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Hyperparasitism by an exotic autoparasitoid: secondary host selection and the window of vulnerability of conspecific and native heterospecific hosts

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Abstract

Encarsia transvena is an 'autoparasitoid' in the hymenopteran family Aphelinidae. In this species, female eggs are laid in whitefly nymphs. Male eggs are laid externally on immature parasitoids enclosed within the whitefly integument, either their own species, or other primary parasitoids. We explored parasitism by *E. transvena* of conspecific female immatures and those of a native primary parasitoid, *Eretmocerus eremicus*, in laboratory experiments. In the first experiment, female *E. transvena* were offered different combinations of two stages of *E. transvena* (late larvae – prepupae (ET2), and early pupae (ET3)), and one stage of *E. eremicus* (prepupae – early pupae (EE2)) in paired choice tests. The results indicated very little parasitism of ET3 relative to the host it was paired with, either EE2 or ET2. However, when EE2 was offered with ET2, there was no statistically significant difference in parasitism. In a no-choice experiment in which oviposition patterns and male progeny development were examined in four stages of both species of wasp, clear differences were observed between the host species. Only one stage of *E. transvena* (ET2) was parasitized and supported development of male *E. transvena* to any significant degree. In contrast, in *E. eremicus*, EE2, EE3 (red-eyed pupae), and EE4 (late pupae) were all parasitized, and male *E. transvena* emerged from all three stages, although fewer males emerged from EE4. In both species, wasp larvae that were still enclosed within the wet whitefly remains (ET1 and EE1) were parasitized at a very low rate. Lastly, an experiment that determined the length of the later developmental stages of *E. transvena* and *E. eremicus* suggested that the duration of the period in which *E. transvena* is susceptible to parasitism by conspecific females is less than half the period of susceptibility of *E. eremicus*. These results taken together suggest the potential for interference of *E. eremicus* by *E. transvena*, but other factors not examined here may also influence the outcome of interactions in the field.

Introduction

Trophic relationships have long been used to guide introductions of parasitoids for classical biological control. Obligate secondary parasitoids (hyperparasitoids) have been excluded from introductions because of their potential to suppress populations of primary parasitoids (Smith, 1916; Huffaker et al., 1976; Luck et al., 1981). It is common, however, to introduce several potential competitors on the same trophic level. The more contentious principle here is that interspecific competition between primary parasitoids does not disrupt biological control (Turnbull & Chant, 1961; DeBach, 1966; Huffaker *et al*., 1976; Force, 1985). The historical record of biological control has suggested that even when one parasitoid species displaces or excludes another, the pest population equilibrium is reduced (Luck & Podoler, 1985; Rose & DeBach, 1991–1992) but some theory suggests an increased pest equilibrium may result under some circumstances (Briggs et al., 1993; Murdoch et al., 1996).

These principles give little guidance, however, when it comes to autoparasitoids, also known as heteronomous hyperparasitoids, or adelphoparasitoids (Walter, 1983; Williams & Polaszek, 1995). In these species, females develop as primary parasitoids on homopteran hosts (here called *primary hosts*), and males develop as obligate hyperparasitoids, either on females

of their own species or on other primary parasitoids (*secondary hosts*). Autoparasitoids have been used for classical biological control, and have been credited with success in several projects including the olive scale, the citrus whitefly, and the citrus blackfly, but their introduction has at times been considered with misgiving (Rosen, 1981; Mills & Gutierrez, 1996).

Theoretical explorations of the effects of autoparasitoid biology on host-parasitoid population dynamics have been ambiguous. Hassell et al. (1983) used a modified Nicholson–Bailey model which predicted that the density-dependent mortality of female autoparasitoids may be strongly stabilizing, and these authors suggested that this attribute may contribute to long term biological control success. In contrast, a simulation model developed by Mills & Gutierrez (1996) predicted that in some circumstances the host density may be suppressed more strongly with a primary parasitoid alone than with both a primary and an autoparasitoid. One might note that although both the assumptions and conclusions of the authors differed with respect to the role of autoparasitoids in biological control, the almost universal tradeoff between stability and pest equilibrium in models (Murdoch, 1990) prompts the speculation that if they had asked the same question, both sets of authors might have reached similar conclusions.

As one might expect, one of the key features in the Mills & Gutierrez (1996) model is whether the autoparasitoid attacks both conspecific and heterospecific hosts to produce males. If the autoparasitoid attacks only developing conspecifics, density-dependent hyperparasitism acts only within the autoparasitoid population, and the two parasitoids together may drive the pest equilibrium to an even lower level than when the two species act alone. Disruption is predicted to occur when the autoparasitoid females oviposit in both the primary parasitoids and the conspecific autoparasitoids at equal rates. Although not investigated by Mills & Gutierrez (1996), one might also expect even greater disruption when the autoparasitoid prefers the heterospecific hosts. More recent explorations of stage-structured models of autoparasitoid – primary parasitoid dynamics predict that not only the autoparasitoid rates of attack on both species, but also the duration of their window of vulnerability is likely to be important in determining the outcome of the interaction (C. J. Briggs & T. R. Collier, unpubl.)

In this paper we explore parasitism by an exotic autoparasitoid *Encarsia transvena* (Timberlake) of conspecific females and a native primary parasitoid, *Eretmocerus eremicus* Rose & Zolnerowich. We examine whether *E. transvena* females exhibit an oviposition preference among two stages of conspecific hosts and one stage of *E. eremicus* in paired choice tests. We then investigate the suitability of four stages of both parasitoid species for oviposition and development of male *E. transvena* eggs. Lastly, we determine the duration of the vulnerable stages of the two species in order to compare their vulnerability to parasitism by *E. transvena*.

The context of these experiments is an ongoing classical biological control program for the sweetpotato whitefly in the southern and western regions of the United States. *Eretmocerus eremicus* is native to the southwestern U.S. (Rose & Zolnerowich, 1997), and is the dominant native parasitoid attacking the sweetpotato whitefly *Bemisia tabaci* (Gennadius) (biotype B, =*B. argentifolii* Bellows & Perring). It also attacks the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood), the banded wing whitefly, *T. abutilonea* (Haldeman) and the iris whitefly *Aleyrodes spiraeoides* Quaintance (Gerling, 1966). The population of *E. transvena* used in these experiments was collected in Murcia, Spain, and cultured at the USDA/APHIS Mission Biological Control Laboratory before being widely released in Texas, Arizona and California. At least one and possibly two populations of *E. transvena* appear to be establishing in the Imperial Valley of California (Roltsch et al., 1998). It is therefore of interest to understand some of the potential interactions between these species that are now sympatric in agricultural areas in California.

Materials and methods

Experiment 1. Preference tests for oviposition of male eggs by E. transvena. This experiment was designed to examine the influence of stage and species on oviposition of male eggs by *E. transvena*. While early pupal parasitoids have been found to be the most suitable for development of male *Encarsia* in some species (Wilk & Kitayama, 1981; Hunter, 1989; Avilla et al., 1991; Williams, 1991; Pedata & Hunter, 1996), *E. transvena* has a melanized pupal sheath. Preliminary observations suggested that melanized pupae of another species, *E. meritoria*, may be relatively impervious to parasitism by the autoparasitoid *E. pergandiella* (Pedata & Hunter, 1996), but little is known about the details of oviposition and development on these different stages in either species. For this reason, two

developmental intervals of *E. transvena* were examined in the preference tests. One of the stages used was early pupae, in which the pupal sheath was deposited within 48 h of the experiment (designated ET3). We also used the stage immediately preceding pupation, when the wasp immature had finished consuming the whitefly and was in a dry environment enclosed only by the whitefly integument; these were late larval and prepupal stages (ET2). We chose these stages because earlier work suggested that *E. transvena* females do not oviposit their ectoparasitic male eggs until the wasp immatures are in a dry environment (Gerling, 1983). In *E. eremicus*, only one developmental interval was used: the prepupal and early pupal immature stages that directly followed the consumption of the host but preceded pigmentation of the eyes (EE2). The three treatments were simply the three pairwise combinations of the three host types.

In order to produce parasitoid immatures for the experiments, green bean seedlings (*Phaseolus vulgaris* L.) were placed in a greenhouse whitefly (*Trialeurodes vaporariorum*) culture cage to be infested with whiteflies for 2–3 days. We used the greenhouse whitefly in these experiments rather than the sweetpotato whitefly because its larger size meant that wasp hosts were slightly larger and more easily manipulated experimentally. Infested plants were moved to a walk-in environmental chamber and incubated at 27 °C (16 h daylength, 35% r.h.). Individual cages were placed over plants and either *E. eremicus* or *E. transvena* adults were introduced 12–14 days following the initial whitefly infestation. Forty eight to 72 h following adult introduction, all of the plants were fumigated for 2 h with a dichlorvos strip to kill adult wasps. This treatment was necessary in the case of *E. transvena* to prevent parasitism of the experimental hosts by the parental females, and was performed on *E. eremicus* plants to remove potential confounding effects. Plants were returned to the environmental chamber and incubated until the immature wasps reached the appropriate stages for use in the experiment.

Experimental females of *E. transvena* were collected at 24 h intervals from a leaf bearing pupae, and were held in a honey-streaked test tube with males overnight before they were used in the experimental arenas. The arenas were 35 mm Petri dishes, lined with moist filter paper, containing 10 leaf disks each bearing one host. Five hosts of each of the two types were presented. One female was introduced to each arena for 24 h and then removed. The arenas were then refrigerated until the hosts could be dissected to look for eggs. Dissections were performed in a drop of saline under a dissecting microscope. The externally-laid male *E. transvena* eggs are sticky and were generally found attached to either the whitefly cuticle or the host.

The analysis of the secondary host choice experiment was performed using a generalized linear modeling technique available in the statistical package GLIM (McCullagh & Nelder, 1983; Crawley, 1993). This technique is especially valuable for proportional data with binomial error distributions because it allows such data to be analyzed without transformation. For each paired choice test, an analysis was performed on the raw data, with the number of hosts parasitized of a given type (e.g. parasitized ET2) as the response variable, and the total number of hosts parasitized (e.g. parasitized ET2 + ET3) as the binomial denominator. A 95% binomial confidence interval was calculated for each mean proportion. A confidence interval that did not include 0.50 indicated that the two hosts were parasitized at significantly different frequencies. The total number of hosts parasitized was also compared among the three treatments, using an analysis of deviance for data with Poisson error distributions, also in GLIM. GLIM uses maximum likelihood techniques to produce a model that best describes the data. For data with normal errors, the analysis of deviance is identical to ANOVA. In models where either binomial or poisson errors are specified, the amount of deviance in the model explained by factors such as host type in this case, is approximately chi-square distributed.

Experiment 2. Suitability of secondary hosts for oviposition and development of male E. transvena. This experiment was designed to measure the acceptability of different host stages of both parasitoid species to ovipositing female *E. transvena* in a no-choice exposure, as well as the developmental success of males in these hosts.

Parasitoid immatures for the experiments were produced as described above. Four different stages of both *E. eremicus* and *E. transvena* were evaluated. Where possible, these were delineated by clear developmental landmarks that could be observed through the whitefly cuticle. EE2, ET2 and ET3 were as described in the previous experiment. The early stages of both *E. eremicus* and *E. transvena* (EE1 and ET1) were those larval stages that were detectable because of the displacement of the whitefly mycetomes, but in which larvae were still enveloped in wet host remains. The third stage of *E. eremicus* was defined as mid-

pupal stage wasps with eye colors that ranged from pale pink to red (EE3). The late pupal *E. eremicus* (EE4) had eye colors that ranged from deep maroon to black. Following the development of a black pupal sheath, the development of *E. transvena* could not be observed directly. For this reason, the early pupal (ET3) and late pupal (ET4) stages of *E. transvena* were distinguished by the amount of time since pupation. Pupae that had deposited their pupal sheath within 48 h of the experiment (at 27 °C) were designated ET3 while those that had pupated between 48 and 96 h prior to the experiment were designated ET4.

Experimental female *E. transvena* were collected as described above (Experiment 1). The females were placed in a Petri dish with abundant whitefly nymphs for 24 h before the experiment, so that they might feed and mature eggs. We gave females an opportunity to feed because we observed relatively low oviposition in the first experiment (see Results), and a subsequent investigation suggested that newly emerged *E. transvena* females may have few mature eggs (T. Collier, unpubl.). Further, these females differed from those of the first experiment in that they were not exposed to males. Because these wasps are haplodiploid and produce males from unfertilized eggs, we did not expect lack of mating to deter females' oviposition of male eggs in the experiment. However, we sought to maximize egg load in these wasps by giving them whitefly hosts on which they could feed but not lay eggs, since only female eggs may develop on these hosts. A preliminary experiment showed unmated *E. transvena* females did not oviposit in whitefly hosts (Hunter & Kelly, unpubl.).

Experimental arenas were set up as described above, each containing ten hosts of a given stage. Two females were introduced to each arena for four h and then removed. The arenas were placed for the following 20 h in an incubator at 25 °C, 65% r.h. Five of the hosts in each arena were then removed individually to 1.2 ml vials and replaced in the incubator to allow the completion of development of the host or male *E. transvena*. The arenas were then refrigerated until the remaining five hosts could be dissected to look for eggs.

The data from the host dissections and host rearings were analysed separately. The number of hosts containing eggs or first instar larvae was compared using an analysis of deviance for data with Poisson error distributions, as described above, with parasitoid host species and stage as factors. From the rearing data, a similar analysis was performed on the number of hosts from which male *E. transvena* emerged. In order to understand the relative impact of attack on the different stages, the proportion of hosts that escaped attack and emerged following exposure to the *E. transvena* females was analysed using binomial errors, as described above. Lastly, male developmental time and size were compared using ANOVA, followed by Tukey's multiple comparisons test (Zar, 1996).

Experiment 3. Duration of the vulnerable stages of the two parasitoid species. Four bean plants were placed in a whitefly culture to be infested for 24 h. After this time, adults were removed, and the plants were placed in individual cages at 27 °C, (35% r.h., 16 h daylength). Thirty female *E. eremicus* and several males were introduced to each of two of the plants 12 days after infestation, when the whiteflies were almost entirely third instar nymphs. Forty *E. transvena* females and a few males were introduced to each of two plants 15 days after infestation, when most of the whiteflies were early fourth instar nymphs. These stages were chosen to reflect the literature that suggests *Eretmocerus* species generally prefer earlier instar whiteflies than do *Encarsia* species (Gerling, 1990). Twenty four h after the wasps were introduced, the plants were fumigated with a dichlorvos strip for 2 h to kill the adult wasps.

Parasitized whiteflies were examined under a dissecting microscope to determine their status. At the first sign of parasitism, plants were checked daily. As each new stage was reached by the developing wasp, a mark was made on the leaf adjacent to the whitefly mummy with a fine-tip marker. The position of the marks relative to the whitefly indicated the stage reached. The color of the marker was used to indicate the date. Thus the data recorded were the dates of transitions between two stages. For *E. transvena* some stages were less than one day, so not all transitions were recorded.

For each parasitoid species, four stages were recorded. For *E. transvena* the stages recorded were (1) larva detectable (2) larva in dry whitefly integument (3) prepupa (meconium cast) and (4) pupa (black pupal sheath). These stages corresponded to those in the previous experiments except that the ET2 stage was divided into two: the stage preceding and following the excretion of the meconium. For *E. eremicus*, the stages recorded were those described in the previous experiment. Thirteen days following *E. transvena* introduction and 17 days following *E. eremicus* introduction, all of the marked pupae were removed from

the leaf on leaf fragments and isolated in 1.2 ml vials marked with the previous stage and date. Vials were checked daily for emergence.

The duration of each stage was calculated by subtracting the dates of the earlier transition from the dates of the later transition. When one transition was missed, as occurred often in *E. transvena*, the recorded interval was divided equally between 2, or in some cases 3 transitions, and these fractions were included in the calculation of the mean duration of each stage. These data give estimates of variance for any particular interval but do not allow the statistical comparison of compound intervals, that is of two or more intervals added together.

We were also interested in the relative amount of variation in total development time between the two parasitoid species because a high degree of variation in development time will effectively widen the window of vulnerability in a species that has any temporal population structure. We used Bartlett's test for homogeneity of variances to make this comparison.

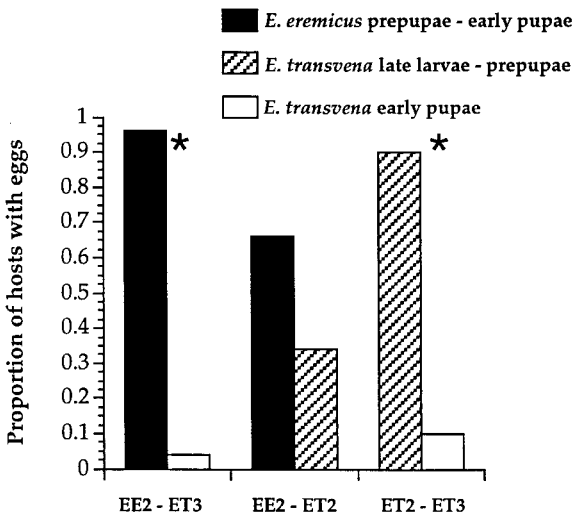

Figure 1. The proportion of hosts of each type containing eggs in three paired choice tests with *Eretmocerus eremicus* prepupae – early pupae (EE2), *Encarsia transvena* late larvae – prepupae (ET2), and *E. transvena* early pupae (ET3). Asterisks indicate statistically significant differences in parasitism of the two host types. N = 15 for each choice test.

Results

Experiment 1. Preference tests for oviposition of male eggs by E. transvena. *Encarsia transvena* females were highly discriminatory when presented with a choice of two host types (Figure 1). When *E. eremicus* (EE2) was presented in combination with the early pupal *E. transvena* (ET3), a mean of 0.96 (95% C.I. = 0.72, 0.99) of the hosts bearing eggs were *E. eremicus*. When late larval or prepupal *E. transvena* hosts (ET2) were presented in combination with early pupal *E. transvena* (ET3), a mean of 0.90 of the hosts bearing eggs were ET2 (95% C.I. = 0.70, 0.97). In contrast, when *E. eremicus* (EE2) were presented in combination with ET2, more of the hosts parasitized were *E. eremicus*, 0.66, but this difference was not statistically significant (95% C.I. = 0.44, 0.79), perhaps because of variation in host preference among the experimental females.

The rate of superparasitism, the proportion of all hosts parasitized that bore more than one egg, appeared slightly higher on ET2 hosts (mean of 0.32 in EE2-ET2, and 0.27 in ET2-ET3) than on EE2 hosts (0.13 in EE2-ET3, 0.17 in EE2-ET2), but an analysis comparing the proportion of superparasitized EE2 with ET2 in the one treatment in which they were paired did not reveal a statistically significant difference (χ^2,1 d.f. = 1.41, P>0.10). Superparasitism did not occur on ET3 hosts.

The mean number of eggs laid in all of the hosts in these arenas was relatively low, between 1.8 ($\pm$ 0.29 SE) in EE2-ET3 and 3.7 ($\pm$ 0.47 SE) in EE2-ET2.The means were marginally significantly different among treatments (χ^2, 2 d.f. = 5.99, P = 0.05); females in the arenas with the two non-melanized host types (EE2 and ET2) tended to lay more eggs overall.

Experiment 2. Suitability of secondary hosts for oviposition and development of male E. transvena. Clear differences in oviposition patterns and developmental success of male progeny were observed when *E. transvena* females were given a no-choice exposure to different host types (Figure 2); both species and stage were statistically significant, as was the interaction between the two (Table 1). Gerling (1983) suggested that *E. transvena* females do not oviposit their ectoparasitic male eggs until the wasp immatures have finished consuming their whitefly host, so the low rate of parasitism of the stages of *E. transvena* and *E. eremicus* that were still in fluid host remains is not surprising. Perhaps the most striking result is that only one of the remaining three stages of *E. transvena* presented appears to be suitable (Figure 2a). In a no-choice exposure, *E. transvena* early pupae are parasitized at a very low rate. No males developed success-

Table 1. Analysis of the host suitability experiment. An analysis of deviance was performed on the no. of hosts parasitized (from the five hosts dissected), and the no. of males produced (from the five hosts reared)

Parameters	Deviance	df	P
No. of hosts parasitized			
Species	7.46	1	<0.001
Stage	26.30	3	<0.001
Species × stage	25.99	3	<0.001
Total deviance	151.79	95	
Residual deviance	92.03	88	
n		96	
No. of males produced			
Species	5.89	1	<0.05
Stage	42.78	3	<0.001
Species × stage	23.49	3	<0.001
Total deviance	131.10	95	
Residual deviance	58.93	88	
n		96	

The analyses assumed Poisson errors, and the deviance is approximately chi-square distributed (see text for details).

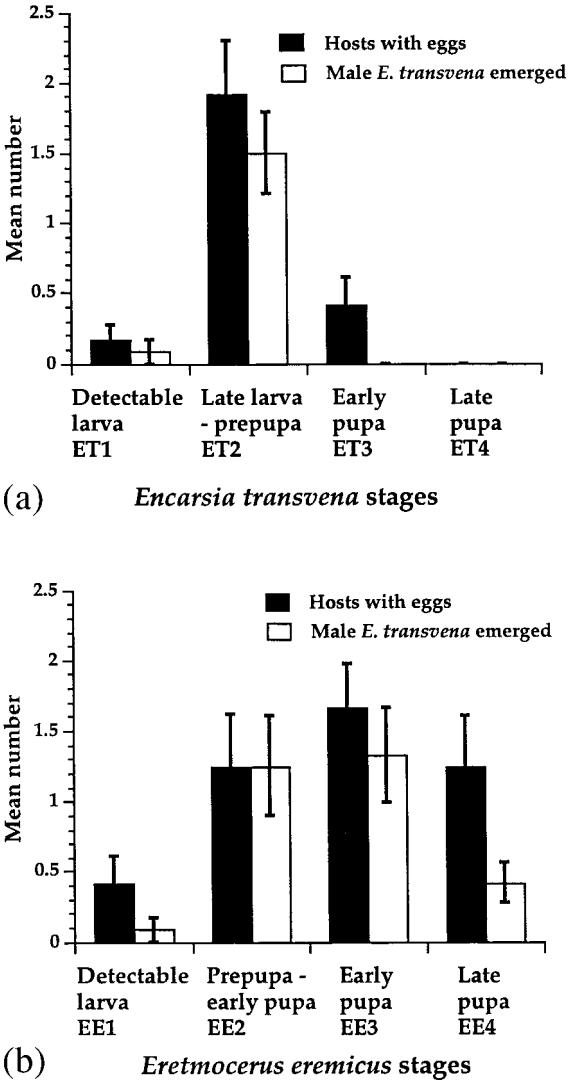

Figure 2. Oviposition and developmental success of male *Encarsia transvena* in four stages of (a) *E. transvena* and (b) *Eretmocerus eremicus*. Ten hosts were provided in each arena. Following exposure to *E. transvena*, 5 of the hosts were dissected for an estimate of oviposition (black bars) and 5 were reared for an estimate of male development (white bars). There were significant differences in both oviposition and developmental success for stage, species and the stage by species interaction. N = 12 for each stage tested. Error bars indicate standard error.

fully on early pupae, but the low rate of oviposition on these hosts makes it difficult to assess developmental success. No eggs were found in dissections of late pupae. In contrast, all of the stages of *E. eremicus* following the point at which the wasp larva is in a dry environment appear to be at least partially suitable (Figure 2b). Eggs were laid in all of these later stages, including the late pupal stage. During one of these dissections, an egg was found on a wasp that was in the process of emerging. Males also emerged from all of the three later stages of *E. eremicus*.

Male development time was significantly different in different hosts (Table 2). This was due to a longer development time of males emerging from late pupal *E. eremicus*; there was no significant difference between the development time on *E. transvena* and either of the two other *E. eremicus* treatments. Males emerging from different treatments also differed significantly in size. The largest wasps emerged from the two *E. eremicus* stages, EE2 and EE3, while the smallest wasps emerged from the late pupal *E. eremicus* hosts and the only suitable *E. transvena* stage, the late larval-prepupal stage.

The proportion of hosts that survived exposure to *E. transvena* females varied with species (χ^2, 1 d.f. = 11.06, $P < 0.001$), stage (χ^2, 3 d.f. = 18.68, $P < 0.001$) and the species by stage interaction (χ^2, 3 d.f. = 13.72, $P < 0.005$) (Figure 3). This was due not only to the greater production of male *E. transvena* in the EE2 and EE3 *E. eremicus* treatments, but to a greater degree of unattributed mortality in the earliest (EE1) and latest (EE4) treatments of this species. The discrepancy between parasitism and successful male emergence in the late pupal treatment (Figure 2b) suggests that in this treatment at least, mortality re-

Table 2. Development time (25 °C) and size of male *E. transvena* emerging from different host types

Host species and stage	Male development time (days) (± SE)[1] *n*	Head width (mm) (± SE)[2] *n*
E. transvena late larva-prepupa (ET2)	12.50 (0.19)a 18	0.164 (0.004)ab 18
E. eremicus prepupa – pupa with colorless eyes (EE2)	12.47 (0.22)a 15	0.175 (0.006)bc 14
E. eremicus pupa with pink – red eyes (EE3)	12.44 (0.25)a 16	0.196 (0.005)c 15
E. eremicus pupa with maroon – black eyes (EE4)	14.40 (0.40)b 5	0.146 (0.003)a 5

[1] $F_{(3,50)} = 7.20$, $P < 0.001$. [2] $F_{(3,48)} = 12.77$, $P < 0.001$. Means followed by a different letter are significantly different (Tukey's multiple comparisons test).

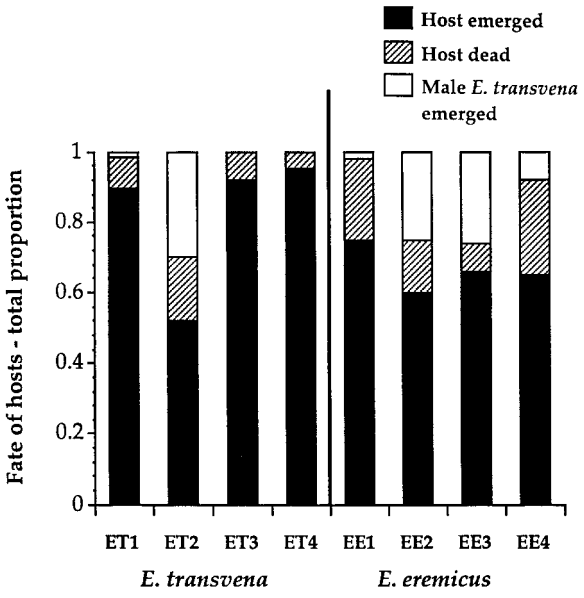

Figure 3. Proportion of all hosts of either *Encarsia transvena* or *Eretmocerus eremicus* that were exposed to *E. transvena* females at different stages and emerged, died, or yielded a male *E. transvena*. See text for description of host stages.

flects unsuccessful parasitism. In contrast, the pupal treatments in *E. transvena* were infrequently parasitized and suffered little from unattributed mortality (Figure 3).

Experiment 3. Duration of the vulnerable stages of the two parasitoid species. The duration of the immature stages of the two wasp species differed mostly in the early stages preceding the first detectable signs of parasitism of the host; *E. eremicus* larvae developed more slowly in these early stages (Table 3). This may have been partially due to the different stages of whiteflies exposed; *E. eremicus* females were introduced to plants bearing third instar *T. vaporariorum* while *E. transvena* females were introduced to fourth instar nymphs. Studies of *Encarsia formosa*Gahan and *Eretmocerus californicus* (=*E. eremicus*) (Rose & Zolnerowich, 1997) indicate that development of these parasitoids does not normally proceed past the first instar until the whitefly has molted into the fourth instar nymph (Gerling, 1966; Nechols & Tauber, 1977). This developmental process is likely to be common across species in these genera, thus wasps that attack earlier stages should have longer development times.

Because *E. transvena* females generally lay external male eggs in a dry environment, a more important comparison might be the development time of immature wasps after the whitefly has been consumed. *Eretmocerus eremicus* actually takes less time to complete development after this point, 5.75 days, compared to 7.4 days for *E. transvena* (Table 3). If both species were equally susceptible during this period, one might expect *E. transvena* to have the marginally wider window of vulnerability. Instead, because of the relatively impervious pupal stage of *E. transvena* the window of vulnerability is restricted to the late larval and prepupal stage, and is only 1.75 days (Table 3), while all of the *E. eremicus* stages following the completion of whitefly consumption may be attacked, and developmental success of males is high when these hosts are prepupae–mid pupae (4.34 days in duration) (Figure 2a, Table 3).

The sex ratio of the 86% of the *E. eremicus* cohort we observed was 0.51 male. Fourteen percent of the *E. eremicus* cohort emerged before they could be isolated and sexed, but observations of the emerging and emerged adults did not suggest a sex ratio bias of these individuals. This sex ratio was not significantly different from the sex ratio of *E. eremicus* emerging following exposure to *E. transvena* in the no-choice experiments, 0.45 (χ^2, 1 d.f. = 1.31, $P > 0.25$). These data suggest that neither sex of *E. eremicus* is preferentially parasitized by female *E. transvena*. The development time of the reared cohort of male and female *E. eremicus* was virtually identical (17.35 d ± 0.09 SE, males; 17.26 d ± 0.08 SE, females).

The variance in total development time for both species was relatively low (Table 3), and there was no statistically significant difference between the variances (χ^2, 1 d.f. = 0.11, $P > 0.75$).

Table 3. Relative duration in days of observer-characterized immature stages of *E. transvena* and *E. eremicus* at 27 °C

Encarsia transvena	Female intro. – detectable larva	Detectable larva – late larva	**Late larva – prepupa**	**Prepupa – early (melanized) pupa**	Early pupa – emergence	Total develop. time
Mean	5.08	1.34	0.87	0.88	5.65	13.95
SE	0.03	0.06	0.03	0.04	0.07	0.08
N	99	94	96	100	96	96
Eretmocerus eremicus	Female intro. – detectable larva	Detectable larva – prepupa	**Prepupa – Pupa with pink eyes**	**Pink-eyed pupa – black-eyed pupa**	Black-eyed pupa – emergence	Total develop. time
Mean	8.72	2.64	2.06	2.28	1.41	17.13
SE	0.05	0.05	0.03	0.03	0.03	0.06
N	215	210	211	202	200	206

Bold face indicates suitability of these stages for production of *E. transvena* males. Stages in bold were oviposited on, and produced greater than an average of one male *E. transvena* per five reared.

Discussion

Secondary host selection. In the choice tests, dramatic differences in parasitism were observed when one of the stages presented was *E. transvena* early pupae (Figure 1); these may be simply explained in light of the suitability studies that indicated extremely low parasitism of pupae in no-choice exposures. Early pupal *E. transvena* are not acceptable hosts for parasitism by conspecific females. In previous studies of secondary host selection and sex allocation behavior by autoparasitoids, early pupal wasps have generally been used as hosts (Hunter, 1989; Avilla et al., 1991; Williams, 1991; Hunter & Godfray, 1995). Further, the presence of a melanized pupal sheath occurs in several species of *Encarsia*, including *E. tricolor* Förster and *E. inaron* (Walker) but does not prevent parasitism of pupae in these species (Avilla et al., 1991; Williams, 1991). However, observations by Pedata & Hunter (1996) suggested that the melanized pupal sheath in *Encarsia meritoria* Gahan deterred parasitism by *Encarsia pergandiella* Howard. The nature of the defense of *E. transvena* pupae against parasitism by conspecific females is unclear. *Encarsia transvena* is unusual among *Encarsia* species (although not among autoparasitoids in general) in having ectoparasitic males (Walter, 1983). One might speculate that ectoparasitic male *E. transvena* larvae may have more difficulty feeding on a melanized host than endoparasitic male *Encarsia* such as *E. tricolor*, but that does not explain why *E. pergandiella*, another species with endoparasitic males, is deterred from parasitizing melanized *E. meritoria* pupae. What was clear in this study was that stage was of critical importance in secondary host selection by *E. transvena*, and that there was no significant preference exhibited when suitable stages of both conspecific and heterospecific hosts were offered (Figure 1).

Although the previous studies of secondary host selection by autoparasitoids have been few, they have suggested that *Encarsia* species prefer to parasitize heterospecific hosts or exhibit no preference. When *E. tricolor* females were presented with choices of conspecific hosts and either *E. inaron* or *E. formosa*, *E. tricolor* females preferred the heterospecific hosts in both cases (Avilla et al., 1991; Williams, 1991). No clear preference in the number of hosts parasitized was seen when *E. pergandiella* was given a choice of conspecifics and either *E. formosa* (Buijs et al., 1981, Pedata & Hunter, 1996) or *E. meritoria*, although more eggs were laid in *E. meritoria* (through superparasitism) than on conspecifics (Pedata & Hunter, 1996). Preference for conspecific hosts by an autoparasitoid has not yet been documented. The hypothesis that has been proposed for this pattern is a kin-selection hypothesis; females ought to prefer to parasitize heterospecific hosts if there is a risk that they may be killing a relative by parasitizing a conspecific (Williams, 1991; Godfray, 1994). No evidence of kin discrimination has yet been discov-

ered (Williams, 1996a), but avoidance of conspecifics may result in greater inclusive fitness of discriminating females. An alternative hypothesis is that immature autoparasitoids may be less vulnerable to parasitism by females of their own species because of selection on immatures to develop resistance to parasitism, particularly to conspecific females. There is the potential for an intraspecific evolutionary arms race in autoparasitoids, with selection for resistance in the immature females and reciprocal selection on adult females and immature males to better circumvent the immature females' defense mechanisms. In such a situation, one might expect to see an asymmetry in the evolutionary contest between immatures and adults, with the 'prey' or immatures being more likely to increase investment in defense than the adult 'predators' are to counter it (Abrams, 1986). These two hypotheses for the avoidance of conspecifics are clearly not exclusive.

Window of vulnerability. In *E. transvena*, adult females have only very brief access to conspecific hosts during the course of their development. Our results suggest that pupae are rarely parasitized, as are larvae that are still in fluid host remains. The window of vulnerability is thus only about $\frac{1}{8}$ of the developmental period. The real window may be even narrower when adult females do not arrest the hosts' development upon oviposition. Casual observation suggests that most, but not all hosts are arrested. If, as we suspect, newly eclosed male larvae have difficulty finding feeding sites on melanized pupae, conspecific hosts that continue development after oviposition by the parasitoid may prove unsuitable by the time the male egg hatches.

The narrow window of vulnerability we observed provides one possible explanation for the observations of extremely female-biased sex ratios of *E. transvena* in the field relative to other autoparasitoid species in the same community (McAuslane et al., 1994). It should be noted, however, that field data on sex ratio of autoparasitoids are generally difficult to interpret without detailed information about relative abundance and vulnerability of all potential primary and secondary host species in the community (Hunter, 1993; Hunter & Godfray, 1995).

The window of vulnerability to parasitism in *E. transvena* is less than half the duration of that in *E. eremicus*, even if the late pupal *E. eremicus* are not considered vulnerable (Figure 2, Table 3). In fact, although few males were produced on late pupal *E. eremicus*, these hosts were parasitized and killed at a comparable rate to the preceding two more suitable stages (Figure 2b, Figure 3). From a population dynamics perspective, what is important is the number of individuals of a given host stage killed, and in this respect late pupae appear as vulnerable to attack by *E. transvena* as early pupae. If one includes the late pupal stage in the window of vulnerability, individuals of *E. eremicus* are susceptible for more than three times as long. However, the mortality patterns observed in these no-choice exposures may well be modified in choice tests or in the field by preference for more suitable stages.

Potential effects on population dynamics. The results of this study suggest the potential for direct interference of *E. eremicus* reproduction by *E. transvena* females. Female *E. transvena* parasitized suitable hosts of both species without exhibiting an apparent preference for either. It is under these conditions that theory predicts that, all else being equal, an autoparasitoid may disrupt biological control (Mills & Gutierrez, 1996) and suppress the primary parasitoid. Further, the results suggest that the window of vulnerability of *E. eremicus* is much wider than that of *E. transvena*, thus reducing the theoretical probability that these two species may coexist, and increasing the probability of disruption (C. J. Briggs & T. R. Collier, unpubl.).

Clearly there are other aspects of the interaction between these two species that may either strengthen or negate the prediction of competitive dominance of the autoparasitoid. Differential abilities to find and successfully convert whitefly hosts to adult parasitoids may influence competitive outcomes between species (Murdoch & Briggs, 1996), as may multiparasitism or selective host feeding on parasitized hosts. The sex allocation behavior of *E. transvena* may also be important if primary and secondary hosts are not always parasitized at a rate equal to their relative abundance (Godfray & Waage, 1990; Hunter & Godfray, 1995). Lastly, the degree of both temporal and spatial overlap of the two species may be critical in determining coexistence (Kakehashi et al., 1984; Comins & Hassell, 1996).

Parasitoid communities that include autoparasitoids are often complex and may be composed of several species with diverse life histories. A review of published examples of these communities suggests that autoparasitoids may be dominant more often than primary parasitoids (Williams, 1996b). However, it is unclear whether this is due solely to their competi-

tive dominance or whether they may simply be more diverse in these communities. In Williams (1996b) review, more of the species with known life histories were autoparasitoids than were primary parasitoids.

In some instances, when the autoparasitoid can attack only conspecific hosts, one would predict that the autoparasitoid would have less of a competitive advantage, and this may promote coexistence. An example is when the competitor to the autoparasitoid is an (ectoparasitic) *Aphytis* species (Williams, 1977). However, other examples exist in which autoparasitoids coexist with primary endoparasitoids that they attack. In a couple of cases, pests have been suppressed below the economic threshold with a parasitoid complex that includes at least one autoparasitoid and a primary parasitoid that is attacked by it (citrus blackfly and citrus whitefly (Rose & DeBach, 1981; Thompson et al., 1987; Argov, 1988)). Lastly, in a greenhouse study that examined the consequences of interspecific interactions of natural enemies on whitefly population densities, no evidence was obtained that the one species of autoparasitoid disrupted control provided by any of the other natural enemies (Heinz & Nelson, 1996).

In conclusion, while there is currently no clear evidence of an autoparasitoid disrupting biological control, no quantitative test of the effects of autoparasitoids on pest population equilibria have yet been conducted in the field. To better determine how to rank prospective whitefly parasitoids in priority for introduction, a more complete understanding of the interactions of these species is required.

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