



## Exposure to competitors influences parasitism decisions in ectoparasitoid fly larvae



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Much theoretical work has been done regarding patch exploitation in insects and several mechanisms have been proposed to describe and predict behaviours under different situations. However, almost no theoretical framework has been developed for parasitoids with host-seeking larvae, even though similar selection pressures are faced by the female of hymenopteran parasitoids and the larvae of dipteran parasitoids. Here we propose and show that factors such as pre-parasitism competition and host physiological state can modulate host orientation and acceptance behaviours in a dipteran parasitoid larva. When larvae were exposed to pre-parasitism competition and then offered different host odours and live hosts, they oriented towards and more readily accepted suboptimal hosts and were more prone to superparasitize. Our results show that the internal state modulates individual decisions that dipteran parasitoids make, confirming the presence of many previously neglected strategies in parasitoids with host-seeking larvae. Hence, comparative studies should be undertaken to form a complete picture of parasitism strategies.

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Patch exploitation strategies in parasitoids have long been studied with both theoretical and experimental approaches. Many theoretical and mathematical models have been developed, with the marginal value theorem (Charnov, 1976), the ideal free distribution (Fretwell & Lucas, 1969) and Waage's (1979) model among the most important. Although useful in starting to understand the principles that rule time allocation to different resource patches by a single individual, the marginal value theorem and the ideal free distribution did not consider behavioural mechanisms mediating patch time allocation or the mechanisms by which animals acquire information about the environment (van Alphen, van Bernstein, & Driessen, 2003; Wajnberg, Bernstein, & van Alphen, 2008). Waage's model attempted to describe the effect of individuals' capacity to obtain information about the quality of the environment leading to increases or decreases in patch time residence (incremental and decremental effects, respectively) after host encounters (van Alphen et al., 2003; Waage, 1979).

Since the publication of these models, many experimental studies have been conducted on insect parasitoids, testing the effects of patch characteristics, female condition, prior visits to host patches and abiotic conditions on patch time allocation (see review by Wajnberg, 2006). Regarding patch characteristics, many studies estimated patch quality by the different number of available hosts, the proportion of healthy hosts, the proportion of different host instars or the presence of competitors in the patch (Wajnberg, 2006). In the majority of studies, patch residence time increased with patch quality. Conversely, when patch quality decreased, behaviour also changed (e.g. shorter patch time residence times and increased acceptance of previously parasitized hosts: Hopper, Prager, & Heimpel, 2013; Outreman, Le Ralec, Wajnberg, & Pierre, 2001).

While this work generated many advances, almost all the theory and experiments were developed for hymenopteran parasitoids where it is the adult female that locates a prospective host and decides whether to use it for ovipositing or host feeding, or to reject it (Godfray, 1994). However, many dipteran and coleopteran parasitoids show a split host-locating strategy where the adult places its eggs near the host and the larvae express active host-seeking behaviour (Brodeur & Boivin, 2004; Feener & Brown, 1997; Godfray, 1994). Since it is the first-instar larvae of dipteran and coleopteran parasitoids that locate the host, they can be viewed as the ecological equivalent of female hymenopteran parasitoids, and

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we expect them to express similar behaviours (Brodeur & Boivin, 2004; Feener & Brown, 1997).

It is well accepted that because there exists a direct relationship between oviposition decisions and fitness, selective pressures should be important in shaping the behavioural mechanisms that determine patch exploitation (van Alphen et al., 2003). Parasitoid females can spread their fitness gain by ovipositing in different hosts. But for a host-seeking larva, the cost of choosing a low-quality host is great because its entire fitness comes from a single host (Brodeur & Boivin, 2004). So, selection pressures might shape the time that larvae spend evaluating host quality much as they do patch searching time for female parasitoids.

The evolution of behavioural mechanisms in parasitoids with host-seeking larvae depends on the distribution of hosts. If hosts are aggregated, the probability of finding more than one host is high. In such conditions, host-seeking larvae may be likely to have evolved discrimination ability (Brodeur & Boivin, 2004). In fact, it has been already shown that host-seeking larvae of different species are capable of locating hosts by means of chemical cues and that host discrimination occurs (Castelo & Lazzari, 2004; Crespo & Castelo, 2009; Goubert, Josso, Lou pre, Cortesero, & Poinso, 2013; L pez, Ferro, & Van Driesche, 1995; Royer, Fournet, Brunel, & Boivin, 1999). In addition to the distribution of hosts, host discrimination could have important adaptive value in species where the host-seeking larvae are long-lived since the probability of finding several hosts in its lifetime is high. However, the effect of host species, size, age, parasitization, instar and nutritional state on host selection by actively seeking first-instar larvae has been little studied and poorly understood.

In addition to patch quality, another source of information that influences patch exploitation is the presence of competitors in the same patch or exposure to competition prior to foraging (Wajnberg, 2006). This information is often used by hymenopteran parasitoids and determines patch residence time and superparasitism depending on its physiological state (Mangel, 1989; Visser, van Alphen, & Nell, 1992). In these cases, a war of attrition is expected where the first female leaving a patch is prone to lose offspring to larval competition if other females remain in the patch and continue to oviposit (Sjerps & Haccou, 1994; van Alphen, 1988). Goubault, Outreman, Poinso, and Cortesero (2005) studied the effect of intraspecific competition in patch residence time in a parasitoid wasp and found that when wasps simultaneously exploited a patch, and hence directly competed, superparasitism increased. They also showed that when wasps had experienced intraspecific competition before the tests, and hence early competition, the proportion of females leaving the patch increased. In the few other studies where the effect of early competition was evaluated, it resulted in an increase of self-superparasitized hosts (Hoffmeister, Thiel, Kock, Babendreier, & Kuhlmann, 2000; Visser, van Alphen, & Nell, 1990; Visser et al., 1992). In the only study where competition has been addressed in host-seeking larvae, the degree of superparasitism increased significantly with the number of foraging conspecifics and the age of the larva when hosts were scarce (Royer et al., 1999).

Given the lack of information on how factors such as host quality (parasitism status and instar) and competition influence individual decisions that host-seeking larvae make, we studied these effects on host location and host acceptance in *Mallophora ruficauda* (Diptera: Asilidae). This solitary ectoparasitoid of the white grub *Cyclocephala signaticollis* (Coleoptera: Scarabaeidae) is a fairly well-studied species with host-seeking larvae. In this species, the adult *M. ruficauda* starts its reproductive stage during early austral summer, but the susceptible host instar (i.e. third larval instar) only becomes available 2 months later (Crespo & Castelo, 2008). Unlike many other parasitoids where the female is responsible for locating

the host, *M. ruficauda* has a split host-location strategy (Castelo, Ney-Nifle, Corley, & Bernstein, 2006). Females lay egg clutches (328 eggs on average) on living plants and also on dry ones in grasslands where adult hosts are present. Females select oviposition sites based on plant height, and parasitism success is highest when eggs are placed on substrates 1.25–1.5 m tall. When the eggs hatch, the larvae are dispersed by the wind and, upon falling to the ground, they bury themselves into the soil. Then, after 1 week in the soil, they moult to the second instar and it is then when the location of the hosts begins (Crespo & Castelo, 2008). *Mallophora ruficauda* parasitizes mainly third-instar hosts of *C. signaticollis* and shows a high preference for this species in the field (Castelo & Corley, 2010). Larvae of *M. ruficauda* can survive 39 days using their own reserves, so the probability of finding several hosts during their life span is high (Crespo & Castelo, 2010). Crespo and Castelo (2009) studied the existence of host discrimination in this species and found that *M. ruficauda* is capable of determining a host's parasitism status (singly parasitized or healthy) by means of chemical cues.

The aim of this study was to determine the effects of hosts of different quality and intraspecific competition on the decisions leading to host location and acceptance. For this, we studied the effect of pre-parasitism competition on the orientation to chemical cues and acceptance of hosts of different quality based on their parasitism status and instar.

## METHODS

### Insects

We used larval *M. ruficauda* obtained from 1750 egg clutches collected from farms near Buenos Aires, Argentina, in 2010 and 2011. Immediately after egg hatching, neonatal larvae were separated either individually (no competition, NC) in 1.5 ml Eppendorf-type tubes or grouped in flasks (diameter = 5.0 cm; height = 10.0 cm), containing a moistened piece of filter paper as substrate. Grouped larvae were kept at a density of 500 larvae per flask (pre-parasitism competition, PPC). Each flask contained 100 larvae from five different egg clutches, and a total of 350 flasks were used throughout. This density was chosen because it is similar to field conditions (Crespo, n.d.). Drops of mineral water were added when necessary to avoid larvae dehydration. Since these larvae live buried in the soil, tubes and flasks were kept in darkness under controlled temperature ( $25 \pm 2^\circ\text{C}$ , 60–70% RH) until larvae were used in experiments. Since larvae can live many days in the absence of hosts or any other food source (39 days on average, Crespo & Castelo, 2010), larval age was considered during experiments and only young larvae between 6 and 12 days after moulting to the second instar were used. Each larva was used only once in the experiments and then reared to be released in the field.

Hosts were either killed and used for extraction of their chemical cues in homogenates (host orientation experiments) or kept alive (host acceptance experiments). Host stimuli used in the experiments were obtained from the hindgut of larvae of *C. signaticollis*, which were collected up to a soil depth of 30 cm in grasslands located in the same localities in Buenos Aires province. Hosts were maintained individually under controlled temperature ( $25 \pm 2^\circ\text{C}$ ) in black tubes filled with clean potting soil and fed weekly with fresh carrot pieces. To obtain the attracting stimulus from the host's hindgut, hosts were frozen and, once killed, a homogenate was made using hexane as the extraction solvent following the procedure outlined in Castelo and Lazzari (2004). An equivalent of 2.5 white grubs/ml was used throughout (Crespo & Castelo, 2008, 2009).

We tested the influence of pre-parasitism competition on the orientation to chemical cues and the acceptance of hosts of

different quality by *M. ruficauda*. In this species, healthy mature third instar of *C. signaticollis* is the preferred host instar and on which it best develops, hence, the optimal host (Castelo & Corley, 2010). Although hosts of different instars and parasitism status are usually encountered, these hosts are suboptimal either because they are not fully developed or because they result in superparasitism. To test the influence of host instar on parasitoid decisions, we used second- and third-instar larvae in host orientation and acceptance experiments. The recency of moulting influences host quality (Chapman, Simpson, & Douglas, 2013), so we offered two types of hosts. Suboptimal hosts were those that had moulted within 24 h, as their cuticles were not yet sclerotized and other physiological processes related to moulting rendered them unsuitable. Optimal hosts had moulted at least 7 days before the tests (Chapman, Simpson, & Douglas, 2013).

We also tested the influence of intraspecific parasitism on the proneness of free-living larvae to orient to and accept parasitized hosts. We performed artificial parasitism in the laboratory to create hosts that had been parasitized for the same period of time and rule out any difference in the nature of the host cue. In brief, we placed a parasitoid larva on the thorax of a healthy host and, after 3 days, we checked the occurrence of parasitism. Parasitized hosts were used either for extraction of chemical cues for orientation experiments or as live parasitized hosts in host acceptance experiments. It has already been established that this procedure does not change the parasitoid development on hosts compared to the natural parasitoid development (Crespo & Castelo, 2010). Hosts that were parasitized as a result of the experiments were raised until the emergence of the adult parasitoids, after which the parasitoids were released in the same localities where the larvae had been collected.

### Experimental Procedures

#### Host orientation

Orientation to host odours was tested in a dual-election air-stationary olfactometer, which consists in an acrylic box divided into three equal-sized zones (one central and two lateral) along the long axis ( $9 \times 6 \times 1$  cm, see Castelo & Lazzari, 2004). We placed a piece of filter paper impregnated with 10  $\mu$ l of either the host extract or hexane as a control in the lateral zones. In each test, an individual larva from either the NC group or from the PPC group (only one larva per flask was used) was released at the centre of the arena, and its position recorded after 90 min. Three possible responses were scored according to the position of the larva in one of the three zones of the arena: choice for the stimulus, choice for the control, or no decision if the larva remained in the middle zone. Table 1 reports the experiments performed in this part. After every test, each individual was discarded and the arena was cleaned up with water, ethyl alcohol and then dried with an air current in order to eliminate any possible remaining cue. All experiments were conducted between 1000 and 1700 hours on days where the barometric pressure was stable or increasing because it has been shown that drops in barometric pressure halt the orientation behaviour of the larvae (Crespo & Castelo, 2012). Experiments were carried out under laboratory conditions ( $26 \pm 1.0$  °C) and in darkness. A piece of damp filter paper at the top of the arena kept the relative humidity high inside the experimental device.

In these experiments, we tested the effect of host instar (second or third), moulting (<24 h (recently moulted third instar) or >7 days (third-instar)) and parasitism status (nonparasitized or singly parasitized) on host orientation and the influence of pre-parasitism competition on the individual choices of the parasitoid larva. For these, we tested orientation to the odours of hosts of different quality versus the extraction solvent as a control. In addition, we

**Table 1**

Treatments tested in the study of parasitism decisions of *M. ruficauda* larva towards *C. signaticollis* (CS) larvae of different quality

Cues used	Behaviour tested	Number of replicates	
		NC	PPC
—	Orientation	97	99
CS2	Orientation/Acceptance	108/13	102/33
CS3	Orientation/Acceptance	347/41	106/72
CS2-CS3	Orientation	95	94
CSrm	Orientation	85	105
CSp	Orientation/Acceptance	312/29	209/97
CSrm-CS3	Orientation	105	82
CSp-CS3	Orientation	106	104

NC: no competition; PPC: pre-parasitism competition; CS2: second-instar hosts; CS3: third-instar hosts; CSrm: hosts recently moulting to the third instar; CSp: hosts that were previously parasitized by another parasitoid (see Methods for details). For orientation experiments, host odour extracts were used, while for acceptance experiments, the host remained live and intact.

performed preference tests in which we offered simultaneously to the parasitoid larvae odours from two hosts of different quality (Table 1). Finally, we performed a control series where we offered only the solvent of extraction on both sides of the olfactometer to detect any asymmetry in choices due to characteristics of the set-up.

#### Host acceptance

For this experiment, we tested the parasitoid's decision of accepting or rejecting a host. We placed an individual larva, either from the NC or PPC group, on the host body, as for the artificial parasitism procedure, and checked for parasitism 3 days later. If the larva attached to the integument of the host, it was considered as host acceptance, while if no attachment had occurred, it was considered as rejection of the host. This procedure enabled us to study only host acceptance because there was no travel necessary to reach the host. The different treatments are outlined in Table 1.

In these experiments, we tested the effect of host instar (second or third) and parasitism status (nonparasitized or singly parasitized) on host acceptance and the influence of pre-parasitism competition on the individual choices of the parasitoid larva. We did not include the effect of moulting in this experiment because we allowed 3 days to elapse between placing the larva on the host and checking whether parasitism had occurred and, during this time, the odour of recently moulted hosts could change.

#### Statistical Analysis

For each treatment, we analysed the proportion of larvae orienting to the host with tests of homogeneity of proportions, which are multiple Tukey-type comparison tests (Zar, 2010). Then, when differences were found, we performed a posteriori contrasts comparing the proportion of individuals that had orientated to the stimulus or that had parasitized a host for every treatment with its corresponding control series in a procedure analogous to the Dunnett's test but applied when proportions are used (Zar, 2010). For analysing the proportion of larvae accepting a host, we conducted a difference of proportion test between the acceptance of suboptimal hosts (different instar or parasitism status) and the acceptance of the best host (healthy third instar).

## RESULTS

### Effect of Suboptimal Hosts on Larva Orientation and Acceptance

The orientation to odours of suboptimal hosts was influenced by previous competition experience. Larvae that experienced pre-

parasitism competition were attracted to odours of suboptimal hosts, whereas larvae that did not experience competition only oriented to odours of the optimal host (Tables 2, 3). Regarding host acceptance, similar results were found. Suboptimal hosts were parasitized at the same level as optimal hosts by larvae from the PPC group while those from the NC group preferentially accepted the best hosts available (Table 4). These results were not influenced by the experimental arena since no asymmetry was found when only the solvent was offered on both sides (proportion of larvae: NC: 0.505,  $\chi^2_2 = 0.010$ ,  $P > 0.9$ ; PPC: 0.515,  $\chi^2_2 = 0.091$ ,  $P > 0.9$ ).

#### Effect of Host Instar on Larva Orientation and Acceptance

Orientation to odours of second-instar hosts when offered alone was only evident in larvae of the PPC group, and the proportion that did so was similar to the proportion of NC larvae that oriented to odours of third-instar hosts (i.e. the control series: 0.598 versus 0.671; Table 2). Furthermore, larvae in the NC group preferred orienting to odours of third-instar hosts, but larvae in PPC group showed no preference for odours of second- or third-instar hosts when offered simultaneously (0.611 versus 0.585; Table 3). Regarding acceptance, NC larvae showed significantly less acceptance of second-instar hosts than they did of third-instar hosts (0.667; Table 4). In contrast, PPC larvae showed similar levels of acceptance of second- and third-instar hosts (0.975; Table 4).

#### Effect of Host Moulting on Larva Orientation

Odours of recently moulted hosts were not attractive to NC larvae but were attractive to PPC larvae (0.471 versus 0.610; Table 2). When odours of recently moulted and third-instar hosts were offered simultaneously, NC larvae oriented to odours of third-instar hosts, whereas PPC larvae were distributed randomly, showing no preference for odours of either host (0.686 versus 0.451; Table 3).

#### Effect of Host Parasitism Status on Larva Orientation and Acceptance

Orientation to odours of parasitized hosts was only evident in PPC larvae (0.617; Table 2). In contrast, NC larvae preferred orientating to odours of third-instar hosts, but PPC larvae showed no preference for either parasitized or third-instar host odours when offered simultaneously (0.604 versus 0.518; Table 3). Regarding acceptance, NC larvae were significantly less likely to accept parasitized hosts (i.e. superparasitize) than they were to accept third-instar hosts (0.610; Table 4). In contrast, PPC larvae accepted superparasitized hosts as often as they did healthy third-instar hosts (0.821; Table 4).

## DISCUSSION

In this work we studied the effect of pre-parasitism competition on orientation towards and acceptance of suboptimal hosts by active host-seeking larvae. Our results show that exposure to conspecifics prior to locating hosts modifies a larva's decisions and lowers its selectivity threshold. In particular we were able to show for the first time that active host-seeking larvae show many characteristics similar to those of adult female parasitoids.

When larvae were raised in an environment with a high density of conspecifics, and hence experienced pre-parasitism competition, they oriented to odours of second-instar hosts and showed no preference when odours of a second-instar host and their normally preferred third-instar host were presented simultaneously. Thus, the larvae lowered their selectivity threshold and oriented to odours of suboptimal hosts. In a separate experiment, larvae exposed to competition accepted poor-quality hosts. This change in the selectivity threshold could be adaptive when competition is likely since it could allow larvae to parasitize hosts that would otherwise not be selected by larvae lacking experience in pre-parasitism competition. Competition is to be expected in the field since females of *M. ruficauda* place eggs continuously from January until March, but second-instar hosts appear in the field in February. So, when second-instar hosts become available, a large number of parasitoid larvae could already be waiting for hosts in the soil. Flexibility in host selectivity would allow larvae to attach to a host that, although suboptimal, is suitable for development because a larva attached to a second-instar host allows the host to feed and reach the third instar before actively feeding on it (Crespo, n.d.). In addition, when larvae were offered odours of recently moulted hosts, only parasitoid larva that experienced pre-parasitism competition oriented to the host. This result could indicate that the source of odour from the host does not change during moulting, but after moulting, hosts become much more attractive.

Finally, when larvae were offered odour extracts from already parasitized hosts as well as live hosts, larvae exposed to pre-parasitism competition were attracted to and accepted suboptimal hosts, whereas larvae that were raised individually maintained their selectivity for the best hosts. These results confirm that this parasitoid is capable of intraspecific host discrimination, as has already been determined (Crespo & Castelo, 2009). In our experiments, host discrimination based on parasitism status was due to changes in the host odours since we observed discrimination based only on extracts of the posterior intestine of hosts. It has been seen for other systems that once parasitism occurs, the chemical profile of an individual is modified into a novel chemical identity (Brodeur & Boivin, 2004; Lebreton, Christidès, Bagnères, Chevrier, & Darrouzet, 2010). Again, the results from the orientation experiments were aligned with choices made by larvae during host acceptance: larvae that experienced pre-parasitism competition superparasitized more readily than larvae raised individually. This

**Table 2**

A posteriori contrasts comparing the proportion of parasitoid larvae that orientated towards the odour of *C. signaticollis* (CS) hosts of different quality when tested with hexane against the control (i.e. proportion of larvae in the no competition group that oriented to healthy third-instar hosts)

	Effect of host instar						Effect of host moulting			Effect of parasitism status		
	CS3			CS2			CSrm			CSp		
	P(st)	Q <sub>2</sub>	P	P(st)	Q <sub>2</sub>	P	P(st)	Q <sub>2</sub>	P	P(st)	Q <sub>2</sub>	P
NC <sup>1</sup>	0.671	(control)		0.537	2.716	<0.01	0.471	3.60	<0.01	0.532	3.350	<0.01
PPC	0.662	0.175	>0.05	0.598	1.480	>0.05	0.610	1.25	>0.05	0.617	1.258	>0.05

CS3: healthy third-instar host odour; CS2: healthy second-instar host odour; CSrm: recently moulted healthy third-instar host odour; CSp: singly parasitized third-instar host odour; P(st): proportion of larvae that oriented to the stimulus offered; NC: no competition; PPC: pre-parasitism competition; Q: analogous to Dunnett's test (critical values:  $Q_{0.05(1),\infty,3} = 1.92$  and  $Q_{0.01(1),\infty,3} = 2.56$ ).

<sup>1</sup> Since the response of NC larvae to CS3 odours was used as a control, there is no comparison against itself (P(st) = 0.671).



**Table 3**  
A posteriori contrasts comparing the proportion of parasitoid larvae that oriented towards the odour of a healthy third-instar *C. signaticollis* host or towards the odour of a suboptimal host offered simultaneously<sup>1</sup>

	Control (CS3 vs Hx)		Effect of host instar (CS2 vs CS3)			Effect of host moulting (CSrm vs CS3)			Effect of parasitism status (CSp vs CS3)		
	P(st)		P(st)	Q <sub>2</sub>	P	P(st)	Q <sub>2</sub>	P	P(st)	Q <sub>2</sub>	P
NC	0.671		0.611	1.221	>0.05	0.686	−0.315	>0.05	0.604	1.373	>0.05
PPC	—		0.585	1.71	<0.05	0.451	4.132	<0.01	0.518	3.117	<0.01

CS3: healthy third-instar host odour; Hx: hexane; CS2: healthy second-instar host odour; CSrm: recently moulted healthy third-instar host odour; CSp: singly parasitized third-instar host odour; P(st): proportion of larvae that orientated to CS3 odours when offered simultaneously with odours of suboptimal hosts; NC: no competition; PPC: pre-parasitism competition; Q: analogous to Dunnett's test (critical values:  $Q_{0.05(1),\infty,2} = 1.63$  and  $Q_{0.01(1),\infty,2} = 2.33$ ).

<sup>1</sup> Responses were compared to the control (i.e. response of NC larvae when offered only CS3 odours; P(st) = 0.671).

result could also have its correlate with a common scenario in the field. Given that dispersion depends on wind conditions, it seems likely that egg clutches that hatch when wind speed is low may fall to the soil in a reduced area, thus increasing the density of conspecifics searching for hosts. In addition, larvae that hatch out early in the season (January) accumulate in the soil as the season progresses, waiting for hosts to appear. In this context, superparasitism could be an adaptive strategy to adopt because, at least in this species, the chance of winning the larval competition when two larvae are attached to the host is approximately 50% irrespective of the time of arrival on the host (Barrantes & Castelo, n.d.). Nevertheless, after the peak of activity of *M. ruficauda* females (i.e. end of February), the scenario is different. Many hosts are already in their third instar and fewer remain as second instars. Also, wind conditions increase, favouring the spread of larvae in the environment from hatching sites (Castelo et al., 2006). Then, parasitoid larvae have a higher probability of finding healthy hosts because they disperse more broadly.

Under this scenario, not being selective could be beneficial for larvae at the start of the season since there would be few suitable hosts. Hence, accepting a second-instar host would assure a host on which to develop and could also provide access to additional resources until a third-instar host becomes available. Being selective at this stage could imply a higher cost in terms of energy expenditure to search for more suitable hosts. However, as the season progresses and more suitable hosts become available, selectivity could be a strategy that would benefit larvae in choosing the best available hosts. Towards the end of the season, when all available hosts are in the field, choosing a low-resource host could result in poor development that would lower fitness. Changes in selectivity of larvae could indicate that it is a dynamic process influenced by environmental factors such as the number of competitors and the number of suitable hosts available. If this is the case, then changes in selectivity should also be reversible, and larvae that lower their selectivity should be able to become more selective again when conditions change. Testing for the reversibility of the process would also help us to understand whether selectivity changes are indeed based on current environmental conditions or whether group

**Table 4**  
Proportion of larvae that accepted suboptimal hosts versus the proportion that accepted healthy third-instar *C. signaticollis* hosts

	Control (CS3)		Effect of host instar (CS2)			Effect of parasitism status (CSp)		
	Prop		Prop	$\chi^2_2$	P	Prop	$\chi^2_2$	P
NC	1.000		0.667	17.378	<0.01	0.610	34.257	<0.01
PPC	1.000		0.975	0.027	>0.95	0.821	3.670	>0.05

CS2: healthy second-instar host odour; CS3: healthy third-instar host odour; CSp: singly parasitized third-instar host odour; Prop: proportion of larvae that accepted hosts; NC: no competition; PPC: pre-parasitism competition;  $\chi^2$ : chi-square test.  $P < 0.05$  denotes a statistically significant difference.

rearing somehow permanently impairs the ability to detect characteristics of high-quality hosts.

*Mallophora ruficauda* larvae express many behaviours that are similar to hymenopteran parasitoids. It is well known that physiological state (e.g. age, eggload) can influence parasitoid exploitation strategies (Charnov, 1976; Mangel, 1989; Outreman, Le Ralec, Wajnberg, & Pierre, 2005; Wajnberg et al., 2008). Similarly, parasitoids with host-seeking larvae are capable of modulating their behaviours given different environmental conditions. Our results show that these parasitoids are capable of acquiring information from their environment and responding in adaptive ways. In fact, *Aleochara bilineata*, a coleopteran parasitoid with characteristics very similar to those of *M. ruficauda*, are capable of host discrimination and kin recognition, recognizing siblings and deciding whether to superparasitize or not based on whether the previous parasite was a sibling (Lize, Carval, Cortesero, Fournet, & Poinso, 2006; Royer et al., 1999). In particular, host selectivity might change in response to the quality of the resource and the level of competition in the environment. This pattern would be explained if animals use the presence of conspecifics as an indication that high-quality resources are present (Stamps, 1987). However, conspecifics could also indicate the level of scramble competition since the presence of competitors on a particular resource will reduce the fitness of individuals that use that resource (Davis, Nufio, & Papaj, 2011). These two mechanisms can be separated if context-dependent experiments are performed since individuals should accept both low- and high-quality resources in presence of competitors if conspecifics are taken as an indication of host quality. In our experiments we were able to show that conspecific pre-parasitism competition informs *M. ruficauda* larvae of the level of competition, not the quality of the resource.

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