Bulletin of Entomological Research (2004) **94**, 473–480 DOI: 10.1079/BER2004318

Two different life-history strategies determine the competitive outcome between *Dirhinus giffardii* (Chalcididae) and *Pachycrepoideus vindemmiae* (Pteromalidae), ectoparasitoids of cyclorrhaphous Diptera

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Abstract

Dirhinus giffardii Silvestri and *Pachycrepoideus vindemmiae* Rondani are solitary parasitoids attacking puparia of many cyclorrhaphous flies. They are not typical ectoparasitoids, as they feed on host pupae within puparia that develop from the exoskeleton of host larvae. *Dirhinus giffardii* did not kill its host until the parasitoid egg developed into a larva, while *P. vindemmiae* permanently paralysed its host at the time of oviposition. As a result, ovipositing into a young host puparium (< 1 day old) in which the host pupa has not yet fully formed resulted in complete death of offspring in *P. vindemmiae*, but *D. giffardii*, although suffering higher mortality than in older host puparia, still showed a level of successful development. In a choice experiment, both parasitoids preferred to attack 2- to 3-day-old puparia in which the host pupae had fully formed, rather than 1-day-old host puparia. *Pachycrepoideus vindemmiae* always prevailed in competition because it injected venom that not only paralysed the host, but also caused the death of *D. giffardii* larvae in multi-parasitized hosts. *Dirhinus giffardii* preferred to attack unparasitized hosts rather than hosts previously parasitized by *P. vindemmiae*, while *P. vindemmiae* did not show a preference between unparasitized hosts and hosts previously parasitized by *D. giffardii*.

Introduction

Competition for host resources may lead one species to eliminate another species through physical attack, physiological suppression or both mechanisms (Salt, 1961; Fisher, 1963; Godfray, 1994; Quicke, 1997). In the case of physical combat, which often occurs among young endoparasitoid larvae, the parasitoid with the most rapid development rate often has an advantage. With physiological suppression, however, older endoparasitoids often have the advantage of eliminating younger competitors through toxic secretions, anoxia or nutrient deprivation. Other factors influencing competitive outcomes may include differences in life-history strategy. In general, an endoparasitic koinobiont (whose larva develops for most of its life inside a host organism, and allows its host to continue developing more or less normally for a period following the parasitization), often loses in competition against an ectoparasitoid idiobiont (whose larva feeds

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externally on its host when the host does not develop further following the parasitization) (Godfray, 1994; Quicke, 1997; Mayhew & Blackburn, 1999). This study documents a case where two slightly different life-history strategies determine the competitive outcome between the two pupal ectoparasitoids, *Dirhinus giffardii* Silvestri (Hymenoptera: Chalcididae) and *Pachycrepoideus vindemmiae* Rondani (Hymenoptera: Pteromalidae).

Both *D. giffardii* and *P. vindemmiae* attack a range of host species in many families of cyclorrhaphous Diptera, including many tephritid fruit flies (Dresner, 1954; Nøstvik, 1954; see Noyes, 2002). *Dirhinus giffardii* is native to West Africa, and has been introduced into more than 20 countries, mainly in the Pacific and Central American regions, while *P. vindemmiae* is widespread over 60 countries around the world (see Noyes, 2002). Both parasitoids have been evaluated for the biological control of pest Diptera. For example, in Hawaii *P. vindemmiae* was introduced from Asia for the control of housefly and horn fly, while *D. giffardii* was introduced from West Africa for the control of tephritid fruit flies during the early 1900s, but both species were subsequently recorded from the same tephritid species (Wharton, 1989). Thus, they may compete for common hosts.

In the literature, both *D. giffardii* and *P. vindemmiae* have been referred to as ectoparasitic idiobionts (Dresner, 1954; Nøstvik, 1954; Podoler & Mazor, 1981). Most egg or larvae-attacking fruit fly parasitoids are typical endoparasitic koinobionts, emerging as adults from host pupae (Wharton *et al.*, 2000; Wang *et al.*, 2003). For example, adult female *Fopius arisanus* Sonan (Hymenoptera: Braconidae), an egg-attacking fruit fly parasitoid, is able to lay over 40 eggs within one day (Wang & Messing, 2003a). In contrast, female *D. giffardii* and *P. vindemmiae* produce only a few but large eggs at once, and have a relatively low fecundity (Podoler & Mazor, 1981; Phillips, 1993; Wang & Messing, 2004a), a characteristic typical of ectoparasitic idiobionts (Godfray, 1994; Quicke, 1997). However, unlike typical ectoparasitoids that attach their eggs to the outside surface of hosts, *D. giffardii* and *P. vindemmiae* attack a fly pupa that is enclosed by a protective puparium formed from the hardened exoskeleton of the fly's last larval stage. Within a young puparium, the fly pupa is not fully formed and separated from the puparium shell, although it can be attacked by pupal parasitoids. Under such circumstances, the hosts are actually attacked during the late larval or prepupal stage, and the pupal parasitoid has to place its egg into the host haemolymph because there is no space between the pupal body and puparium shell. When the parasitoids attack old puparia in which the fly pupae have separated from the puparia they lay eggs in the space between the pupa and puparium shell (Dresner, 1954; Nøstvik, 1954).

This study first examined the host age preference of *D. giffardii* and *P. vindemmiae* and the effects of host puparium age on the offspring survival of both parasitoids. Because it was found that *D. giffardii* does not kill its host until the parasitoid egg hatches (i.e. not a typical idiobiont but acting like a koinobiont), while *P. vindemmiae* permanently paralyses its host at the time of laying an egg inside a host puparium (i.e. a typical idiobiont), this model system was then used to test a hypothesis that relates to the competitive superiority of idiobionts over koinobionts. In the test of this hypothesis, the competitive mechanism and interspecific host discrimination by both parasitoids were also considered.

Materials and methods

Host and parasitoids

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) was used as the common host species for both parasitoids throughout this study. *Ceratitis capitata* was provided by the USDA-ARS Pacific Basin Agricultural Research Center in Honolulu, Hawaii, where it was reared using standard wheat-based artificial diets (Tanaka *et al.*, 1969). Fly eggs were incubated on diets in a plastic container (20 × 12 × 4 cm) and shipped weekly from the rearing laboratory to the Kauai Agricultural Research Center in Kauai, Hawaii, where this study was conducted. The fly eggs were reared under laboratory conditions (23 ± 1°C, 65 ± 10% RH, 12:12 LD, 3500 lux) at the Kauai Agricultural Research Center. When fly larvae started to pupate, the rearing container was placed into a fibreglass box (45 × 30 × 15 cm) containing 2 cm of sand, so that fly puparia could be easily collected for experiments or cultures of *D. giffardii* and *P. vindemmiae*.

Laboratory populations of both *D. giffardii* and *P. vindemmiae* were maintained on *C. capitata* puparia at the Kauai Agricultural Research Center. *Dirhinus giffardii* was initially established in a laboratory at the University of Hawaii at Manoa, Honolulu, from field collections of parasitized fruit fly puparia from the Big Island, Hawaii, and was later trans-shipped to the Kauai Agricultural Research Center. *Pachycrepoideus vindemmiae* was established from field collections of parasitized fruit fly puparia in Kauai. Adult *D. giffardii* (3–5 mm) were held in large cages (30 × 30 × 30 cm) while adult *P. vindemmiae* (1.5–2 mm) were held in small cages (9.5 × 10.5 × 13 cm), each in an approximately equal proportion of females to males, with water and honey provided following emergence.

Pilot observations were conducted to determine the rate of development of *C. capitata* within puparia under the laboratory conditions described above. *Ceratitis capitata* puparia undergo changes with age, both externally and internally. A newly formed puparium is yellowish-white while the fly inside is still in the late larval stage. After one day its shell becomes hardened and turns yellowish-brown, while the fly is in the prepupal stage and there is still no space between the fly body and the puparium shell. After 2–3 days the puparium becomes reddish-brown and the colour no longer changes with age, while the internal tissues have undergone histolysis, histogenesis and differentiation to form adult organs and appendages. The appendages of a fly pupa within a 2- to 3-day-old puparium can be seen under a dissection microscope, and there is an obvious space between the pupa and the puparium shell. After 5–6 days, red eyespots appear. Adult flies are ready to emerge from 8- to 9-day-old puparia.

Four experiments were conducted under the above laboratory conditions. All experiments used 7-day-old female wasps of both species that were taken from the holding cages of adult wasps. Thus, the experimental wasps were presumed to be mated, naqve (without oviposition experience), and with a high mature eggload.

Host age preference

Newly formed host puparia were collected twice a day, and two distinctly different age classes of puparia (< 12 h and 2 days old) were selected to test puparia age preference

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by both parasitoids in a choice experiment. The experimental setting for both parasitoid species was similar but used different sized containers and different numbers of host puparia, because *P. vindemmiae* is much smaller than *D. giffardii*. Also, *D. giffardii* normally holds 4–6 mature eggs and can lay 4–6 eggs within 12 h (Wang & Messing, 2004a) while *P. vindemmiae* normally holds 12–14 mature eggs and can lay up to 10 eggs within 12 h (Phillips, 1993).

For the test with *D. giffardii*, a wet tissue paper was spread over the bottom of a 7 cm diameter Petri dish and covered with 1 cm of sand to keep it moist to prevent desiccation of puparia. Four puparia of each age group were placed in a random arrangement on the sand. The Petri dish was placed into a cage ($9.5 \times 10.5 \times 13$ cm), and a female parasitoid wasp was released into the cage with water and honey provided for 12 h. Thus, the actual host ages for the test were 12 h to 1 day old or 2 to 2.5 days old (hereafter referred to as < 1 day or 2–3 days old). The test for *P. vindemmiae* was conducted in a small plastic container ($3 \times 5 \times 4$ cm) with air holes on the container's lid. After 1 cm sand was spread over wet tissue in the container and six puparia of each age group were randomly arranged on the sand, a single female wasp was released into the container for 12 h. After these exposures, all hosts were immediately dissected to determine the presence or absence of parasitoid eggs in the hosts. Each test was replicated 20–23 times.

Effect of host puparia age on offspring survival

No-choice experiments were conducted to determine the effect of host puparia age on the offspring survival of each of the two parasitoid species. As in the above experiment, host puparia of the two different age groups were prepared and used. About 40 puparia of each age group were placed in a Petri dish and exposed to 20 *D. giffardii* females or 10 *P. vindemmiae* females in cages ($9.5 \times 10.5 \times 13$ cm) for 12 h. Half of the exposed puparia were dissected two days later to determine the survival and development of both parasitoid egg and host pupae, while the other half were reared until the fly or parasitoid adults emerged. A control of 20 unparasitized puparia of each age group was reared simultaneously. It was observed that both parasitoid eggs hatched within two days if they were still alive, and the appendages of 2- to 3-day-old hosts appeared if they were not killed by the parasitoids. Each exposure was repeated 10 times. Adult emergence rate of each parasitoid species was first estimated as the percentage of emerged adults to the total hosts parasitized based on the results of dissection, and was then corrected according to the control mortality, which was 11.3% and 0.05% for the < 1 day and 2–3 days host puparia, respectively.

Competitive outcome

This experiment manipulated exposure order of host puparia to both parasitoids in succession, as well as the exposure interval in order to determine the outcome of competition and the mechanism that one parasitoid used to eliminate the other. Two-day-old host puparia were used in this experiment, as oviposition by each parasitoid in 2- to 3-day-old host puparia resulted in no apparent egg mortality (see results of above experiment). Two tests were conducted, each consisting of two different exposure intervals to both species in succession followed by two different dissection times after the second exposure.

In the first test, hosts were exposed first to *D. giffardii* and then to *P. vindemmiae* either immediately or two days later, by which time *D. giffardii* eggs had developed into first instar in the hosts. Following these exposures, half of the hosts were dissected immediately while the other half were dissected two days later to determine the survival of both parasitoid individuals in multi-parasitized hosts. The four treatments of the second test were similar to that of the first test, except that the hosts were exposed first to *P. vindemmiae* and then to *D. giffardii*. In this way, it was possible to determine if adult females of each species could directly destroy the individuals of the other species during multi-parasitism, or if any toxic or paralysing factors released by the adult female or her eggs or larvae killed the other species in multi-parasitized hosts.

To obtain hosts parasitized by the first species, about 100 puparia were placed in a Petri dish, and exposed to about 100 female wasps in a cage for 6 h. This exposure time resulted in about 60–80% parasitism by *D. giffardii* and 80–100% parasitism by *P. vindemmiae*, with a relatively high rate of superparasitism for the latter species. Both parasitoids laid eggs in the space between the pupa and puparium shell inside 2- to 3-day-old puparia. When a host puparium was slightly brushed with water it became sufficiently transparent to make the egg of *D. giffardii* clearly visible through the cuticle under a microscope. Thus, only those puparia parasitized previously by *D. giffardii* were chosen for the exposure to *P. vindemmiae* by placing them in a 3 cm diameter Petri dish; the dish was moved into a cage holding *P. vindemmiae* females at a ratio of one wasp to three hosts for 6 h.

It was difficult to determine whether or not a host puparium was parasitized by *P. vindemmiae* by external examination. Therefore, all of the hosts first exposed to *P. vindemmiae* were used for the second test. During dissections those puparia that had more than one individual of *P. vindemmiae* or *D. giffardii* within the same puparium were discarded in order to avoid any possible effects of superparasitism on mortality. Only the cases with a single *P. vindemmiae* or *D. giffardii* individual were counted in the results.

These two tests were repeated until at least 20 individuals of each treatment for each test were dissected. The size and shape of eggs and larvae of these two parasitoid species were distinctly different. *Pachycrepoideus vindemmiae* eggs were small (0.2–0.25 mm) and elongate-ovate; while *D. giffardii* eggs were large (0.6–0.7 mm), hyaline and crescent-shaped, with a slight dorsal arch and a greater diameter at the cephalic than at the caudal end (Dresner, 1954). Newly hatched *D. giffardii* larvae are of the caudate type while *P. vindemmiae* larvae are hymenopteriform in all instars. Thus, it was easy to distinguish the two species during the dissection.

Additional experiments were conducted by exposing about 100 2-day-old puparia to 100 female wasps of both species one immediately followed by the other for 6 h each. After the exposure, half of the hosts were dissected to determine the percentage parasitism and multi-parasitism, while another half were reared until fly or parasitoid adults emerged. In total, ten additional exposures were conducted. The mean percentage parasitism at dissection was compared with the percentage emergence of each wasp species obtained from rearing.

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Interspecific host discrimination

Naqve female wasps of each species were first individually provided with two 2-day-old unparasitized host puparia to obtain oviposition experience 12 h prior to the experiment. The experienced wasps were then used for the experiment. All host puparia used in this experiment were 2 days old.

A single *D. giffardii* female was provided with four unparasitized puparia and four puparia previously parasitized by *P. vindemmiae* for 24 h, while a single *P. vindemmiae* female was provided with six unparasitized puparia and six puparia previously parasitized by *D. giffardii*, also for 24 h. The number of hosts provided was high relative to the eggload of their parasitoids in order to maximize the possibility of preferred host selection.

Hosts previously parasitized by *D. giffardii* were prepared using the same method as in the previous experiment and were selected through external examination under a microscope, while hosts parasitized by *P. vindemmiae* were obtained through direct observation of ovipositions under a microscope. Preliminary observations and dissections showed that *P. vindemmiae* rarely self-superparasitize, but often repeatedly probe the same host several times before foraging for other *C. capitata* puparia even when searching alone. The time leading up to an actual oviposition (5.4 ± 0.7 min, $n = 10$) was significantly longer than probing during non-oviposition (1.6 ± 0.3 min, $n = 10$); therefore, parasitized hosts were determined by noting oviposition times > 5 min.

The test for both parasitoid species was conducted as follows: unparasitized and parasitized hosts were placed in a random arrangement over sand in a container (3 × 5 × 4 cm) with air holes on the lid, with a droplet of diluted honey provided as food inside the container. A single female of either species was released into the container. After 24 h exposure, all the exposed hosts were dissected within 1–2 days to determine the presence of both parasitoid species.

Each test was repeated 30 times. In a few cases, some of the hosts were not parasitized by the first parasitoid species, leading to a slightly unbalanced presence of parasitized vs. unparasitized hosts; these replicates were excluded from the analysis.

Data analysis

All comparisons of mean values between two different treatments within one species or two different species were performed using Student t-test (JMP 4.1, SAS Institute, Cary, North Carolina). All proportional data were transformed by arcsine square root before an analysis of variance.

Results

Host age selection and its effect on offspring survival

Both *D. giffardii* and *P. vindemmiae* could attack < 1-day-old *C. capitata* puparia in choice and no-choice experiments. However, they both preferred to attack 2- to 3-day-old rather than < 1-day-old host puparia (*D. giffardii*, $t_{44} = -6.3$, $P < 0.001$; *P. vindemmiae*, $t_{38} = -6.0$, $P < 0.001$) (fig. 1).

Attacks on young puparia resulted in about 20% egg mortality in both parasitoid species, while all eggs of both species survived in the 2- to 3-day-old puparia (table 1). A sub-sample dissected immediately following exposure of young puparia to each parasitoid found that 43.9% of *D. giffardii* eggs ($n = 39$) and 21.2% of *P. vindemmiae* eggs ($n = 33$) had obvious black spots on the surface of their chorion when bathed in the host haemolymph. It was observed that some of these blackened eggs of *D. giffardii* successfully hatched in the young puparia. Thus, the actual egg mortality for *D. giffardii* based on dissection (20.7%, table 1) two days later (by which time all surviving eggs had hatched) was low.

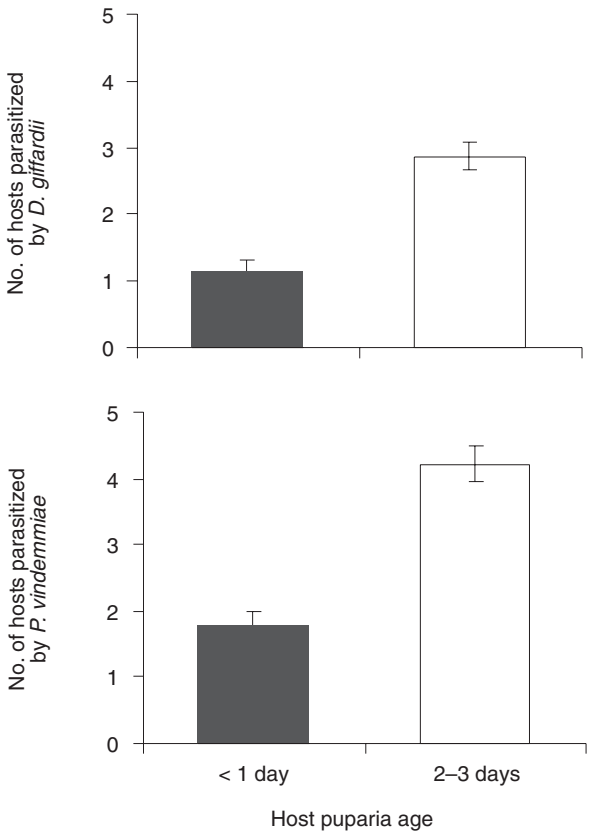

Fig. 1. Preference of host puparia age of *Ceratitis capitata* by *Dirhinus giffardii* and *Pachycrepoideus vindemmiae*. Bars refer to mean and standard error.

It was observed that in young host puparia containing *D. giffardii* eggs the host pupae become fully formed two days following an attack by *D. giffardii*. Once *D. giffardii* eggs hatched and the first instar larvae started feeding, the host ceased development. This indicated that *D. giffardii* did not paralyse its host at the time of attack. In contrast, *P. vindemmiae* permanently paralysed its hosts during parasitism, as it was observed that young hosts no longer continued to melanize after the parasitoid's oviposition.

Rearing results also showed that oviposition into young puparia resulted in higher mortality of parasitoid offspring than oviposition into old puparia for *D. giffardii* ($t_{18} = -8.0$, $P < 0.001$) (table 1). Attacks of young puparia by *P. vindemmiae* resulted in complete death of all parasitoid offspring (table 1). Although most *P. vindemmiae* eggs successfully hatched (table 1), the parasitoid larvae were unable to develop into adults because they were embedded into dead and dried host tissues.

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Table 1. Effect of host puparia age of *Ceratitis capitata* on the offspring survival of *Dirhinus giffardii* and *Pachycrepoideus vindemmiae*.

Parasitoid species	Host puparia age (days)	Dissection		Rearing
		n	% Parasitoid eggs died	% Emergence of adults
D. giffardii	< 1	10	20.7 ± 3.58 a	40.0 ± 5.82 a
	2–3	10	0.0 ± 0.0 b	94.0 ± 2.43 b
P. vindemmiae	< 1	10	22.9 ± 3.73 a	0.0 ± 0.0 a
	2–3	10	0.0 ± 0.0 b	93.3 ± 2.54 b

Values (mean ± SE) were compared with the same species between the two different host age groups; different letters within the same column indicate a significant difference (Student t-test, $P < 0.05$). Percentage emergence of adults was corrected based on the control mortality of unparasitized hosts for each host age group.

Interspecific competition

Regardless of the exposure order to *D. giffardii* and *P. vindemmiae*, immediate dissection following the exposures showed that both parasitoid eggs were still alive in multi-parasitized hosts (table 2), suggesting that both parasitoids were unable to directly kill the other species during multi-parasitism.

When hosts parasitized by *D. giffardii* were immediately exposed to *P. vindemmiae*, dissections two days later showed that all *D. giffardii* and *P. vindemmiae* eggs hatched, but almost all first instar *D. giffardii* larvae were dead in the presence of *P. vindemmiae* larvae, while no *P. vindemmiae* larvae were killed in the presence of *D. giffardii* larvae (table 2). In the cases where *D. giffardii* eggs had hatched in the parasitized hosts before they were exposed to *P. vindemmiae*, dissections both immediately and two days later showed that almost all *D. giffardii* larvae were dead in the presence of *P. vindemmiae* eggs or larvae, while all *P. vindemmiae* eggs or larvae were alive (table 2). These results suggest that the paralysing chemicals injected by *P. vindemmiae* during oviposition kill developing *D. giffardii* larvae, while venom released by *D. giffardii*, if any, did not kill *P. vindemmiae* larvae.

When hosts parasitized by *P. vindemmiae* were immediately exposed to *D. giffardii*, dissections two days later showed that all hatched *D. giffardii* larvae were dead. Even when *P. vindemmiae* eggs had already hatched in the parasitized hosts, further exposure of these hosts to *D. giffardii* showed that, while *D. giffardii* eggs could survive, first instar larvae died immediately in the presence of *P. vindemmiae* larvae. These results further confirm that *P. vindemmiae* unconditionally wins in competition against *D. giffardii*.

Dissection did not show that any supernumerary eggs were killed by conspecific adult females in either species. In both parasitoid species, all eggs hatched but the supernumerary larvae were eliminated. Direct fighting between two *D. giffardii* larvae was frequently observed in dissected hosts, and rearing results showed that in all cases only one parasitoid adult emerged from each host. In *P. vindemmiae* it was often observed that two live, large host larvae occurred within a host, and in a few cases two adults emerged from the same host, suggesting that the venom injected by *P. vindemmiae* is not poisonous to conspecific individuals, and competition within the same species may be by physical attack.

There was no difference in the resultant percent parasitism of hosts between *D. giffardii* and *P. vindemmiae* when the hosts were exposed to one species immediately followed by the other species ($t_{18} = -1.9$, $P > 0.05$) (fig. 2). However, a very low percentage of *D. giffardii* adults

Table 2. Competitive outcome between *Dirhinus giffardii* and *Pachycrepoideus vindemmiae* under different exposure orders and intervals of host *Ceratitis capitata* puparia to both parasitoids.

Exposure order		Exposure interval (h)	Dissection time after the 2nd exposure (h)	No. dissected	*D. giffardii*		*P. vindemmiae*	
1st species	2nd species				Stage	No. killed	Stage	No. killed
D. giffardii	*P. vindemmiae*	0	0	36	Egg	0	Egg	0
			48	28	Larvae	25	Larvae	0
		48	0	78	Larvae	63	Egg	0
			48	35	Larvae	34	Larvae	0
P. vindemmiae	*D. giffardii*	0	0	20	Egg	0	Egg	0
			48	26	Larvae	26	Larvae	1
		48	0	20	Egg	0	Larvae	0
			48	36	Larvae	36	Larvae	0

All hosts were exposed to the first parasitoid species for 6 h, then after either 0 h or 48 h they were exposed to the second parasitoid species for another 6 h. Stage refers to the parasitoid developmental stage within host puparia at the time of dissection. Number of killed parasitoids refers to cases in which one species individual died in the presence of another species individual within multi-parasitized hosts that were dissected.

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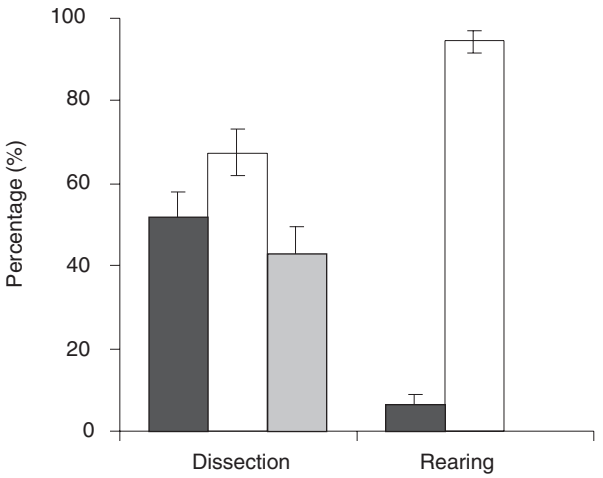

Fig. 2. Comparison between percentage parasitism observed during dissection and percentage emergence of adult wasps from reared hosts of *Ceratitis capitata* puparia that were exposed to *Dirhinus giffardii* (■) and *Pachycrepoideus vindemmiae* (□) in succession. Bars refer to mean and standard error; (▨) multi-parasitism.

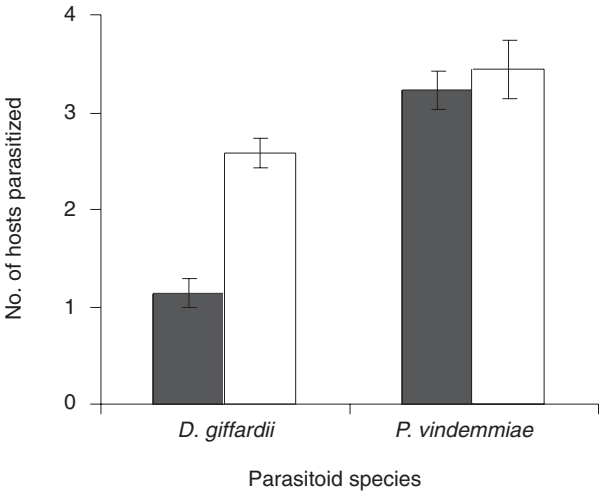

Fig. 3. Interspecific host discrimination by *Dirhinus giffardii* and *Pachycrepoideus vindemmiae*. Bars refer to mean and standard error; ■, hosts previously parasitized by other species; □, unparasitized hosts.

successfully emerged from the rearing of the hosts exposed to both parasitoids ($t_{18} = -6.2$, $P < 0.001$) (fig. 2). Comparison of the results between the dissection and rearing further confirmed that *P. vindemmiae* always won in competition against *D. giffardii* (fig. 2).

Interspecific discrimination

On average, *D. giffardii* laid 3.7 ± 0.2 eggs ($n = 29$), while *P. vindemmiae* laid 6.7 ± 0.4 eggs ($n = 27$) over 24 h in a choice experiment given both unparasitized hosts and hosts previously parasitized by the other species. *Dirhinus giffardii* attacked more unparasitized hosts than hosts previously parasitized by *P. vindemmiae* ($t_{56} = -7.0$, $P < 0.001$) (fig. 3); while *P. vindemmiae* showed no preference for unparasitized vs. previously parasitized hosts ($t_{56} = -0.6$, $P > 0.05$) (fig. 3). The results suggest that *D. giffardii* can and does discriminate against hosts previously parasitized by *P. vindemmiae*; while *P. vindemmiae* either cannot or does not discriminate.

Discussion

The results showed that *D. giffardii* does not kill its hosts until the first instar larvae hatch about two days after oviposition, while *P. vindemmiae* permanently paralyses its hosts during the process of oviposition. In terms of this trait, *D. giffardii* acts more like a koinobiont while *P. vindemmiae* is a typical idiobiont (Godfray, 1994; Quicke, 1997). Although both parasitoids are similar in many other respects, the slight difference in life-history strategy overwhelmingly affected the competitive outcome.

Some parasitoid species physically kill the eggs or larvae of other parasitoid species already present in a host when they multi-parasitize the host (e.g. Leveque *et al.*, 1993; Pedata *et al.*, 2002). There was no evidence of this physical killing by either *D. giffardii* or *P. vindemmiae*. However, death of *D. giffardii* larvae occurred soon after multi-parasitization by *P. vindemmiae*. Thus, the killing of *D. giffardii* larvae in the presence of *P. vindemmiae* eggs resulted from the toxic venom injected by *P. vindemmiae* at the time of oviposition, that not only paralyses the host but also interspecific competitors within the host. In many hymenopterous ectoparasitoids of lepidopterous and coleopterous larvae, female wasps inject venom into the host at the time of oviposition (Godfray, 1994; Quicke, 1997). It is well known that the maternal venom may disrupt host feeding, moulting, movement and /or viability (Shaw, 1981; Coudron & Puttler, 1988; Coudron *et al.*, 1990; Quistad *et al.*, 1994; Weaver *et al.*, 1997). However, virtually nothing is known about the effects of ectoparasitic venom on other individuals of the parasitoid itself, or other competing parasitoid species. The present results suggest that the venom injected by *P. vindemmiae* resulted in specific physiological changes within the fly host and adverse impacts were limited to individuals of *D. giffardii* rather than conspecific individuals, as supernumerary larvae of *P. vindemmiae* were not immediately killed. In several cases, more than one adult *P. vindemmiae* developed to the adult stage within a single host puparium.

It appears that first instar larvae of *D. giffardii*, rather than the adult ovipositing females, secrete venom that paralyses the host. However, even when *D. giffardii* larvae paralysed and killed a host, *P. vindemmiae* still survived and eliminated *D. giffardii*, suggesting that the latter is a physiological generalist, an important trait for host expansion in many ectoparasitoids (Wang & Messing, 2004a,b).

In general, prior possession of a host is an important factor in competitive outcomes, especially among fruit fly endoparasitoids (Wang & Messing, 2002, 2003b; Wang *et al.*, 2003). When competition between an egg-pupal and a larval-pupal parasitoid occurs, the early acting egg-pupal parasitoid *F. arisanus* always prevails through physiological suppression of the eclosion of the later attacking larval-pupal parasitoid species. However, in the case of pupal ectoparasitoids, the competitive outcome seems to depend more on whether or not the parasitoid can releases toxic

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factors that not only perturb host haemocytes, but also prevent the development of other competing species feeding on the same host, although the sources and chemistry of this adult parasitoid-derived venom by *P. vindemmiae* are currently unknown.

Interspecific discrimination is mainly observed among phylogenetically close competitors (Vet *et al*., 1984), or parasitoid species that have sympatrically co-evolved for many generations (Gauthier *et al*., 1999). Being taxonomically distinct was thought to make a parasitoid less likely to discriminate between healthy hosts and hosts parasitized previously by other species, particularly among ectoparasitoids (Godfray, 1994; but see Zaviezo & Mills, 2001). In this study it was found that *D. giffardii* was able to discriminate against *P. vindemmiae* although these two species are not phylogenetically close. Both theoretical and empirical studies suggest that it is advantageous for an inferior competitor to avoid multi-parasitism when there is a low chance of survival in the same host as a superior competitor (van Alphen & Visser, 1990; van Baaren *et al*., 1994; Wang & Messing, 2002, 2003b). In this case, both the acceptance of previously parasitized hosts by *P. vindemmiae* and the rejection of previously parasitized hosts by *D. giffardii* is adaptive; in terms of offspring survival there is no cost for *P. vindemmiae* but no chance for *D. giffardii*. It suggests that interspecific host discrimination even in ectoparasitoids is often adaptive and is related to the low survival chances of its offspring or low fitness consequences of interspecific interaction (van Alphen & Thunnissen, 1983; Gauthier *et al*., 1999; Zaviezo & Mills, 2001).

Unlike typical ectoparasitoids whose eggs do not necessarily face internal host defences, the eggs of *D. giffardii* and *P. vindemmiae* suffer the same complex host immune responses as faced by a typical koinobiont endoparasitoid (Vinson, 1990) if they attack young host puparia. Therefore, host age is a major factor determining offspring survival of both parasitoids, and the selection of different age host puparia by adult females has important consequences for offspring mortality. In general, younger host pupae are preferred for parasitoid oviposition (e.g. Vinson & Iwantsch, 1980; Wang & Liu, 2002), as younger hosts may offer nutrition of higher quality for parasitoid development (assuming that there is no trade-off between host age and offspring survival) (Charnov & Stephens, 1988). However, both *D. giffardii* and *P. vindemmiae* preferred to attack older rather than younger host puparia. Because attacking young puparia resulted in a high juvenile mortality in *D. giffardii*, and complete death in *P. vindemmiae*, the selection of old host puparia is probably an adaptation to reduce juvenile mortality in both species.

The slightly different life-history strategies reflect a compromise between the two syndromes from typical koinobiont to typical idiobiont. The advantage for *D. giffardii* is that it can attack relatively young hosts, as it allows them to continue growing after parasitism, but might be unsuccessful attacking older hosts, that could emerge before eclosion of the parasitoid's eggs. Dresner (1954) observed that when *D. giffardii* attacked an old host puparium in which the host was close to emergence, no parasitoid developed, probably due to the fact that the fly had emerged before eclosion of the parasitoid egg. However, the advantage for *P. vindemmiae* is that it can also attack relatively older host puparia. It was observed that the parasitoid could successfully develop even from 5-day-old pupae of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) in which the fly was close to emergence (Phillips, 1993) and in 15-day-old pupae of *Asobara tabida* Nees (Hymenoptera: Braconidae) where the wasp was in its pharate stage within the host puparium (van Alphen & Thunnissen, 1983). Although *P. vindemmiae* kills hosts during attack and may not suffer from the host's immune response following parasitism, the parasitoid cannot survive when attacking a young host because the unformed host pupa dies quickly and the parasitoid egg or larva becomes trapped inside the dead host tissues.

Acknowledgements

The authors wish to thank T. Moats for assistance and the USDA-ARS Pacific Basin Agricultural Research Center for providing fruit fly eggs. The authors also thank the two anonymous reviewers and the subject editor for their very useful comments on an earlier version of this manuscript. This research was supported by USDA-ARS grant 5853208147 to R.H.M. This is Paper 4683 of the UH College of Tropical Agriculture and Human Resources Journal Series.

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(Accepted 1 June 2004)

https://doi.org/10.1079/BER2004318 Published online by Cambridge University Press

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