to the outside of the cricket through a hole in the body wall (Adamo et al. 1995b; Vinson 1990). After 7 to 10 d, the larvae emerge from the host (Adamo et al. 1995a). The emerged larvae then pupate, remaining in the pupal stage for 10-14 d before eclosion to adult flies. Larval emergence usually results in the death of the host cricket (Adamo 1999). In natural populations, parasitized male crickets typically harbor 2 parasitoid fly larvae (Adamo et al. 1995b; Kolluru & Zuk 2001).

During the fall of 2008, while collecting G. texensis (formerly G. integer; Cade & Otte 2000) in the field by acoustically locating calling males, we found that a significant proportion of the crickets collected in the vicinity of calling G. texensis were either G. assimilis (Fabricius 1775), or the here-tofore undescribed species, G. sp. #45 (D. Weismann, pers. comm.). Given that G. assimilis and G. sp. #45 are in the vicinity of calling G. texensis males that attract O. ochracea, they may be at risk of parasitism by O. ochracea because gravid females can parasitize any crickets nearby calling males (Cade 1975). However, it is unknown whether these potential host species are capable of supporting parasitism.

Adamo et al. (1995a) investigated parasitism success rates between species and found that there was no difference in the number of larvae emerging from 2 natural hosts, G. texensis and G. rubens, and a third species that is not parasitized in the wild, G. bimaculatus (De Geer 1773). Adamo et al.’s (1995a) study was primarily focused on the behaviors of the hosts and parasitoids. While it is unknown if G. sp. #45 and G. assimilis are parasitized by O. ochracea in the wild, we tested whether O. ochracea larvae are capable of successfully surviving in these two sympatric potential host species. We compared the rates of parasitoid establishment, emergence, and eclosion in these two potential host species to one of their natural host species, G. texensis.

**Materials and Methods**

Field crickets (G. texensis, G. assimilis, and G. sp. #45) and parasitoid flies (O. ochracea) were collected in Bastrop County, Texas, United States, between 15 and 24 Sep 2008. Crickets were collected at lights in parking lots, or by acoustically locating them in fields. Parasitoid flies were collected using an acoustic sound trap modelled after the Walker (1989) slit-trap design. A compact disc player was placed underneath the trap and broadcast a call at a dominant frequency of 4.6 kHz that had been recorded from a laboratory-reared male G. texensis. The call was broadcast through an amplified speaker (model AMX 18, Radioshack Corp., Fort Worth, Texas) at an intensity of 61dB SPL from 30 cm measured with a digital sound level meter measuring root mean square amplitude (Extech Instruments, Waltham, Massachusetts).

Parasitoid flies and field crickets were brought to our laboratory at Carleton University, Ottawa, Canada. Specimens were housed separately in ventilated plastic bins (61 cm x 40 cm x 42 cm) in a greenhouse with a 12:12 h L:D cycle. The temperature was maintained at $X \pm SE = 26 \pm 2$ °C and crickets were given ad libitum water and food (Tekland Rodent diet 8604, Harlan Laboratories, Madison, Wisconsin). Parasitoid flies were housed in terrariums in a temperature, humidity and light controlled incubator (26 °C, 75% RH, and 14:10 h L:D; see Vincent & Bertram (2009b) for housing and rearing details). Flies were fed ad libitum liquid hummingbird feed (Instant Nectar, Yule-Hyde Associates Co., Brampton, Ontario) at a concentration of 1 mL feeder solution per 7 mL water.

We used third and fourth generation laboratory-reared female O. ochracea to manually parasitize first generation laboratory-reared crickets from all 3 species. The numbers of crickets parasitized (subdivided by species and sex) were: G. assimilis: females = 37, males = 26; G. sp. #45: females = 16, males = 17; G. texensis: females = 24, males = 31. Due to the restriction in the number of flies available at any given time, the parasitisms were carried out in 2 blocks, with roughly equal sex ratios of each cricket species being parasitized in each block. The first block was in Jan 2009 using third-generation gravid female flies ($N = 3$); the second was in Feb 2009 using fourth-generation flies ($N = 2$). Each block contained all 3 cricket species to eliminate effects related to fly fitness. There were no significant differences in parasitism rates between blocks across males and females of each species.

Detailed methods of parasitism can be found in Vincent & Bertram (2009b). Briefly, the abdomen of a gravid female O. ochracea was teased apart exposing planidia in the reproductive tract. Two planidia were transferred to the articular sclerite (soft tissue surrounding wing attachment) located at the anterior end of the thorax of each cricket. We chose this location because we could easily see the planidia enter the cricket; thereby increasing the chances of successful parasitism.

Once parasitized, crickets were housed individually in 500 mL plastic containers, given ad libitum food and water, and checked daily for larval emergence. Upon larval emergence and puation,
pupae were housed in separate compartments in the same conditions as the adult flies. Pupae were checked daily to quantify eclosion success. Following larval emergence and the resulting death of the host, each cricket was frozen for future dissection. If a larva did not emerge after 14 d of parasitism, 2 things could have occurred. First, the larva may have died before it had a chance to fully establish in the cricket host, either of natural causes or as a result of the cricket’s immune response. Second, the larva may have established in the cricket host as evidenced by the presence of a breathing tube only to die of natural causes or succumb to the cricket’s immune response prior to emergence from the host. We therefore dissected all cricket hosts to ascertain whether breathing tubes had been established. The presence of a breathing tube confirmed that the larva did not die prior to establishing in the host (e.g. during parasitization).

We compared parasitoid success across host species and host sex using the following measurements: the total number out of the 2 possible larvae that established, emerged, and eclosed, and the proportion of crickets in which larvae successfully established, emerged, and eclosed. Only crickets that survived beyond the first day following parasitism were included in the study.

All data were analysed using JMP 9.0.0 statistical software (SAS Institute Inc., Cary, North Carolina). We used Chi-Square to test for differences in the proportion of crickets with at least 1 larvae established, emerged, and eclosed. We used analyses of variance (ANOVA) to determine whether cricket species differed and Tukey’s post-hoc analyses to determine differences between species in the number of parasitoids establishing, emerging, and eclosing. We analyzed sex differences within each species separately using ANOVA. We analyzed sexes in separate analyses instead of combining them into a two-way ANOVA. This was done because males are naturally parasitized much more often than females (Walker & Wineriter 1991) and may have an evolved response that differs from that of the females, which may have resulted in a sex by species interaction that would negate our ability to discuss species differences.

RESULTS

Cross Species Comparisons: Females

Proportion of Female Crickets with Parasitoids Established, Emerged, and Eclosed

Species did not differ statistically in the proportion of female crickets with parasitoids that established and emerged ($\chi^2; P > 0.1800$ for both analyses; Table 1). The proportion of females in which at least 1 larva established itself averaged 64% in all species (mean = 0.64; range = 0.59-0.71), with the highest success at establishment observed in G. texensis. The proportion of females from which at least 1 larva emerged averaged 39% for all species (mean = 0.39, range = 0.31-0.54), with the highest emergence success observed in G. texensis. Species differed statistically in the proportion of females with larvae eclosed; no parasitoids eclosed from female G. assimilis, while the proportion of females from which pupae eclosed was 24% in G. sp. #45 and 42% in G. texensis ($\chi^2 = 22.421, df = 2, P = < 0.0001$; Table 1).

Number of Parasitoids Established, Emerged, and Eclosed from Female Crickets

The identity of the host species did not significantly affect the total number of larvae (0, 1 or 2) that established or emerged from female hosts (ANOVA: $P > 0.1400$ for both analyses; Fig. 1, Table 2). However, the identity of the host species significantly affected the total number of pupae eclosed (0, 1 or 2). The number of pupae hosted by female G. texensis that successfully eclosed was greater than female G. assimilis, although not significantly different from G. sp. #45 (ANOVA: F = 10.6076, df = 2, 74, $P < 0.0001$; Fig. 1, Table 2).

Cross Species Comparisons: Males

Proportion of Male Crickets with O. ochracea Parasitoids Established, Emerged, and Eclosed

The Gryllus species differed in the proportion of male crickets with larval establishment ($\chi^2 = 9.340, df = 2, P = 0.0094$; Table 1). Gryllus sp. #45

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Established Larvae</th>
<th>Emerged Larvae</th>
<th>Eclosed Pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. assimilis</td>
<td>Female</td>
<td>0.59</td>
<td>0.32</td>
<td>0</td>
</tr>
<tr>
<td>G. assimilis</td>
<td>Male</td>
<td>0.54</td>
<td>0.35</td>
<td>0.08</td>
</tr>
<tr>
<td>G. sp. #45</td>
<td>Female</td>
<td>0.63</td>
<td>0.31</td>
<td>0.24</td>
</tr>
<tr>
<td>G. sp. #45</td>
<td>Male</td>
<td>0.29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G. texensis</td>
<td>Female</td>
<td>0.71</td>
<td>0.54</td>
<td>0.42</td>
</tr>
<tr>
<td>G. texensis</td>
<td>Male</td>
<td>0.74</td>
<td>0.61</td>
<td>0.35</td>
</tr>
</tbody>
</table>
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had 29% of males with 1 or more larvae established within them, which was a significantly less than both *G. assimilis* and *G. texensis* with 54% and 71%, respectively. The species also differed in the proportion of male crickets from which at least 1 larva emerged ($\chi^2 = 22.421$, df = 2, $P < 0.0001$; Table 1). No larvae emerged from male *G. sp.* #45, whereas in *G. assimilis* and *G. texensis* 35% and 61% of the *O. ochracea* larvae emerged, respectively. *Gryllus assimilis* had 8% of males that had at least 1 pupa eclose successfully, which was significantly smaller than *G. texensis* males with 35% ($\chi^2 = 14.360$, df = 2, $P = 0.0008$; Table 1). Given no parasitoids successfully emerged from *G. sp.* #45, they could not have had any parasites eclosed and were, therefore, excluded from this analysis.

Number of *O. ochracea* Parasitoids Established, Emerged, and Eclosed from Male Crickets

The identity of the host cricket species had a significant effect on the total number of *O. ochracea* larvae that successfully established, emerged, and eclosed from *Gryllus* male hosts (0, 1, or 2). More *O. ochracea* larvae established in male *G. texensis* than in both male *G. assimilis* and *G. sp.* #45 hosts (ANOVA: $F = 8.3256$, df = 2, 67, $P = 0.0006$; Fig. 2, Table 2). More larvae emerged from male *G. texensis* hosts than from males of the other two species (ANOVA: $F = 9.9436$, df = 2, 67, $P = 0.0002$; Fig. 2, Table 2). Also, male *G. texensis* hosted a greater number of pupae that successfully eclosed than male *G. assimilis* and *G. sp.* #45 (ANOVA: $F = 9.4872$, df = 2, 67, $P = 0.0002$; Fig. 2, Table 2).

Cross Sex Comparisons

With the exception that fewer female *G. assimilis* had *O. ochracea* pupae eclose than male *G. assimilis* ($\chi^2 = 1.675$, df = 1, $P = 0.0489$; Table 1), males and females did not differ in the proportion of crickets that had larvae establish, emerge, and eclose in either *G. assimilis* or *G. texensis* ($\chi^2$: all significance values $P > 0.6000$; Table 1). For *G. sp.* #45, the proportion of males and females with at least 1 *O. ochracea* larvae established was not significantly different; however, there were significantly fewer *G. sp.* #45 males (none) to have larvae emerge ($\chi^2 = 7.863$, df = 1, $P = 0.0050$; Table 1) and eclose ($\chi^2 = 6.119$, df = 1, $P = 0.0134$; Table 1) when compared to the proportions of females with emerged, and eclosed larvae.

Table 2. Post-hoc Tukey-Kramer Tests used to determine differences between species in the numbers of parasitoids established, emerged, and eclosed. $P$-values for males are above each diagonal, while those for females below each diagonal.

<table>
<thead>
<tr>
<th></th>
<th><em>G. assimilis</em></th>
<th><em>G. sp #45</em></th>
<th><em>G. texensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. assimilis</em></td>
<td></td>
<td>0.2157</td>
<td>0.0430</td>
</tr>
<tr>
<td><em>G. sp #45</em></td>
<td>0.7207</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. texensis</em></td>
<td>0.4158</td>
<td>0.9566</td>
<td></td>
</tr>
<tr>
<td><em>G. assimilis</em></td>
<td></td>
<td>0.1060</td>
<td>0.0374</td>
</tr>
<tr>
<td><em>G. sp #45</em></td>
<td>0.9052</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. texensis</em></td>
<td>0.1236</td>
<td>0.4476</td>
<td></td>
</tr>
<tr>
<td><em>G. assimilis</em></td>
<td></td>
<td>1.0000</td>
<td>0.0008</td>
</tr>
<tr>
<td><em>G. sp #45</em></td>
<td>0.1581</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td><em>G. texensis</em></td>
<td>&lt;0.0001</td>
<td>0.1169</td>
<td></td>
</tr>
</tbody>
</table>

# parasitoids established

# parasitoids emerged

# parasitoids eclosed

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Number of O. ochracea Parasitoids Established, Emerged, and Eclosed

Males and females did not differ in the number of O. ochracea larvae established, emerged, and eclosed for G. assimilis and G. texensis (ANOVA: P > 0.3700 for all analyses; Figs. 1 and 2). Male G. sp. #45 had significantly fewer larvae establish (ANOVA: f = 7.3482, df = 1, 30, P = 0.0110; Figs. 1 and 2) emerge (ANOVA: f = 6.000, df = 1, 30, P = 0.0204; Figs. 1 and 2) and eclose than females (ANOVA: f = 5.000, df = 1, 30, P = 0.0329; Figs. 1 and 2).

DISCUSSION

We parasitized 3 cricket species and found that larvae of the tachinid parasitoid, O. ochracea, had the greatest success in its natural host, G. texensis. Cricket species was a good predictor of whether a host would experience larval establishment, larval emergence, and pupal eclosion. Cricket species was also a good predictor of the total number (0, 1, or 2) of larvae that would establish, emerge, and eclose from the cricket. Larvae emerged from all species and sex combinations, with the exception of male G. sp. #45. Only 29% of male G. sp. #45 had any signs of larval establishment (Table 1), and the larvae in all 29% died prior to emergence. Parasitoids in the remaining 71% of male G. sp. #45 must have died at an early instar since no evidence of larvae or breathing tubes were found upon dissection. Additionally, even though O. ochracea larvae successfully established in over 50% of both female and male G. assimilis, in none of the females and only in 8% of the males did pupae successfully eclose (Table 1). The greatly reduced emergence and eclosion success of parasitoids hosted within G. assimilis and G. sp. #45 suggest that neither species would be an ideal host for O. ochracea larvae when compared with G. texensis.

It is unknown why no larvae emerged from male G. sp. #45 and no flies eclosed from female G. assimilis. When faced with larval infection, crickets will initiate an immune response to kill the larvae (Vinson 1990). Perhaps the immune response in G. assimilis and G. sp. #45 is capable of successfully encapsulating or otherwise neutralizing the parasitoid larvae, and thereby preventing emergence. Our study did not measure host immune response so the possibility that immune response differences drive the observed variation between species and sexes cannot be excluded. Quantifying the crickets’ immune response in the future may clarify the efficacy of the defense mechanisms used by crickets of different species and sex.

In 3 previous studies unnatural cricket hosts were parasitized with O. ochracea. Our results are somewhat consistent with those of Wineriter and Walker (1990), who parasitized G. rubens, a natural host, and Acheta domesticus, an unnatural host. Wineriter and Walker (1990) found that O. ochracea experienced the highest success in its natural hosts. Conversely, Adamo et al. (1995a) parasitized natural hosts, G. integer and G. rubens, and an unnatural host, G. bimaculatus, and found no significant difference in the number of larvae emerged. Additionally, Vincent and Bertram (2009a) found that rates of establishment, emergence, and eclosion in laboratory reared juvenile G. texensis, an unnatural host instar, were not significantly different than the rates in adults. Our study investigated parasitoid success in two unnatural hosts that occupy the same mating territories as the natural host. These species therefore have the potential to come into contact with O. ochracea and may have developed defenses against parasitism.

In Texas, G. texensis is the only documented natural host of O. ochracea (Gray et al. 2007). Our findings show that in the population studied, O. ochracea experiences limited survival in some unnatural host species. Given the dramatically reduced offspring survival rates compared with those in G. texensis, O. ochracea should be under strong selection to avoid parasitizing G. assimilis and G. sp. #45. Ormia ochracea is capable of behavioral host specificity (Walker 1993; Gray et al. 2007), but it is unknown whether O. ochracea in Texas are behaviorally avoiding parasitizing G. assimilis and G. sp. #45. During and parasitoid fly collections in 2008 in Texas, we tested fly call preferences by using calls of G. assimilis and G. sp. #45 along with the standard calls of G. texensis. Gryllus texensis broadcasts attracted 13-
124 flies per 2 h, *G. assimilis* broadcasts attracted 1-7 flies per 2 h, while *G. sp. #45* failed to attract any flies at all. While our sample sizes were small (three 2-h sessions per species), our anecdotal observations suggest that *O. ochracea* in Bastrop, Texas, display some degree of behavioral host specificity. Our results agree with those of Gray et al. (2007) who also found that *O. ochracea* flies in Texas prefer the calls of their local natural host, *G. texensis*.

Even though calling males are the target host of *O. ochracea*, we found that larvae would typically experience success in both males and females. Walker & Wineriter (1991) found that levels of parasitism in Florida between natural and unnatural cricket hosts, and between male and female hosts, were similar. Since *O. ochracea* lay live larvae around calling males (Cade 1975), all individuals in the vicinity of calling males are at risk. Several authors have reported finding female crickets infected with *O. ochracea* larvae in the wild (Walker & Wineriter 1991, Zuk et al. 1993, Adamo et al. 1995a). The population of *O. ochracea* that we studied might therefore benefit from depositing planidia around calling males with the hopes of parasitizing females as well. With changes in ecosystems, climate cycles, and the extirpation and introduction of species, it is of vital importance to study not only the natural hosts of parasites and pathogens, but the potential hosts as well. Studying potential hosts of *O. ochracea* has implications for host switching and the evolution of this host-parasite system in general. We provide evidence that in this population, *O. ochracea* displays limited success in potential hosts, *G. assimilis* and *G. sp. #45*, when compared with its natural host, *G. texensis*.

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