

## RESEARCH ARTICLE

### Discrimination by *Coptera haywardi* (Hymenoptera: Diapriidae) of hosts previously attacked by conspecifics or by the larval parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)

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*Coptera haywardi* (Oglobin) is an endoparasitoid of fruit fly pupae that could find itself in competition with other parasitoids, both con- and heterospecific, already resident inside hosts. In choice bioassays, ovipositing *C. haywardi* females strongly discriminated against conspecifically parasitised *Anastrepha ludens* (Loew) pupal hosts. They also avoided pupae previously attacked by *Diachasmimorpha longicaudata* (Ashmead), a larval–prepupal koinobiont endoparasitoid, and the degree of larval–parasitoid superparasitism had no effect on this avoidance. There was no difference in the number of ovipositor insertions when hosts previously parasitised by a conspecific and *D. longicaudata* were exposed simultaneously. As females aged the degree of host discrimination declined. An ability to discriminate against pupae previously attacked as larvae suggests low levels of both conspecific and heterospecific competition in the field.

**Keywords:** host discrimination; multi-parasitism; superparasitism; competition; Diapriidae; Tephritidae

#### Introduction

Niche separation is fundamental to the coexistence of natural enemies that use the same host population (May and Hassell 1988; Borer, Murdoch, and Swarbrick 2004). Separations can be maintained by various ‘exclusion mechanisms’ that include microhabitat preferences, expansion or contraction of host ranges and responses to physical and chemical cues that indicate the presence of a potential competitor (Pijls, Hofker, van Staalduinen, and van Alphen 1995; Roriz, Oliveira, and Garcia 2006; Mehrnejad and Copland 2006; Mahmoud and Un Taek 2008). Cues indicative of potential competition can be employed in ‘host discrimination’, the ability of parasitoids to select unparasitised hosts for oviposition and progeny development (van Lenteren 1981; Godfray 1994; Agboka et al. 2002; Adams and Six 2007). In general, it is during the final part of the search process that a parasitoid discriminates and so minimises the chances of super- or multi-parasitism (Brodeur and McNeil 1992; Mahmoud and Un Taek 2008). On a population level, discrimination by parasitoids plays an important role in the regulation of host numbers (van Dijken

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and van Alphen 1998; Cusson et al. 2002) as it leads to parasitism of more hosts over a larger area and in less time than would occur with indiscriminate foraging (Quicke 1997; van Baaren et al. 2009).

The use of more than one parasitoid species to suppress a pest population has resulted in successful examples of biological control (Paine, Paine, Hanks, and Millar 2000; Denoth, Frid, and Myers 2002; Cusson et al. 2002; Snyder et al. 2004; Pedersen and Mills 2004). However, in other cases, the addition of a new natural enemy has not resulted in better biological control (Pijls et al. 1995; De Moraes, Cortesero, Stapel, and Lewis 1999; Tian, Zhang, Yan, and Wang 2008). The establishment of successful guilds has often been through trial and error which in the end may have formed less optimal natural enemy combinations than could have been generated by informed initial candidate choices (Force 1974; Mackauer 1990; García-Medel, Sivinski, Díaz-Fleischer, Ramirez-Romero, and Aluja 2007). In theory, one way to enhance niche separation among natural enemies is through the use of parasitoids capable of inter-specific discrimination (Waage and Mills 1992; De Moraes et al. 1999; Harris and Bautista 2003).

Historically, there have been efforts to identify discriminating parasitoids of frugivorous tephritids (García-Medel et al. 2007). Inter-specific competition was considered an important deficiency in the release of 32 species of natural enemies to control *Bactrocera dorsalis* (Hendel) in Hawaii (van Den Bosch and Haramoto 1953; Bess, van den Bosch, and Haramoto 1961), and research to identify compatible combinations has continued to the present. For example, it was argued that host mortality would be increased if larval-pupal braconids such *Diachasmimorpha tryoni* (Cameron) and *Diachasmimorpha longicaudata* (Ashmead) could recognise and reject hosts previously attacked by the egg-prepupal parasitoid *Fopius arisanus* (Sonan). In the case of *D. tryoni*, females were significantly less likely to oviposit in hosts already parasitised by *F. arisanus* (Bautista and Harris 1997; Wang and Messing 2003) and under field conditions, Stark, Vargas, and Thalman (1991) and Vargas, Stark, Uchida, and Purcell (1993) and Vargas, Leblanc, Putoa, and Piñero (2012) found additional mortality inflicted by *D. longicaudata* from larvae unparasitised by *F. arisanus*. The use of larval and pupal parasitoids was also proposed (Dresner 1954), but low host specificity and a tendency towards hyper-parasitism in the largely idiobiont pupal parasitoids of Diptera prevented the development of this alternative (Dresner 1954; Xin-Geng and Messing 2004).

Recently, the apparent specificity of the endoparasitic diapriid pupal parasitoid *Coptera haywardi* (Oglobin) to Tephritidae suggested that the environmental concerns confronting the introduction/augmentation of pupal parasitoids might be overcome (Sivinski et al. 1998). It is a relatively efficient forager (Guillén, Aluja, Equihua, and Sivinski 2001) that can inflict substantial mortality on more shallowly buried pupae under semi-natural conditions (Baeza-Larios, Sivinski, Holler, and Aluja 2002). If in addition to host specificity, it recognises and avoids hosts previously attacked by other parasitoid species, it could be a valuable addition to a multi-species guild of tephritid biological control agents.

*Diachasmimorpha longicaudata* was introduced to Mexico from a strain reared under laboratory conditions in Hawaii and rapidly established in *Anastrepha* spp. (Schiner) populations with parasitism rates ranging from 1 to 20% (Jiménez 1956; Aluja et al. 1990; Ovruski, Aluja, Sivinski, and Wharton 2000; Schliserman, Ovruski, and De Coll 2003). It is native to the Indoaustralian region, where it develops in

larvae of *Bactrocera* spp. (Hendel) (Wharton and Gilstrap 1983). In Mexico, it is mass-reared and released to suppress *Anastrepha* spp. populations in support of 'fly-free' and 'low-prevalance' agricultural zones (Montoya et al. 2000; Aluja et al. 2009).

It has been observed that even under very high release densities of *D. longicaudata*, a portion of the fruit fly larval population escapes parasitism (Montoya et al. 2000, 2003). Therefore, it is possible that these individuals would be vulnerable to subsequent attack by a pupal parasitoid such as *C. haywardi*. Our aim in this study was to identify the ability of *C. haywardi* to discriminate pupae parasitised previously by conspecifics or by *D. longicaudata*.

### Materials and methods

The research took place in the Biological Control Laboratory of the Moscafrut Program SAGARPA-IICA, located in Metapa de Domínguez, Chiapas, Mexico. The evaluations were carried out at  $21 \pm 2^\circ\text{C}$ . *Coptera haywardi* were obtained from colonies that originated from insects collected in Veracruz State, Mexico (Aluja et al. 2009), maintained under mass-rearing conditions (Cancino and Montoya 2008) and had been in the laboratory for 75–85 generations. Adults of *D. longicaudata* were taken from a colony reared under laboratory conditions for over 300 generations. Pupae of *Anastrepha ludens* (Loew) from the Moscafrut mass-rearing facility were used as hosts. Pupae previously parasitised by *D. longicaudata* were obtained by exposing *A. ludens* larvae in artificial diet to adult wasps (2♀:1♂) in Petri dishes. The exposed larvae were maintained in larval diet for 2 more days and then placed on vermiculite to complete pupation. The pupae parasitised by *C. haywardi* were obtained from young pupae exposed 6–10-day-old adults (1♀:1♂). After the exposition the pupae were placed in a plastic container with vermiculite. These rearing procedures are those used to mass-rear these species at the Moscafrut facility and are described by Cancino and Montoya (2008) and Domínguez, Artiaga-López, Solís, and Hernández (2010).

### Discrimination of parasitised pupae

In choice experiments, individual females of *C. haywardi* were exposed to the following alternatives: (1) a pupa previously parasitised by *D. longicaudata* and an unparasitised pupa; (2) a pupa parasitised by *C. haywardi* and an unparasitised pupa; (3) a pupa previously parasitised by *D. longicaudata* and a pupa parasitised by *C. haywardi*; (4) both pupae previously parasitised by *D. longicaudata*; (5) both pupae parasitised by *C. haywardi*; and (6) two unparasitised pupae. Only 3–5-day-old pupae were used in all treatments, since this is the optimal range for parasitisation (Aluja et al. 2009). A total of 50 individual females per each treatment were observed. All females were tested only once.

### Observation arena and recording of behavioural activities

Discrimination evaluations were conducted in an arena consisting of a 14.5 cm diameter Petri dish containing a 2 mm thick vermiculate layer. To observe host selection, two pupae with different parasitism histories (i.e. unparasitised or parasitised by *D. longicaudata* or *C. haywardi*), were placed 7 cm apart at one end

of the Petri dish. A 5-day-old *C. haywardi* female was released at the other end. The Petri dish was maintained inside a 30 × 30 × 30 cm Plexiglas chamber. Five sides were covered by black cardboard, one side was open so as to maintain a light intensity of 8 lux in the chamber (measured with a photometer Sper Scientific<sup>®</sup>) and to serve as a window for observations. These were made from the time the adult parasitoid was placed in the chamber until the time when ovipositor insertion was finished or until 20 minutes passed without any movement. In all experiments, searching behaviour was divided into four parts, which were timed with a stopwatch. These parts were: (1) time to first encounter, which refers to the period from the start of observation to the time when the female moved towards and reached one of the host options; (2) host examination previous to final choice, which refers to antennation and oviposition attempts by the female on a puparium which was ultimately rejected; (3) final host examination, which refers to walking on the puparium, and antennation prior to oviposition; and (4) oviposition insertion time, period from insertion to withdrawal of the ovipositor. Ovipositor insertion was considered to have occurred when the female inserted her ovipositor into a pupa for more than 10 minutes. This oviposition period was based on previous observations and oviposition was corroborated as described below.

#### ***Discrimination of superparasitised pupae***

Because cues to previous attacks might accumulate, we compared the oviposition attempts of *C. haywardi* females on hosts with different levels of *D. longicaudata* superparasitism, relative to an unparasitised control. Superparasitism was recognised by multiple oviposition scars on the puparial cuticle and categorised into three levels by the numbers of scars: one scar, 2–5 scars and 6–10 scars. There is a significant relationship between the number of scars and the level of superparasitism ( $\sim r^2 = 70\%$ ) (González, Montoya, Pérez-Lachaud, Cancino, and Liedo 2007). Choice bioassays were as described above and duplicated for each superparasitism category. A total of 50 individual females were observed for each scar category.

#### ***Host discrimination as affected by oviposition experience***

Because oviposition experience might affect female capacity to discriminate, we compared the oviposition behaviour of *C. haywardi* females previously exposed and unexposed to unparasitised hosts. Samples of 100 sexually mature females and 100 males of *C. haywardi* were maintained in separated 25 × 25 × 25 cm Plexiglas chambers with water and honey as food. There were two groups in different chambers, one where females were host deprived (no oviposition experience at the time of evaluation) and another one where females were provided daily with one hundred 3-day-old pupae of *A. ludens* (experienced females). From both, experienced and non-experienced groups, every day random and independent samples of individual females were taken during the first 15 days of age. Individual evaluations of discrimination between pupae parasitised by *D. longicaudata* and unparasitised pupae were performed as described above. Thirty 1–15-day-old females, both experienced and unexperienced, were evaluated daily.

### ***Dissection of pupae and parasitoid offspring emergence***

In order to corroborate female oviposition in the first two bioassays, two evaluations were carried out. First, the number of scars per host puparium was counted in a sample of 20 female-selected pupae. These scars were caused by ovipositions or oviposition attempts either by one or both species of parasitoids. Three days after the discrimination observations, the pupae were dissected to count the number and species of immature parasitoids inside each. At this time pupae were 5–7 days old and it was possible to find first instar larvae of both parasitoid species. Pupal dissection was done under a stereoscopic microscope at 2.5X. Second, the emergence of adult parasitoids was used as an indicator of oviposition. After discrimination observations, 30 female-selected pupae were individually placed in 2 cm high by 1.5 cm diameter cylindrical cells with vermiculite. The emergence of *D. longicaudata* and adult flies began 15 days after the observations, while *C. haywardi* emergence began 30 days after oviposition. Immature stages found in the pupae and emergence data were compared with the oviposition observations.

### ***Data analysis***

The numbers of females that chose unparasitised pupae were compared to those that attacked parasitised pupae in each treatment were analysed by a contingency table using Chi-square test (Zar 1974). Because of non-normal distributions, the mean duration times of behavioural activities were compared with non-parametric Kruskal–Wallis tests and multiple comparisons of means with minimum significant difference test (Sprent 1993). The statistical software JMP Version 5.7 (2005) and Minitab Version 15 (2006) were used. Daily discrimination data of females with and without oviposition experience were analysed applying a logistic regression with multinomial response (Hosmer and Lemeshow 2000). The logistic regression was obtained with software R Version 2.13.0 (R development core TEAM 2011). All tests were interpreted with  $\alpha = 0.05$ .

## **Results**

### ***Discrimination of parasitised pupae***

*Coptera haywardi* females were significantly less likely to choose pupae previously parasitised by *D. longicaudata* or *C. haywardi* than they were unparasitised alternatives (Figure 1;  $\chi^2 = 40.49$ ,  $p < 0.0001$  for pupa parasitised with *C. haywardi* and non-parasitised pupa;  $\chi^2 = 61.08$ ,  $p < 0.0001$ , for pupae parasitised with *D. longicaudata* and non-parasitised pupa). When pupae parasitised by *C. haywardi* and *D. longicaudata* were presented simultaneously there were no significant differences in the numbers of females that attempted to oviposit in either species (Figure 1;  $\chi^2 = 1.0$ ,  $p = 0.05$ ).

The time spent in the three stages of host searching behaviour: (1) host encounter, (2) host examination (attention, touching host puparia) and (3) ovipositor insertion, were compared among the six combinations of hosts (Table 1). The time required to find a host ranged from 1.5 to 4 minutes, with significant difference among treatments ( $df = 5$ ,  $H = 12.13$ ,  $p = 0.03$ ). When females were exposed simultaneously to hosts parasitised by *D. longicaudata* and *C. haywardi* the time

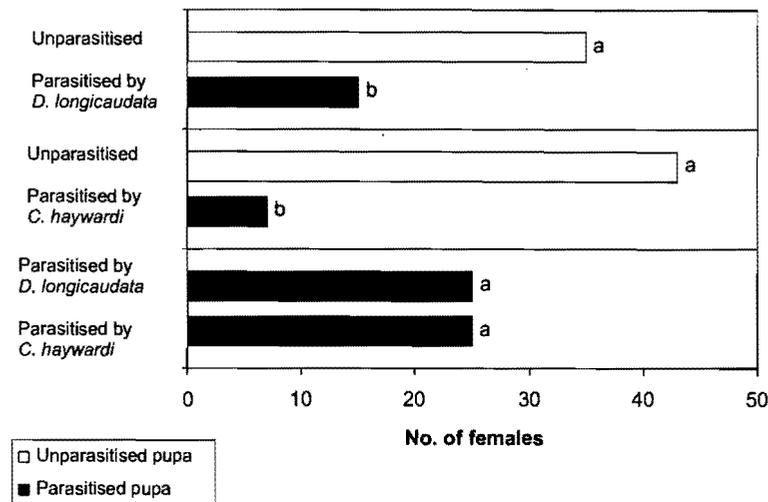


Figure 1. Number of *C. haywardi* females that chose the various alternatives in paired host exposures. The same letter next to both options within a pair indicates no significant difference.

spent searching for hosts where no oviposition attempts were made was 17.7 minutes, significantly higher than in any of the other treatments ( $df = 4$ ,  $H = 13.69$ ,  $p = 0.008$ ). Females did not select any pupae previously parasitised by *D. longicaudata* when an unparasitised alternative was available. Examination time previous to oviposition attempts was relatively short in all treatments and did not exceed 2 minutes. The time females spent examining pupae parasitised by *C. haywardi* was longer than the time spent on unparasitised ones. When the pupa was previously parasitised by *D. longicaudata*, the females took more time examining the unparasitised pupa. The examination time varied significantly among parasitised ( $df = 6$ ,  $H = 75.31$ ,  $p = 0.0001$ ) and unparasitised pupae ( $df = 2$ ,  $H = 78.03$ ,  $p < 0.0001$ ). The shortest examination time was on unparasitised pupae when the alternative was a conspecific parasitised pupa. The longest examination time was when both pupae were parasitised by *C. haywardi*. The oviposition insertion time varied from 11 to 27 minutes (all over the 10 minutes threshold) and there were significant differences among treatments ( $df = 5$ ,  $H = 175.77$ ,  $p < 0.0001$ ). The longest oviposition time was recorded when both pupae were parasitised by *C. haywardi*.

The statistical analyses were carried out considering only females that responded, either to unparasitised or parasitised pupae. The proportions of females that did or did not respond to either of the pupae varied with the nature of the pupae presented (Figure 2). When presented with an unparasitised pupa as an option, between 40 and 50% of females did not respond within the 20 minutes time limit. When both pupae were parasitised, whether by *D. longicaudata* or *C. haywardi*, the percentage of females that did not attempt oviposition was over 60%. Only 32.1% of females did not attempt oviposition when both pupae were unparasitised, significantly less than the other proportions ( $\chi^2 = 1031.83$ ,  $p < 0.0001$ ).

Table 1. Mean time ( $\pm$ SE) in minutes of different *C. haywardi* searching behaviour components when provided various host choices.

Treatments (options)	Time to first encounter	Selection of pupa not oviposited	Examination of parasitised pupa	Examination of unparasitised pupa	Oviposition insertion
Parasitised by <i>D. longicaudata</i> and non-parasitised pupa	2.3 $\pm$ 0.1ac		1.0 $\pm$ 0.1b	1.2 $\pm$ 0.0a	11.8 $\pm$ 0.4e
Parasitised by <i>C. haywardi</i> and non-parasitised pupa	2.3 $\pm$ 1.3ac	4.4 $\pm$ 0.7a	1.3 $\pm$ 0.2ab	0.9 $\pm$ 0.0a	15.3 $\pm$ 0.9cde
Parasitised by <i>D. longicaudata</i> and parasitised by <i>C. haywardi</i>	1.7 $\pm$ 0.1c	17.7 $\pm$ 5.0b	1.2 $\pm$ 0.0D. 1. b 1.2 $\pm$ 0.1C. h. b		14.0 $\pm$ 0.9de
Both pupae parasitised by <i>D. longicaudata</i>	3.8 $\pm$ 0.4a	2.8 $\pm$ 0.3a	1.5 $\pm$ 0.1ab		18.7 $\pm$ 1.0bc
Both pupae parasitised by <i>C. haywardi</i>	2.7 $\pm$ 0.2abc	4. $\pm$ 0.8a	1.9 $\pm$ 0.1a		27.9 $\pm$ 1.7a
Both pupae unparasitised	2.2 $\pm$ 0.1c			1.73 $\pm$ 0.08a	21.9 $\pm$ 0.9b

Notes: Means followed by the same letter in each column are not significantly different. Kruskal–Wallis non-parametric test and means comparison with the minimum significant difference ( $\alpha = 0.05$ ).

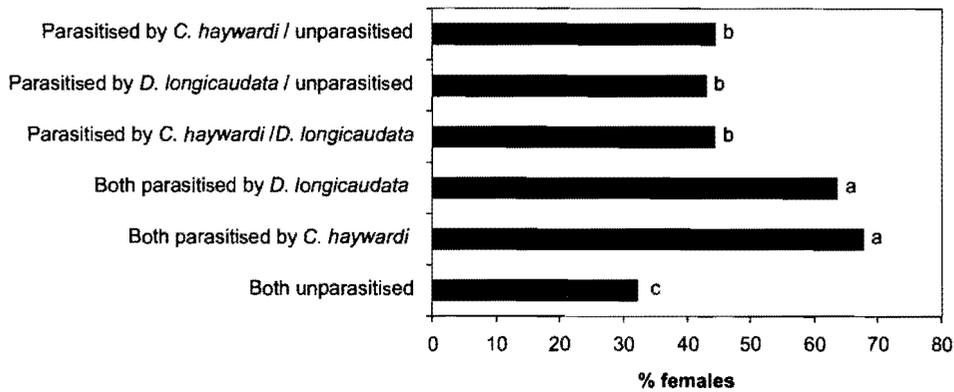


Figure 2. Proportions of *C. haywardi* females that did not respond within 20 minutes to either host pupa in paired exposures. Bars followed by the same letter were not significantly different.

#### Discrimination of superparasitised pupae

Overall, superparasitism by *D. longicaudata* was not a factor that modified female discrimination ability (Figure 3). In all cases, females selected the unparasitised pupa with higher frequency than the parasitised pupa, regardless of the number of oviposition scars (one scar:  $\chi^2 = 71.84$ ,  $p < 0.0001$ ; 2–5 scars:  $\chi^2 = 65.72$ ,  $p < 0.0001$ ; 6–10 scars:  $\chi^2 = 61.06$ ,  $p < 0.0001$ ). The level of superparasitism, represented by the number of scars, had no effect on the discrimination capacity of *C. haywardi*.

The presence of pupae superparasitised to different degrees had no effect on the time to host encounter ( $df = 2$ ,  $H = 2.38$ ,  $p = 0.305$ ), the time for host examination, whether parasitised ( $df = 2$ ,  $H = 1.10$ ,  $p = 0.57$ ) or unparasitised ( $df = 2$ ,  $H = 1.20$ ,  $p = 0.54$ ), or ovipositor insertion time ( $df = 2$ ,  $H = 0.19$ ,  $p = 0.91$ ), (Table 2). As in the previous case, when there was a choice between a *D. longicaudata* parasitised pupa and an unparasitised pupa, no parasitised pupae were chosen.

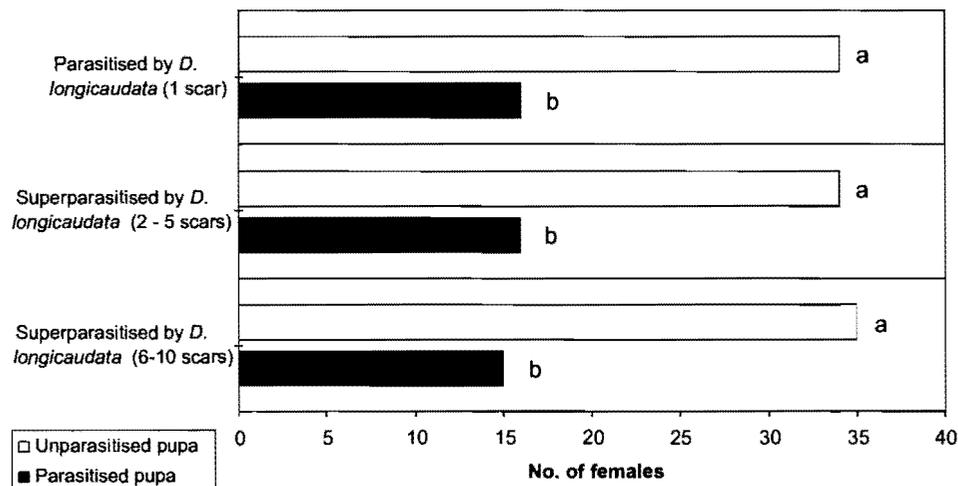


Figure 3. Number of *C. haywardi* females that oviposited into either unparasitised pupae or pupae that had been superparasitised to different extents by *D. longicaudata*.

Table 2. Mean duration ( $\pm$ SE) in minutes, of *C. haywardi* host searching components when presented with host pupae having different levels of *D. longicaudata* superparasitism.

Treatments (options)	Time to first encounter	Examination of parasitised pupa	Examination of unparasitised pupa	Oviposition insertion
Parasitised (1 scar) and non-parasitised pupa	4.4 $\pm$ 0.3a	1.1 $\pm$ 0.2a	0.7 $\pm$ 0.1a	15.5 $\pm$ 0.6a
Superparasitised (2–5 scars) and non-parasitised pupa	3.4 $\pm$ 0.3a	1.5 $\pm$ 0.2a	1.3 $\pm$ 0.1a	18.1 $\pm$ 0.7a
Superparasitised (6–10 scars) and non-parasitised pupa	3.5 $\pm$ 0.3a	1.6 $\pm$ 0.2a	1.2 $\pm$ 0.1a	15.6 $\pm$ 0.6a

Notes: Means followed with the same letter in each column were not significantly different. Kruskal–Wallis non-parametric test and means comparison with the minimum significant difference ( $\alpha = 0.05$ ).

#### *Host discrimination as affected by oviposition experience*

Females were significantly more likely to choose unparasitised hosts, whether they had previous oviposition opportunities ( $\chi^2 = 55.48$ ,  $p < 0.0001$ ) or not ( $\chi^2 = 50.91$ ,  $p < 0.0001$ ). Overall, experienced females were significantly more like to oviposit (t-Wald =  $-3.06$ ,  $p = 0.002$ ) but there was no difference in the degree of discrimination against parasitised pupae (t-Wald =  $-1.30$ ,  $p = 0.19$ ) (Figure 4). Oviposition activity increased with age during the first 8 days in females without experience (host deprived), but this tendency was not observed in females with experience. Females without experience selected only non-parasitised pupae during their first 7 days but subsequently they oviposited into a greater number of parasitised pupae. Experienced females were significantly more like to oviposit into unparasitised hosts with increasing age (t-Wald =  $4.09$ ,  $p = 0.000041$ ).

#### *Dissection of pupae and emergence*

The above results, obtained by direct observation, were corroborated by dissections and adult emergence. There was 88.1% correspondence between the arena observations of oviposition and the presence of parasitoid larva inside the host, and a 62.4% correspondence between the arena observations and adult emergence. When both parasitoid species attacked the same pupa, we only found *D. longicaudata* larvae, and in  $\sim 50\%$  of the cases, we also found some remains of *C. haywardi* larvae.

#### **Discussion**

*Coptera haywardi* were able to discriminate between parasitised and unparasitised *A. ludens* pupae (Figure 1). Whereas rejection of hosts previously attacked by conspecifics is wide spread (Visser et al. 1992b; Godfray 1994), the capacity to recognise prior heterospecific parasitism, as in *C. haywardi*, is much less commonly encountered. All other things being equal, multi-parasitism is more frequently observed than superparasitism (Godfray 1994; Cusson et al. 2002; Javad Ardeh,

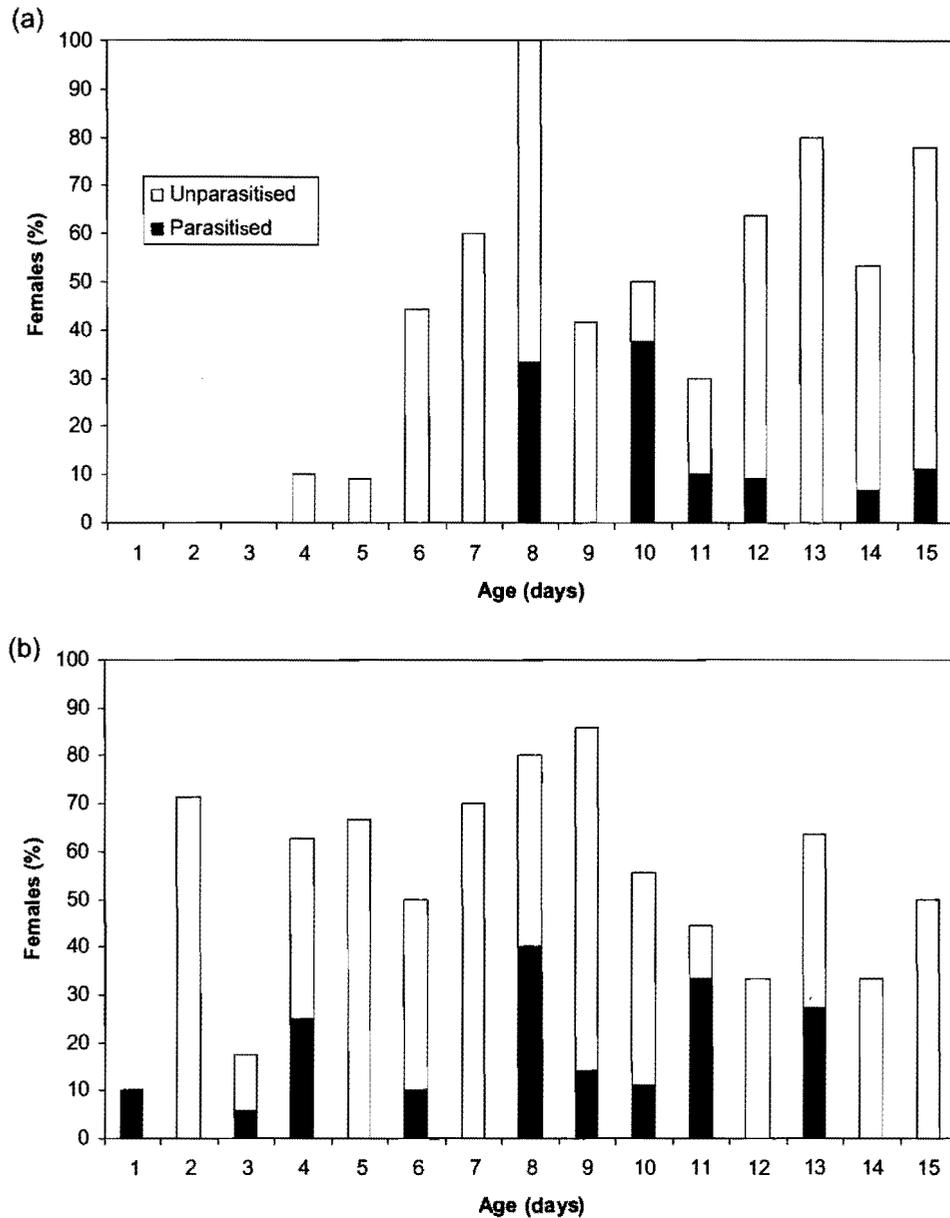


Figure 4. Proportions of *C. haywardi* females of different ages that oviposited into either previously parasitised or unparasitised pupae. (a) Females without previous exposure to hosts; (b) females with previous exposure to hosts.

de Jong, and van Lenteren 2005), and when parasitoids avoid heterospecifically parasitised hosts, discrimination is often greatest among closely related species (Vet, Meyer, Bakker, and van Alphen 1984; Agboka et al. 2002; Pedata, Giorgini, and Guerrieri 2002). In the present case, not only are *C. haywardi* and *D. longicaudata* distantly related, belonging to different superfamilies, but they

share no selective history having occurred sympatrically for only ~50 years in Mexico (Ovruski et al. 2000).

Perhaps *C. haywardi* faces predictable competitive situations that other parasitoids do not and as a result has developed an unusually general capacity for discriminating parasitised hosts. Conflict between heterospecific larvae would seem unimportant to most dipteran pupal parasitoids which are typically ectoparasitic, idiobiont generalists that readily act as hyperparasitoids (Hawkins 1994). However, as an endoparasitoid, *C. haywardi* faces dangers from intrinsic competitors already present in the body of the host. Our observation that *D. longicaudata* eliminates *C. haywardi* when the two species co-occur is evidence of these risks. Since the first of two endoparasitoids is often the winner in within-host conflicts (Vinson 1972; Bai 1991; Visser et al. 1992a; Cusson et al. 2002; Agboka et al. 2002; Harvey, Gols, and Strand 2009), and *C. haywardi* will most likely be the last endoparasitoid to occupy a host, there may be unusually strong selection to recognise previously parasitised pupae.

When faced with a lack of unparasitised hosts, some *C. haywardi* females oviposited on parasitised hosts. However, the fraction of females that did not attempt oviposition was significantly greater under this condition (Figure 2). This suggests that females can make oviposition decisions based on egg load, previous experience or host availability, and those that did lay eggs on heterospecific parasitised pupae may have been 'making the best of a bad situation'. According to Heinz (1996) and Rivero (2000) female decisions might be influenced by age or experience. For example, older females with higher egg loads and histories of low host encounters might choose to risk ovipositing in less than optimal, previously parasitised pupae (Visser et al. 1992a; Islam and Copland 2000; Outreman, Le Ralec, Wajnberg, and Pierre 2001; Baeder and King 2004).

The underlying mechanisms that *C. haywardi* females use to discriminate parasitised hosts are not known. These could be chemical, physiological or acoustical. Oviposition deterrent pheromones have been proposed as a possible mechanism for intra-specific discrimination in larval parasitoids (Hoffmeister 2000; Rivero 2000; Darrouzet, Lebreton, Gouix, Wipf, and Bagneres 2010). However, in our case, superparasitised pupae were rejected by *C. haywardi* at the same rate as those that had been attacked only once, so accumulation of chemical cues seems not to have an effect here.

The times required for the different stages of host searching behaviour of *C. haywardi* did not vary significantly across the various choice situations. The most dramatic difference was the relatively long period it took to approach and then abandon a puparium when both potential hosts were parasitised, one by a conspecific and the other by a heterospecific (Table 1). However, the variances in examination times were lower than oviposition times which could indicate that *C. haywardi* has a well-defined period during which it decides to oviposit, guided by specific factors such as host shape, a very common feature among pupal parasitoids (Vinson 1976; Romani, Isidoro, Bin, and Vinson 2002; Goubault et al. 2004). Females normally made a linear movement between each end of the puparium while investigating and this could result in an estimate of size (Heinz 1996; Goubault et al. 2004). It is also possible that *C. haywardi* females obtained information on the parasitisation status of potential hosts during this stage (Fischer, Samietz, and Dorn 2004; Wang and Messing 2004; Chow and Heinz 2005).

The period of ovipositor insertion of *C. haywardi* was relatively lengthy (between 10 and 30 minutes). In contrast, female *D. longicaudata* invests  $29 \pm 11.7$  seconds in oviposition (Montoya et al. 2003). Since *C. haywardi* attacks sheltered hosts (buried pupae) perhaps the dangers associated with oviposition are minimal and females may not be exposed to the risks associated with long host-handling times on exposed hosts. There could be other reasons for this longer oviposition time. For example, the puparium could be harder to pierce than larvae or ovipositor morphology may make rapid penetration difficult. No difference in the oviposition time into parasitised or unparasitised hosts was found, suggesting that host discrimination is an unlikely explanation for this long period of host-handling.

In conclusion, *C. haywardi* seems to have good discrimination ability and deserves further investigation to assess its potential as a natural enemy in fruit fly biological control programmes. By avoiding pupae previously attacked by *D. longicaudata*, it would be expected to provide supplemental mortality to the fruit fly population.

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