

Age-related pupal defenses against congeneric internecine activity in *Encarsia* species

Dan Gerling* & Neli Rejouan

Department of Zoology Tel-Aviv University, Ramat Aviv 69978, Israel

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Abstract

We examined whether the young pupae of three parasitoid species, *Encarsia inaron* (Walker), *E. lutea* (Masi), and *E. sophia* (Girault and Dodd) (Hymenoptera: Aphelinidae), all attacking the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), exhibited different susceptibility to host feeding and to autoparasitism than older pupae. These parasitoid species differ as follows: *E. lutea* is autoparasitic and has non-melanized pupae, *E. inaron* is not autoparasitic and has melanized pupae, whereas *E. sophia* is auto-parasitic and has melanized pupae.

The results showed that the younger pupae were always more susceptible to internecine action (host feeding or autoparasitism) than the older pupae. The relative immunity of the older pupae was not dependent upon pupal melanization, as it was the same for pupae of *E. lutea* that have unmelanized pupae as the other melanized species.

The findings increased the range of the 'window of opportunity' for male development discovered by Hunter & Kelly (1998, *Entomologia Experimentalis et Applicata* 89: 249–259) to more *Encarsia* species and to the phenomenon of host feeding, and suggest that relative pupal resistance to damage may be widespread among these parasitic Hymenoptera.

Introduction

Immature parasitoids are subjected to various causes of mortality during their development. These include intrinsic factors that are generated by the host's defense system and extrinsic factors, such as parasitism by hyperparasitoids, and predation of the host, and of the parasitoid that develops therein. In whitefly parasitoids of the genus *Encarsia* Foerster (Hymenoptera Aphelinidae), congeneric females, due to their unique life history, may prey upon a whitefly nymph or hyperparasitise it. Predation through host feeding occurs when the females pierce the cuticle of immature whiteflies to obtain proteins necessary for developing their ovaries and feed on the oozing body fluids, thereby killing the host (van Lenteren et al., 1987). Most species also exhibit heteronomous development of the sexes, with females developing as primary whitefly parasitoids, while the males exhibit autoparasitism, i.e., they develop hyperparasitically upon developing immature whitefly parasitoids of their own species (Gerling, 1990; Williams, 1991; Hunter & Woolley, 2001). Both Gerling (1983) and Hunter & Kelly

(1998) noted that the autoparasitism in *Encarsia sophia* (Girault and Dodd) occurred on developing parasitoids that were mature larvae or in their early pupal stages.

The life cycle of immature *E. sophia* takes roughly 15–20 days at 25–26 °C. Of that time, 40–50% is spent in the pupal stage (Hunter & Kelly, 1998; Rejouan, 2000). Thus, the latter is exposed to the environment for a relatively long period, during which it could be killed by congeneric females searching for hosts, and serve either as food for adult *Encarsia*, or as hosts for male development.

The differential production of the sexes and the susceptibility of *E. sophia* pupae to autoparasitism have been studied by Hunter & Kelly (1998). They reported that the *E. sophia* pupa was susceptible to attack by ovipositing females searching for hosts in which to lay their male progeny only as long as the developing pupae were young, and had not been melanized. Once the *E. sophia* developed a black pupal skin they were no longer susceptible to attack. It therefore appeared that the either the age and/or melanization of the pupa might play a role in defense against oviposition by conspecifics.

It is interesting to determine if the putative defensive properties, such as those shown for *E. sophia* pupae are also shared by other species of *Encarsia*. Indeed, Pedata &

*Correspondence: Tel.: +972 3 640 8611; Fax: +972 3 640 7830 or 9403; E-mail: dangr@post.tau.ac.il

Table 1 Characteristics of the *Encarsia* females used in our experiments

<i>Encarsia</i> species	Melanization	Autoparasitism
<i>E. sophia</i>	+	+
<i>E. inaron</i>	+	–
<i>E. lutea</i>	–	+

Hunter (1996) mentioned that they observed the black pupae of *E. meritoria* (Gahan) to be ‘relatively impervious to *E. pergandiella* (Howard) parasitism’. These findings raise the following questions: does age-related pupal defense extend to other *Encarsia* species? Is it limited to autoparasitic species or does it extend beyond autoparasitism? For example, because both host feeding and autoparasitism require ovipositor punctures, are pupae that are immune to one susceptible to the other? Moreover, is defense necessarily associated with the melanization of the pupae, i.e., are pupae of *Encarsia* species that do not become melanized as protected as melanized ones? We set out to examine these questions using three species of *Encarsia*, each exhibiting a different combination of male development and pupal coloration (Table 1).

Materials and methods

The parasitoids

We chose three species of *Encarsia*, *E. lutea* (Masi), *E. inaron* (Walker), and *E. sophia*. The pupa of each is light colored and has a relatively thin cuticle at pupation. However, the pupal skin of the two latter species becomes melanized during the 2 days following pupation, whereas that of *E. lutea* remains light. Of the two species with melanized pupal skins, *E. inaron* is not autoparasitic and its males and females develop as primary parasitoids of whiteflies, whereas *E. sophia* is autoparasitic. *Encarsia sophia* has been cultured in our laboratory from a stock imported from Florida about 10 years ago. The two other species are native to Israel. All three readily attack the nymphs of *Bemisia tabaci* (Gennadius), which was used as a host. The cultures were each kept in a separate environmental chamber at 26 ± 1 °C, L14:D10, and about 60–70% r.h. All whiteflies were grown on Acala-type cotton plants in the same soil mixture. Parasitoids were collected from the culture by placing the leaves with whitefly pupae in black ‘emergence bottles’, where the emerging adults congregated in a scintillation vial placed at the top (Gerling & Fried, 1997). They were collected from the vials within 24 h following emergence, and either placed on hosts or used in the studies. Whitefly infested cotton plants were obtained by releasing several hundred adult whiteflies onto potted

cotton seedlings with four true leaves each. The whitefly adults were removed 24 h later, and the plants were kept for ca. 10 days until a mixture of 2nd and 3rd instar whitefly nymphs was present on the leaves.

Autoparasitism age in *E. sophia*

Gerling (1983) and Hunter & Kelly (1998) reported that the male-producing eggs were always laid in parasitized hosts in which the developing parasitoids had finished devouring their whitefly host, i.e., where the hosts had reached at least the very late stages of the third instar larva. In contrast, Reese (1994) reported finding male-producing eggs of *E. sophia* present 2–3 days following oviposition of the female-producing eggs. This finding was critical for us, as our experiments included the determination of autoparasitic, male-producing activities. Therefore, we re-examined the possibility that *E. sophia* will produce males by ovipositing in whiteflies that had only been parasitized for 48 h and would contain first instar female larvae. This was done by infesting four leaves of cotton with whiteflies, two leaves each per plant. When these reached the late second instar, each leaf was isolated for 24 h in a leaf cage with about 20 females of *E. sophia*, after which the parasitoid females were removed. Twenty-four hours later (i.e., 48 h after the initiation of the experiment) several females were introduced into two of the leaf cages for 24 h and then removed. The experiment was repeated with the two remaining leaves 4 days later (i.e., with 4–5-day-old *Encarsia* larvae). The leaves were then left to develop within the cages for 26 days, after which the parasitoids that had since emerged and died within the cages were counted and sexed.

Host feeding

Host-feeding studies were conducted in ‘experimental units’ each of which consisted of a Petri dish in which a moist filter paper was placed, together with 30 pieces of cotton leaves each bearing one whitefly immature. We used ‘protein starved’ parasitoid females belonging to the three species. They had been kept for 3 days following emergence in vials with a piece of wet filter paper and honey only. On the fourth day, females were individually introduced into the experimental unit, and kept there for 8 h. Parallel parasitoid-free dishes were kept as controls to determine baseline mortality. Three kinds of hosts were used: unparasitized whitefly 4th instar pharate adult, parasitized whitefly containing ‘young’ (up to 12 h post-pupation), or ‘old’ (ca. 12 days after oviposition and about 2–3 days following pupation) parasitoid pupae. As previously stated, the integument of all pupae was unmelanized in the ‘young’ material, but melanized in ‘old’ *E. inaron* and *E. sophia*, and unmelanized in *E. lutea*. Each

of the nine exposures (three species of parasitoids on three types of hosts) was run in eight replicates. Experimental units for each parasitoid species were always run in units of three, one for each host species, in order to minimize any possible variability caused by environmental conditions. Once the results indicated a refraining from host feeding on old pupae, we conducted qualitative observations on the foraging behavior of protein starved females. In these, we studied whether a lack of host feeding resulted from unsuccessful attempts to penetrate the pupal skin or from refraining to attack these pupae altogether.

Autoparasitism

Host pupae were prepared by exposing whitefly-bearing leaves (3rd instar nymphs) to mated young *Encarsia* females of each of the three species for about 50 h. Leaves were used at two parasitoid ages: 'young' and 'old' pupae as previously defined. On each leaf, we released a 24 h-old virgin female of either *E. sophia* or *E. lutea* for 24 h. A total of 12 combinations was used: two species of autoparasitic females, each on two ages of pupae belonging to each of three parasitoid species. Forty replicates were run of each combination (averaging between 48 and 66 host pupae per leaf). At the same time, we kept an identical leaf to that of each experimental combination as a control, without exposing it to a parasitoid virgin. The differences between the frequencies of attack on the different kinds of pupae were compared using χ^2 -tests.

Results

Results of the autoparasitism experiment upon young larvae of *E. sophia* showed that males may be produced even from just-hatched larval hosts (48 h exposure: leaf 1, 15 ♀ + 7 dead pupae; leaf 2, 1 ♂, 18 ♀ + 4 dead pupae. Five day exposure: leaf 1, 14 ♀, + 8 dead pupae; leaf 2, 1 ♂ + 18 ♀).

Observations of the host-feeding parasitoids showed that they examined the whitefly containing both young and old parasitoid pupae and tried to insert their ovipositors in both groups. The resulting mortality was not significantly different from that in unparasitized hosts and in hosts containing young and/or unmelanized parasitoid pupae, and it always significantly exceeded that of the controls, in which no parasitoid was present (Table 2). There was occasional host feeding in old pupae, and exposure to the parasitoid females resulted in more dead individuals than in the controls, although the difference was not statistically significant (Table 2).

Autoparasitism, i.e., oviposition by virgin females within parasitoid-containing hosts, was performed by the two autoparasitic species on all three species of parasitoids tested. Males emerged from young and old pupae, from pupae that were subjected to heteronomous parasitism, and from the controls. However, significantly more males emerged from the young pupae than from the older ones. This was true for the melanized *E. sophia* and *E. inaron*, as well as for the *E. lutea* pupae that were never melanized (Table 3). The low rate of male production from the material that had been exposed as old pupae did not differ from the rate produced by the controls of both the young and old pupae. In addition to parasitoid production, we also observed the mortality of parasitized whiteflies, which was significantly higher in all six combinations when we exposed young rather than old pupae (Figures 1 and 2).

Discussion

Immature mortality up to ca. 24% was observed in the parasitoid pupae of the controls (Table 2 and Figures 1 and 2). Higher mortality rates were observed for young pupae, while the mortality of old pupae was lower, reaching only 10% or less. The rates of immature mortality in both cases

Table 2 Numbers of pupae failing to emerge to adulthood resulting from 8 h exposure to protein-starved conspecific adult *Encarsia* females. Eight replicates of 30 individual pupae (240 pupae in total) of each host or stage were used

Parasitoid	Host pupae			χ^2 (b vs. c)
	Unpar (a)	Para – young (b)	Para – old (c)	
No exposure	17	33	25	ns
<i>E. sophia</i>	71	88	40	*
χ^2	*	*	ns	
No exposure	18	37	27	ns
<i>E. lutea</i>	73	70	39	*
χ^2	*	*	ns	
No exposure	16	35	26	ns
<i>E. inaron</i>	77	73	44	**
χ^2	*	*	*	

Unpar = unparasitized, Para = parasitized.

*P < 0.05; ** χ^2 = 3.6501, P = 0.053, ns = not significant.

Table 3 χ^2 -values comparing the proportions of parasitoid males emerging from parasitized whiteflies in the autoparasitism experiments. The percentage data are presented in Figures 1 and 2

(A) Young vs. old pupae; comparing the test (with a parasitoid female) vs. the control (without a parasitoid female)

		Host (parasitized whitefly)					
		<i>E. sophia</i>		<i>E. inaron</i>		<i>E. lutea</i>	
Parasitoid		test	control	test	control	test	control
<i>E. sophia</i>	χ^2	78.4966*	0.0121	131.5029*	0.0101	107.7589*	0.3365
<i>E. lutea</i>	χ^2	104.3966*	2.6468	68.1662*	0.0373	109.2649*	1.0854

(B) Pupae that were exposed (test) vs. unexposed (control) to a parasitoid female; comparing young vs. old pupae

		Host (parasitized whitefly)					
		<i>E. sophia</i>		<i>E. inaron</i>		<i>E. lutea</i>	
Parasitoid		young	old	young	old	young	old
<i>E. sophia</i>	χ^2	64.3178*	1.2479	91.8936*	1.323	104.5742*	0.7257
<i>E. lutea</i>	χ^2	144.86*	0.6229	55.4812*	0.561	146.0849*	0.0093

All tests have 1 degree of freedom.

*Refers to a significance level of $P < 0.001$. Lack of a * refers to insignificant differences between the test and the control treatments.

probably represent the natural mortality of parasitoids under our handling conditions, being somewhat lower in the host feeding experiment and higher in the hyperparasitism experiments. In the latter, they probably include about 10–20% mortality from young pupae to mature pupae, and an additional 10% before parasitoid emergence. These figures resemble those obtained by Gerling & Sinai (1994) for *E. luteola* (as *E. deserti*): 20%, and 27% total immature mortality in two experiments.

Our studies examined mortality factors affecting the developing *Encarsia* pupae, including host feeding and autoparasitism. The results with *E. lutea*, whose pupal skin is not melanized but is as protected from feeding and oviposition as the other species, indicate that the defensive properties against internecine action by parasitoids do not depend upon melanization of the parasitoid pupae; neither had pupal resistance to attack developed in association with autoparasitism, which is absent in *E. inaron*.

The relationship between host feeding and host age was studied by Rosenheim & Rosen (1992) with *Aphytis lingnanensis*, Tran & Takasu (2000) with *Diadromus subtilicornis*, and van Alphen et al. (1976) with *Encarsia formosa*. The overall results were that parasitoids preferred to feed on host stages that were less suitable for development, thus having minimal deleterious effects upon the host stages that are most suitable for oviposition by the parasitoid. In the greenhouse whitefly this was evidenced by *E. formosa* preferring to feed upon the very young whitefly instars and the late 4th instar nymph (van Alphen et al., 1976).

The success of predator and parasitoid development as functions of host age was also considered. Physiological

and physical factors were mentioned in many studies (e.g., Godfray, 1994; Eslin & Prevost, 2000; Roger et al. (2001; Tran & Takasu, 2000; Ueno, 1997; Wang & Liu, 2002). In most of the latter, the cuticle thickness of the particular host stage was considered as an important factor, although no direct experimental evidence had been furnished.

Our studies were conducted with parasitized or healthy late 4th instar whiteflies, which are unsuitable hosts for normal parasitism, but suitable or even preferable for host-feeding. Indeed, host feeding and autoparasitism were readily performed on both unparasitized and young parasitized material. Thus, the age dependent defence reactions seem to be the overriding factor determining the suitability of the parasitized whitefly nymph as food for the parasitoid female and its male progeny.

Considerations concerning the possible role of a cuticular shield in the defense of parasitoid pupae include the impracticality of developing a thick cuticle during larval development when the immature parasitoid has to grow, absorb nutrients and oxygen, and move within the host. Once the pupal stage has been reached, and the developmental changes of the immature parasitoid do not require the same degrees of mobility and interaction with the immediate environment, the development of a heavier and more protective cuticle might become feasible. This would increase the chances of the sessile parasitoid pupae surviving while on the leaf. Moreover, examination of parasitoid life history indicates that parasitoid pupae may benefit more from being defended against attack by foraging parasitoid females than the younger, larval stages. The reason being that many whitefly species develop gregariously on

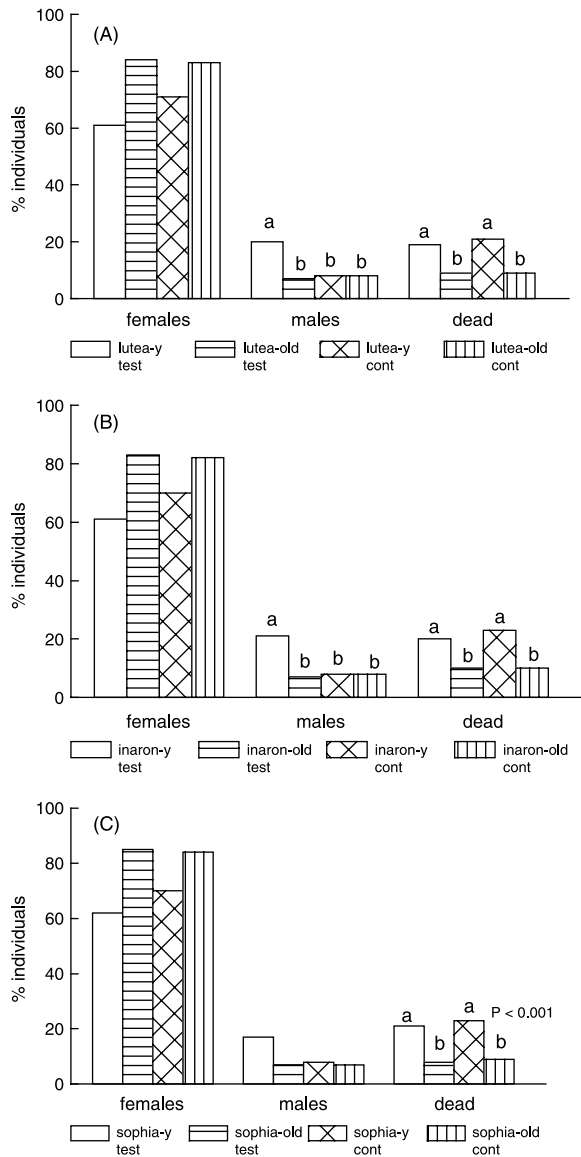


Figure 1 Results of the exposure of young and old pupae of three *Encarsia* species to a female of *E. sophia* for 24 h. The exposed pupae are: (A) *E. sophia*, (B) *E. inaron*, and (C) *E. lutea*. y = young. Different letters designate a significant difference in the same group of columns (χ^2 -test, $P < 0.001$)

the same leaf and may therefore be parasitized contemporaneously. The first parasitoid females emerging from parasitized whiteflies will forage upon their leaf of emergence for food (host feeding) and the autoparasitic species may also attempt to lay male-producing eggs upon the already parasitized hosts.

Hunter & Kelly (1998) discussed the 'window of vulnerability' during which the immature parasitoids can serve as hosts for parasitoid attack. They considered this 'window'

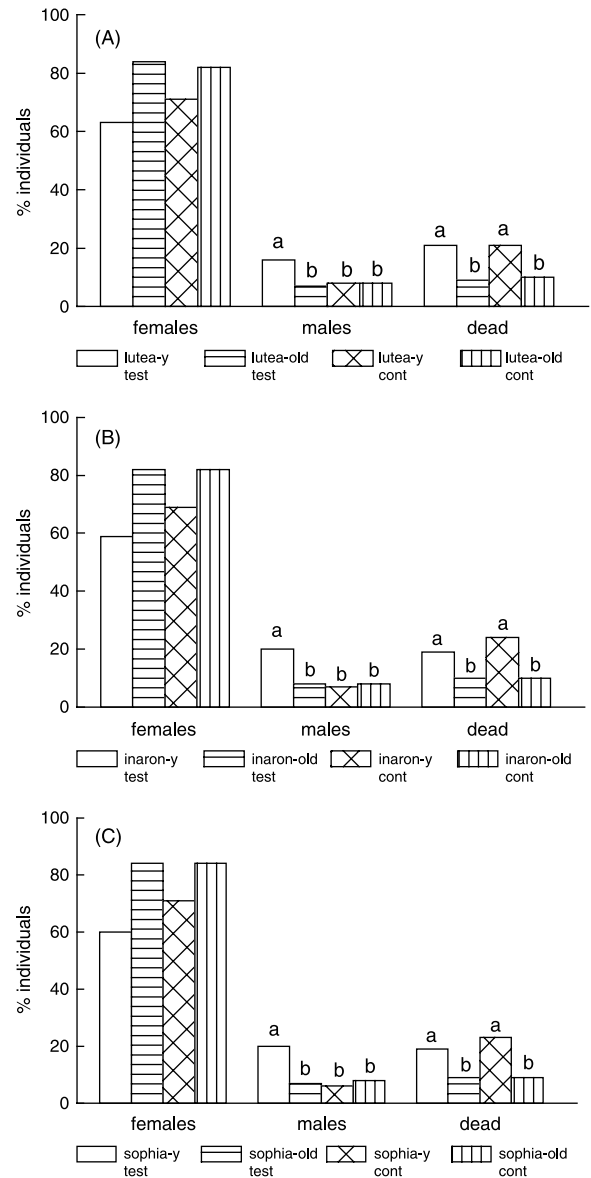


Figure 2 Results of the exposure of young and old pupae of three *Encarsia* species to a female of *E. lutea* for 24 h. The exposed pupae are: (A) *E. sophia*, (B) *E. inaron*, and (C) *E. lutea*, y = young. Different letters designate a significant difference in the same group of columns (χ^2 -test, $P < 0.001$).

to be delimited in *E. sophia* on one side by the lack of oviposition of male-producing eggs into hosts that are younger than late third instar larvae (in agreement with Gerling, 1983) and on the other, by black parasitoid pupae. Our present findings confirm those of Hunter and Kelly, but extend the 'window' to the entire duration of immature development from egg hatch to pupation. However, the late third instars and early pupae are probably preferred for the deposition of haploid eggs. This could occur

whenever the ovipositing female would have a choice of ages into which to lay its male-producing eggs.

Our experiments are insufficiently thorough to assume the generality of mature pupal protection against inter-necine activity, extending it to all *Encarsia* species. However, they do indicate the possibility of a more widely distributed phenomenon. Several authors have examined the vulnerability of different *Encarsia* stages to autoparasitism (Hunter, 1989; Avilla et al., 1991; Williams, 1991; Pedata & Hunter, 1996; Hunter & Kelly, 1998). However, with the exception of the latter, pupal parasitoids offered as hosts for male production were young, i.e., pupating or recently pupated individuals, and the susceptibility of pupae that are 2 or more days old was not determined. The apparent inconsistency found in the results of Hunter & Kelly (1998), who showed that pupae of *E. sophia* were unacceptable, against those of Hunter (1989), who showed that pupae of *E. pergandiella* were acceptable for secondary male production, is interesting in this connection. This might be explained by considering the variation in the durations of pupal maturation in the different parasitoid species. It is possible that the time necessary for the pupal shell to harden and confer unsuitability to the pupa varies between parasitoid species, as well as among the host species that are utilized. Some parasitoids, like *E. sophia*, may become resistant to attack earlier, whereas others like *E. pergandiella* may require longer.

The value of melanization in the defense of parasitoid pupae was not elucidated in our study, and the data available on this topic indicate great variety. At least three situations with respect to melanization exist: *Encarsia formosa*, while parasitizing *T. vaporariorum*, causes its host cuticle to melanize, but the parasitoids pupal skin remains transparent. Melanization occurs during the late larval stages of the parasitoids and does not protect them from the deposition of haploid eggs by *E. pergandiella* (Pedata & Hunter, 1996). *Encarsia lutea* and *E. pergandiella* have transparent pupal cases, whereas *E. inaron*, *E. sophia*, and *E. meritoria* have melanized ones. All five reside in the transparent cuticle of their hosts. Thus, no correlation between melanization and defense against ovipositor-induced damage by other parasitoids is apparent. However, although melanization is not functional in protecting against attack by parasitoid females, it may serve against other mortality factors and represent one of the varying methods of parasitoid defense.

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