The effect of the age of the serpentine leafminer *Liriomyza trifolii* (Diptera: Agromyzidae) on parasitism by the parasitoid wasp *Gronotoma micromorpha* (Hymenoptera: Figitidae: Eucoilinae)

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Key words. Host suitability, host acceptance, ovipositional preference, host feeding, host stinging

Abstract. *Gronotoma micromorpha* is a solitary egg-pupal and larval-pupal parasitoid. In a previous study, the developmental time of this parasitoid decreased with the age of its leaf miner host, *Liriomyza trifolii*, being significantly longer when the host was 0-day old (= 0–24 h old egg) than when 4 days old (mature larva) at the time of parasitization at 25°C. In the present study, the suitability and acceptability of 0 and 4 day old *L. trifolii* as hosts for *G. micromorpha*, and the ovipositional preference of this parasitoid for these two developmental stages of the host were examined. No significant difference was found in the size of the parasitoid offspring that emerged from hosts parasitized at these two developmental stages. There was no significant difference in the acceptability of the two developmental stages of *L. trifolii* in no choice tests, but 4 day old larvae were preferred to 0 day old eggs in choice tests. Moreover, parasitization of eggs by *G. micromorpha* did not appear to result in more of the eggs dying before they hatched. Non-reproductive killing (host feeding and host stinging without oviposition) of host eggs by this parasitoid was also not detected.

INTRODUCTION

A female parasitoid can influence the fitness of her offspring by her choice of a host, since the quality of the host can affect offspring fitness (Luck & Nunney, 1999). Since parasitoid females appear to select hosts according to the effect they have on their offspring's fitness, the ovipositional preference of the parasitoid is thought to be correlated with the suitability of hosts for juvenile parasitoid development (Hopper & King, 1984; Brodeur et al., 1998; Neveu et al., 2000). Host suitability is an important determinant of successful parasitism by parasitoids (Doutt, 1959; Vinson & Iwantsch, 1980) and the age of a host influences its suitability (Vinson, 1976; Godfray, 1994). Vinson (1998) pointed out that, compared to idiobionts, koinobionts attack a wider variety of host ages, because after oviposition the hosts are able to grow sufficiently to provide for the parasitoids' development. However, even in koinobionts the age of the host influences host suitability (Godfray, 1994). The relationship between parasitoid fitness and the age or size of its host is described for some koinobiont parasitoid species (e.g., Nicol & Mackauer, 1999; Harvey et al., 2000; Chau & Mackauer, 2001; Colinet et al., 2005).

Larval-pupal parasitism occurs in the subfamily Eucoilinae (Figitidae) and egg-pupal parasitism in *Gronotoma micromorpha* (Perkins) (Abe, 2001). Egg-pupal parasitism is restricted to some species of Braconidae and a few species of Ichneumonidae (Gauld & Bolton, 1988; Quicke, 1997). To my knowledge *G. micromorpha* is the only hymenopterous parasitoid with both modes of parasitism (Abe, 2001). The serpentine leafminer, *Liriomyza trifolii* (Burgess), develops from egg to mature larva in 4 days, and the age of the host at the onset of parasitism

does not significantly influence the percentage of *G. micromorpha* offspring that survive to the adult stage. However, the developmental time of the parasitoid decreases with increase in host age, and is more than 1.5 days shorter when *L. trifolii* are parasitized when 3 to 4 days old compared to 0 day old at 25°C (Abe, 2001). Five different relationships between parasitoid development time and host age at the commencement of parasitism are recognized, and the negative relationship reported for *G. micromorpha* parasitizing *L. trifolii* occurs in some other parasitoids (Colinet et al., 2005). In another eucoilid, *Trybliographa rapae*, which can successfully parasitize all larval stages of its host, the first instar larvae of the parasitoid remain at this stage until the host pupates (Wishart & Monteith, 1954). The longer developmental time of *G. micromorpha* when it parasitizes the eggs of its host may be due to prolongation of the time spent in the first instar larva as in *T. rapae*. Although body size and developmental time (or development rate) are considered to be primary indicators of fitness in parasitoids (Roitberg et al., 2001), the latter may not be as good as an indicator as the former in *G. micromorpha*.

Gronotoma micromorpha is a solitary parasitoid, which reproduces thelytokously because it is infected with *Wolbachia* (Arakaki et al., 2001). This parasitoid occurs in subtropical regions, i.e., Okinawa, Indonesia, Florida, Hawaii, Guam and Tahiti (Yoshimoto, 1963; Beardsley, 1988; Rauf et al., 2000; Abe & Konishi, 2004). On Okinawa and Guam, *G. micromorpha* appears to be the dominant parasitoid of *L. trifolii* (Johnson, 1993; Konishi, 1998) and in Indonesia is an important parasitoid of the pea leafminer *L. huidobrensis* (Blanchard) (Rauf et al., 2000; Prijono et al., 2004). Under laboratory conditions,

G. micromorpha has a high net reproductive rate when it parasitizes *L. trifolii* (Abe & Tahara, 2003) and the tomato leafminer *L. bryoniae* (Kaltenbach) is also a suitable host for this parasitoid (Abe, 2006). Thus, *G. micromorpha* may be a useful biological control agent of some pest species of *Liriomyza*.

A previous study (Abe, 2001) indicates that a mature larva of *L. trifolii* is more suitable than an egg as a host for *G. micromorpha*, based on the developmental time of the parasitoid's offspring. The present study compared the size of *G. micromorpha* offspring emerging from *L. trifolii* parasitized at the egg and mature larval stages, because body size is another important index of parasitoid fitness. To examine egg-pupal and larval-pupal parasitism in *G. micromorpha* in more detail, host acceptance (in no-choice tests) and ovipositional preference (in choice tests) of this parasitoid were assessed in the laboratory. The tiny and fragile eggs of the host are susceptible to injuries caused by oviposition, host feeding and host stinging without oviposition. Thus, the survival to hatching of eggs exposed to parasitism was determined.

MATERIAL AND METHODS

General methods

All insects were maintained and experiments conducted at 25°C under a 15L: 9D photoperiod. A laboratory culture of *G. micromorpha* was established using adults that emerged from leafminer infested kidney bean, *Phaseolus vulgaris* L., leaves collected at Itoman, Okinawa Prefecture, in April 1998. The culture of the parasitoid was maintained on *L. trifolii* larvae. A laboratory culture of *L. trifolii* was established using larvae collected at Itoman in April 1998. *Phaseolus vulgaris* was used as the host plant for maintaining the leafminer colony and for experiments (for rearing details, see Abe, 2001).

The base of the stem of one or two host plants infested with 0 day old (0–24 h old) or 4 day old *L. trifolii* was immersed in water in a 10-ml glass vial placed inside and at the bottom of a cylindrical glass tube (6.4 cm diameter, 22 cm high). The top of the glass tube was covered with organdy and the bottom with Kim-wipe®. Undiluted honey was streaked on the inside of the glass tube as a source of food for the wasps. Immediately after, one naive (0–24 h old, no oviposition experience) female *G. micromorpha* was released into the tube and allowed to parasitize the host insects for 24 h. Preliminary observations (Abe, 2001) indicate that the eggs of *L. trifolii* hatch after approximately 48 h and the larvae take 2 to 3 days to mature.

Emergent parasitoid body size and host age

To compare the suitability of 0 and 4 day old *L. trifolii* as hosts the sizes of the parasitoids that developed in these hosts were determined. One *G. micromorpha* female was provided with more than 40 either 0 or 4 day old *L. trifolii* on a host plant. These hosts were reared until wasps emerged. Each host stage trial was replicated 20 times. The hind tibial length of one wasp, randomly selected from each replicate, was measured under a binocular stereomicroscope using an ocular micrometer.

Host acceptance

To compare the host acceptance of 0 and 4 day old *L. trifolii* by *G. micromorpha*, each female parasitoid was provided with either sufficient (40 and above) 0 or 4 day old hosts infesting the first two true leaves of a host plant. The mean (± SE) numbers of 0 and 4 day old hosts were similar, 57.0 ± 2.7 and 54.5 ± 2.8, respectively (two-tailed Student's t-test, t = –0.643, d.f. = 38,

P > 0.05). There were a total of 20 trials for each host stage. The 0 day old hosts were reared for three days after removal of the wasp from the tube. They were then transferred from the leaves into Ringer's solution and dissected with minute pins under a binocular stereomicroscope to determine whether they were parasitized. The 4 day old hosts were transferred from the leaves into Ringer's solution and dissected shortly after removal of the wasp from the tube.

Ovipositional preference

To assess whether *G. micromorpha* preferred to oviposit in 0 or 4 day old *L. trifolii*, each female parasitoid was provided with 0 day old *L. trifolii* on a leaf of one host plant and 4 day old *L. trifolii* on a leaf of another host plant. The parasitism of these *L. trifolii* hosts by *G. micromorpha* was determined as in the host acceptance experiment. There were 20 replicates of each host stage.

Data analysis

A two-tailed Student's t-test was used to detect differences in the hind tibial length of *G. micromorpha* offspring that emerged from the two host groups. The influence of the age of the host on the percentage parasitized by *G. micromorpha* was analyzed using two-tailed Student's t-tests after arcsine transformation of the data. Ovipositional preference of *G. micromorpha* was analyzed by paired two-tailed Student's t-test after weighting data for proportional availability of hosts, as there were different numbers of hosts available to the parasitoids (eggs: 18–44, mature larvae: 12–30). The weighting was done as follows: the number of *L. trifolii* eggs parasitized was divided by eggs / (eggs + mature larvae); the number of *L. trifolii* mature larvae parasitized was divided by mature larvae / (eggs + mature larvae). The significance level for all statistical tests was set at P = 0.05.

RESULTS

Effect of host age on the body size of the adult parasitoid

There was no significant difference between the mean (± SE) hind tibial length of the *G. micromorpha* offspring from hosts parasitized when 0 (0.399 ± 0.005 mm) and 4 days old (0.387 ± 0.007 mm) (two-tailed Student's t-test, t = –1.387, d.f. = 38, P > 0.05).

Host acceptance

The mean (± 95% confidence interval) percentages of 0 and 4 day old *L. trifolii* parasitized by *G. micromorpha* were 42.6 ± 7.1 and 45.2 ± 6.1%, respectively. There was no significant difference in the percentage parasitism of these two host stages of *L. trifolii* (two-tailed Student's t-test, t = –0.532, d.f. = 38, P > 0.05).

Ovipositional preference

The mean (± SE) weighted numbers of the 0 and 4 day old *L. trifolii* parasitized by *G. micromorpha* were 18.9 ± 1.8 and 27.5 ± 2.2, respectively. The latter host stage was parasitized significantly more than the former (paired two-tailed Student's t-test, t = –2.507, d.f. = 19, P = 0.02).

Host egg-mortality

In the host acceptance experiment, a total of 105 dead eggs and 16 dead first instar larvae of *L. trifolii* were found after rearing 1,139 0 day old *L. trifolii* previously exposed to *G. micromorpha*. The 105 dead host eggs con-

tained no *G. micromorpha* eggs or larvae, but 2 of the 16 dead first instar host larvae contained parasitoid eggs. Of the 1,139 0 day old *L. trifolii*, 472 were parasitized. Thus, the percentage of the parasitized *L. trifolii* eggs that hatched was 100%, and it was inferred that 84.3% of those that were not parasitized hatched. In the oviposi-tional preference experiment, a total of 62 dead eggs and 6 dead first instar larvae of *L. trifolii* were found after rearing 568 0 day old *L. trifolii* previously exposed to *G. micromorpha*. Two of the 62 dead *L. trifolii* eggs con-tained parasitoid eggs, but the 6 dead first instar larvae did not. Of the 568 0 day old *L. trifolii* 209 were parasi-tized. Thus, the percentage of parasitized *L. trifolii* eggs that hatched was inferred to be 99%, and that of unparasi-tized eggs 83.3%.

DISCUSSION

No significant difference was detected in the hind tibial length of female *G. micromorpha* that resulted from egg-pupal and larval-pupal parasitism. Large female parasi-toids are generally more fecund than small ones (Hurlbutt, 1987; King, 1987; Rosenheim & Rosen, 1991; Visser, 1994; Vet et al., 1994; Kazmer & Luck, 1995; West et al., 1996; Bennett & Hoffmann, 1998; Ellers et al., 1998; Luck & Nunney, 1999; Harvey et al., 2000). Thus, the present results indicate that eggs and mature larvae of *L. trifolii* are equally suitable hosts for the development of the offspring of this parasitoid. However, these results are not consistent with a previous study (Abe, 2001), which showed that the developmental time of this parasitoid was significantly shorter when *L. trifolii* was parasitized at the mature larval stage compared to the egg stage, indicating that the mature larva is a more suit-able host than the egg (Abe, 2001). In many cases, the developmental time of koinobiont parasitoid offspring decreases when the host is parasitized at a later develop-mental stage (e.g., Neveu et al., 2000). As shown in *T. rapae* by Wishart & Monteith (1954), *G. micromorpha* probably delays its development when it parasitizes host eggs in order to allow the host to grow larger and thus provide more resources. Therefore, developmental time may not be a good means of evaluating fitness in *G. micromorpha*. The age of *L. trifolii* at the time of oviposi-tion did not significantly influence the percentage emer-gence of *G. micromorpha* adults from *L. trifolii* (Abe, 2001). Percentage emergence is a combination of host acceptability and offspring survival. In the present study, there was no significant difference in the acceptability of eggs and mature larvae of *L. trifolii* in the no-choice tests. Hence, the offspring survival of *G. micromorpha* is inde-pendent of whether it oviposits in eggs or mature larvae of *L. trifolii*. Body size and developmental time are the primary traits used to evaluate fitness of parasitoids, and offspring survival is a secondary trait (Roitberg et al., 2001). These biological traits are affected by the host instar parasitized (Hopper & King, 1984). In *G. micro-morpha*, however, the relationships between develop-mental time, the other two traits and fitness differ. Thus,

it is better to use more than one trait when evaluating parasitoid fitness.

It is predicted that foraging female parasitoids that encounter hosts of different stages should select the most suitable. In terms of developmental time (development rate), mature larvae of *L. trifolii* are more suitable than eggs for *G. micromorpha* (Abe, 2001), although in terms of offspring body size or survival (present study) they are equally suitable. *G. micromorpha* exhibited a significant preference for mature larvae of *L. trifolii*. Compared to larvae, the eggs of *L. trifolii* are motionless, small and concealed in leaf tissue. Thus, it may be more difficult for a parasitoid to recognize and subsequently utilize eggs than larvae. This may account for why this parasitoid pre-fers to oviposit in larvae rather than the eggs of its host.

Egg-pupal parasitism is a rare case among parasitoids (Gauld & Bolton, 1988; Quicke, 1997), presumably because it is difficult for an immature parasitoid to sur-vive the many host stage changes from egg to pupa. As mentioned above, the eggs of *L. trifolii* are less con-spicuous than the larvae. The difficulty of locating eggs may be an additional constraint on the evolution of egg-pupal parasitism in hymenopterous parasitoids.

Almost all of the *L. trifolii* eggs that were parasitized by *G. micromorpha* hatched. As in other egg-pupal parasi-toids (Clausen, 1954; Quicke, 1997), it is thought that *G. micromorpha* oviposits in host eggs that are at an advanced stage of development. In a previous study, 82.5% of the eggs of *L. trifolii* that had not been exposed to parasitoids hatched under the same conditions as used in the present study (Tokumaru & Abe, 2003). The per-centage of unexposed and exposed eggs of *L. trifolii* that hatched appears to be almost the same. Therefore, the eggs of *L. trifolii* that are parasitized by *G. micromorpha* are not less likely to hatch. Non-reproductive killing of host eggs by this parasitoid was not detected. Embryonic development probably did not occur in those eggs of *L. trifolii* that did not hatch, and *G. micromorpha* females may not oviposit in such eggs. Further study is needed to determine how *G. micromorpha* females detect whether the eggs of *L. trifolii* are viable.

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