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Behavioural plasticity induced by intraspecific competition in host orientation in a parasitoid

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Abstract. 1. Accurate measurement of external conditions is fundamental for survival. For parasitoids, in particular, sensing the environmental conditions is key because they are short-lived animals that must acquire information shortly after emergence.

2. This study investigated whether conspecifics during larval growth can influence and modify the decision to orient to different quality hosts in a parasitoid with an active host-seeking larva. How the density of conspecifics during growth modifies these decisions was also studied.

3. When larvae were submitted to increases in the intensity of pre-parasitism competition and then offered different host odours, they increased the orientation to poor-quality hosts likewise. It was also found that this behaviour is general to orientation to hosts in different physiological states.

4. These results show that pre-parasitism competition can influence and modulate orientation to poor-quality hosts when high-quality hosts are not available.

Key words. Asilidae, density dependence, host location, pre-exposition competition.

Introduction

Environmental stochasticity can lead to the expression of different phenotypes with different degree of success. Genotypes responsible for the expression of these phenotypes should be at a disadvantage against genotypes that reduce temporal variation in fitness (Rajon *et al.*, 2014). Reduced variation in fitness can be a result of an accurate measurement of external conditions, but can also occur by random expression of several phenotypes, each successful in a given environment (strategy known as bet hedging) (Hopper, 1999; Rajon *et al.*, 2014).

Effective sensing of the environmental conditions is of paramount importance because it not only dictates the speed of response to change, but it will also allow the individual to seize the best available conditions, contributing to its success in a particular environment (Chown & Terblanche, 2006). In parasitoids, in particular, sensing the environmental conditions is central because, in the adult stage, they are short-lived animals that must acquire information shortly after emergence, as opposed to long-lived animals which can learn in time and obtain information from the environment across seasons (van Alphen *et al.*, 2003). For parasitoids, assessing efficiently the best available host is directly linked to their fitness. Failing to correctly determine the identity or current host state can result in parasitising a low-value host.

The resource value of a habitat for a parasitoid is determined by: (i) the characteristics of the habitat; (ii) the decisions of the parasitoid; and (iii) the decisions of conspecifics (Visser *et al.*, 1992). The characteristics of the habitat include the time travel between patches, the number of hosts in the patches and the number of competitors (Visser *et al.*, 1992). Regarding the decisions of the parasitoids, host selection and patch time may influence habitat value (Visser *et al.*, 1992). Hence, habitat value influences behavioural decisions parasitoids make, but in turn, these decisions also modify habitat value. Finally, the decisions of conspecifics might also modify habitat value as many competitors can rapidly deplete a patch, lowering its value. However, a high number of competitors in a patch can be an indicator of a high-value habitat.

These models and experimental studies showed that individual decisions may determine not only individual fitness but also habitat value impacting directly at a population level. However,

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all of them assume that the individual can somehow acquire or estimate information on the current state of the habitat. A habitat with high availability of healthy hosts should imply a higher habitat value than habitats with low abundance of healthy hosts. One aspect of the way in which parasitoids can perceive information on host abundance that has received less attention is the influence of conspecifics prior to foraging or parasitising a host. The few studies that have addressed this issue show that pre-parasitism competition can influence foraging decisions (Visser *et al.*, 1992; Goubault *et al.*, 2005; Crespo *et al.*, 2015). Pre-parasitism competition can modify parasitoid strategies like retreating from a patch, increased superparasitism or acceptance of poor-quality hosts (Royer *et al.*, 1999; Goubault *et al.*, 2005; Crespo *et al.*, 2015). Retreating from a patch can lead to exploiting a new patch, but accepting poor-quality hosts can incur similar risks to superparasitism.

Superparasitism of a host incurs a higher risk for solitary than for gregarious parasitoids because only one individual per host will be able to reach the adult stage. In this context, mechanisms that help parasitoids determine and evaluate the nutritional and parasitism status of a host are highly beneficial (van Alphen & Visser, 1990; Godfray, 1994). However, errors in determining the host status incur a higher risk for parasitoids with host-seeking larvae than for parasitoids where the female chooses a host. If a female were to misplace an egg on a poor-quality host whenever there is a better host, it would diminish its fitness, but it may still have more opportunities to lay eggs due to its large egg load. However, a larva seeking a host usually only gets one decision regarding which host to accept. If that decision turns out to be wrong, the larva may die before reaching the adult stage.

A well-studied case of split host location behaviour and host discrimination is the dipteran parasitoid *Mallophora ruficauda* Wiedemann (Diptera: Asilidae). This species is an endemic robber fly of Argentina, particularly of the Pampas region inhabiting open grasslands near bee farms (Castelo, 2003). It is a solitary koinobiont ectoparasitoid of scarab beetle larvae which are also known as white grubs. *Mallophora ruficauda* has a high preference for parasitising the third-instar larvae of *Cyclocephala signaticollis* Burmeister (Coleoptera: Scarabaeidae) in the field (Castelo & Capurro, 2000; Castelo, 2003; Castelo & Corley, 2010). In this species, females place their eggs on tall grasses away from hosts that live underground. Parasitoid larvae are dispersed by the wind, they bury themselves once they reach the soil and, after moulting to the second instar, actively locate and parasitise hosts. Once moulted to the second instar, the parasitoid larva orientates to the host guided by chemical cues arising from the hosts' fermentation chamber (Castelo & Lazzari, 2004; Groba & Castelo, 2012). As eggs are placed on tall grasses away from the host and the wind is the main agent of dispersion, the number of larvae on any given portion of soil is very variable. This situation may lead to some environments where a high number of larvae are searching for hosts, increasing the probability of superparasitism (Castelo *et al.*, 2006).

Theory predicts that the acceptance of superparasitism in solitary parasitoids should be strongly influenced by the number of other females searching on the same patch and also by the probability of survival of their offspring (Vet *et al.*, 2002). As for parasitoids with host-seeking larvae, superparasitism might occur if the probability of winning a contest with a parasitoid that is already present is higher than the probability of survival until finding an unparasitised host (Godfray, 1994; Brodeur & Boivin, 2004). In the case of *M. ruficauda*, it has been seen that the order of parasitism events do not determine which larva wins competition (Barrantes, 2009). Also, superparasitism might occur if larvae are unable to detect a parasitised host. *Mallophora ruficauda* can discriminate its host regarding its parasitism status and indeed they can recognise but are not attracted to parasitised hosts when raised individually. However, when larvae are raised in an environment with a large amount of competitors, orientation and acceptance of poor-quality hosts increase (Crespo & Castelo, 2009; Crespo *et al.*, 2015). Despite the fact that *M. ruficauda* is a solitary parasitoid and shows high levels of host specificity, data from the field show that superparasitism is common, representing almost 50% of the parasitised hosts (Castelo, 2003).

Previous studies of *M. ruficauda* indicate that the density of conspecifics could be an important determinant for plasticity in orientation and acceptance of different quality hosts because when larvae fall to the ground after being dispersed by the wind, they can accumulate in the soil before starting to orient to the host. However, the functional relationship between the intensity of intraspecific competition and host orientation and selectivity is unknown. In this work we studied whether orientation to and acceptance of poor-quality hosts are plastic behaviours and whether they are influenced by past experiences such as increasing physical contacts with conspecifics when the levels of intraspecific competition increase. In order to achieve this, we first studied which hosts types (different stage, parasitism status or time since moulting) are poor-quality hosts for *M. ruficauda*. Then, we chose one host type of poor-quality host odour and tested the influence of increasing levels of intraspecific competition on orientation and acceptance behaviours. Finally, we tested whether orientation to odours of each of the poor-quality host types would be equally influenced by intraspecific competition alike.

Materials and methods

Insects

We used larvae of *M. ruficauda* obtained from 195 egg-clutches collected from bee farms near Buenos Aires, Argentina, during January and February from 2011 to 2015. Egg-clutches were brought to the laboratory and, once the eggs hatched, larvae were separated in flasks (diameter = 6.8 cm, height = 12.3 cm), containing 100 ml of potting soil as substrate. In order to modify the intensity of intraspecific competition, we stored larvae in flasks at different densities from birth. In total, seven densities were used: 1, 100, 200, 300, 400, 500 and 600 larvae per flask. Except for the treatment with only one larva, each flask contained only five larvae per egg-clutch. In this way, for instance, in the 100 larvae per flask treatment, 20 egg-clutches were used to make up a flask. A minimum of 22 and a maximum of 28 larvae per flask were used in the different experiments. In total, for every treatment, a maximum of five different flasks were used in order to account for possible variability. The density range used was chosen because it reflects natural scenarios that occur in the field (Castelo & Capurro, 2000; Castelo & Corley, 2010).

Flasks containing larvae were kept in darkness under controlled temperature (25 ± 2 °C, 65% ± 1% RH) until larvae reached the appropriate age. Only larvae between 20 and 25 days old were used because the survival rate is higher than 50% in larvae that are less than 32 days old, hence we guaranteed a good physiological state (Crespo & Castelo, 2010). Each larva was used only once in the experiments and then reared to be released in the field.

Hosts were collected in grasslands located in the same fields where parasitoids were collected. Using a shovel, we dug down to a soil depth of 30 cm and collected hosts manually. They were then brought to the laboratory for species identification and transferred individually to black tubes filled with clean potting soil. Hosts were then stored under controlled temperature (25 ± 2 °C) and fed weekly with fresh pieces of carrot until they were used for stimuli extraction or directly in an experiment.

Host stimuli were obtained by making a homogenate from the posterior body part of *C. signaticollis* larvae. Hosts were frozen and, once dead, cut between the third and fourth spiracles. Only the posterior part was put in a pestle and crushed using hexane as the extraction solvent, following the procedure outlined in Castelo and Lazzari (2004). A concentration equivalent to 2.5 white grubs ml^{-1} was used.

Throughout the study, we used hosts in different states in the experiments. First we categorised hosts as high or poor quality. A high-quality host was one whose state allows the parasitoid larva to develop better than on other hosts, i.e. healthy and well developed third-instar *C. signaticollis*. Regarding poor-quality hosts, we assume that any host in a different state (such as instar, time since moulting, time since parasitism or parasitism state) would be of diminished quality because either they are not fully developed or they are already parasitised. Hence, we used second-instar hosts (CS2), hosts that had moulted to third instar within the previous 24 h (CSrm), well-developed third-instar hosts (CS3), third-instar hosts parasitised for <7 days (CSrp) and third-instar hosts parasitised for >60 days (CSp). For the CSrm hosts, we checked second-instar hosts every day until moulting occurred and then performed the extracts. In the case of CSrp and CSp hosts, artificial parasitism was performed.

Artificial parasitism consists in placing a parasitoid larva on the thorax of the host and checking after 3 days whether the parasitoid larva was attached to the host integument. In cases where a larva was found attached to the host, positive parasitism was considered and then those hosts were used for extracts. In order to avoid the influence of the parasitoid larva on the extracts, we used only hosts that were parasitised in the fore half, thus excluding any cue from the parasitoid's body itself.

Host orientation

In order to evaluate the orientation response, we performed experiments in a static air two-way olfactometer. The same arena as in Crespo and Castelo (2008) was used. The rectangular arena ($9 \times 6 \times 1\ \mathrm{cm}^3$) was divided into three zones of equal size (one middle and two laterals) along the long axis. A piece of filter paper ($1 \times 1\ \mathrm{cm}^2$) was placed on each lateral zone of the arena. In these papers, 10 μl of either the stimulus or the control solution (solvent) was added at the beginning of every trial. In each assay, only one larva from a given treatment was gently released with a paintbrush at the centre of the arena and its position was 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. The larvae that remained in the middle zone were excluded from the analysis. 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. New filter papers were placed at the beginning of every trial. All experiments were conducted between 10.00 and 17.00 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 ± 1.0 °C) and in darkness.

Experimental procedures

Proportion of individuals that oriented to host odours was tested in different situations (Table 1).

Poor-quality hosts. In order to determine which host state represents a poor-quality host, orientation of parasitoid larvae

Table 1. Treatments tested in the present study.

	Larvae condition	Cues used	Number of replicates
Poor-quality host	1	CS2	108/33
		CSrm	85
		CS3	179
		CSrp	96
		CSp	93
Experience and level of intraspecific competition	1	CS2	108
	100		80
	200		142
	300		81
	400		88
	500		78
	600		80
Intraspecific competition and orientation to poor-quality hosts	600	CS2	80
		CSrm	105
		CS3	136
		CSp	108

For orientation experiments, host odour extracts were used, while for acceptance experiments, the host remained live and intact. CS3, third-instar hosts; CS2, second-instar hosts; CSrm, hosts recently moulted to the third instar; CSrp, third-instar hosts that were recently parasitised by another larva; CSp, third-instar hosts that were parasitised for a long time by another larva (see Materials and methods section for details). Larvae rearing conditions: individually, 1; in groups, 100–600 larvae per flask.

raised alone (one larva per flask) was tested against odours of second-instar hosts (CS2), those recently (less than 24 h) moulted to third instar (CSrm), well-developed third-instar hosts (CS3), recently (between 5 and 7 days) parasitised third-instar hosts (CSrp) and parasitised third-instar (CSp) hosts. These experiments allow us to study the effect of host instar, moulting and parasitism on the orientation of *M. ruficauda*. From these experiments, host odours that were not attractive were classified as odours from poor-quality hosts and those attractive were classified as odours from high-quality hosts.

Experience and level of intraspecific competition. From the results of the previous section we selected CS2 host odours to use in this section as poor-quality host. Orientations of parasitoid larvae raised alone (one, with no experience of intraspecific competition) or in groups under increasing conspecific densities (exposed to intraspecific competition, from 100 to 600 larvae per flask with increasing densities of 100 larvae at a time) were tested against odours of second-instar hosts (CS2). We also tested acceptance of second-instar hosts of larvae raised in the same conditions.

Intraspecific competition and orientation to poor-quality hosts. In order to determine if the effect of intraspecific competition on orientation to CS2 host odours is a general response to any poor-quality host, we tested orientation of larvae raised under the highest intensity of intraspecific competition (600 larvae per flask) to the odours of all poor-quality hosts types identified in the previous section (poor-quality hosts) independently in different assays.

Statistical analysis

For orientation experiments, the proportion of larvae orienting to host odours was analysed by means of generalised linear mixed models (GLMMs) assuming a binomial distribution of error variances and a logit link function in package *lme4* (Bates *et al.*, 2015). The results of 'poor quality hosts', 'experience and level of intraspecific competition' and 'intraspecific competition and orientation to poor-quality hosts' were analysed separately, generating three different models. Factors included instar, time since moulting, time since parasitism and intraspecific competition. Flask where larvae were raised was a random effect in every model.

For the first experiment, 'poor-quality hosts', we estimated the effect of instar, time since moulting and time since parasitism [factorial predictor variable with the levels second-instar host (CS2), recently moulted third-instar host (CSrm), developed third-instar host (CS3), recently parasitised third-instar host (CSrp) and parasitised third-instar host (CSp)] on the proportion of solitary-reared larvae orienting to host odours (suboptimal model). Orientation to CS2 odours was used as the reference level. Then, after analysing the results of the previous sections, in the 'experience and level of intraspecific competition' experiment we estimated the effect of increasing levels of intraspecific competition (predictor numerical variable) on the proportion of group-reared larvae orienting to CS2 host odours (competition model). Finally, in the 'intraspecific competition and orientation to poor-quality hosts' experiment, we tested the prediction that the effect of intraspecific competition is general in the orientation to every poor-quality host's odour. We estimated the effect of instar, time since moulting and parasitism [factorial predictor variable with the levels second-instar host (CS2), recently moulted third-instar host (CSrm) and parasitised third-instar host (CSp)] on the proportion of larvae reared under high intensity of competition orienting to host odours (generalisation model). In this model, we used CS3 odours as the reference level.

For every model we estimated the parameters and calculated the confidence interval. If the exponentiated confidence interval contained 1 then that variable/factor was rendered as not statistically significant from the reference level (Caffo, 2015). The significance of the variables/factors in the models was further evaluated by the *P*-value obtained from the GLMM and the Bayesian information criterion (BIC). A high value of BIC (>10) indicates a very strong relationship (Zuur *et al.*, 2007).

Finally, for the competition model, we generated predictions of the orientation response with increasing intensity of intraspecific competition in order to obtain testable predictions with the function *predict()*. All the analyses were performed in R 3.2.4 (R Core Team, 2016). Graphics were performed using the library 'ggplot2' in R (Wickham, 2009).

Results

We found that orientation to different quality host odours was dependent on the level of intraspecific competition in which larvae were raised. In general, our results support the hypothesis that increasing intraspecific competition lowers the selectivity thresholds of the parasitoid larva (Table 2).

Poor-quality hosts

The orientation response of larvae to host odours in different conditions was heterogeneous. In particular, this experiment showed that when solitary larvae are exposed to second-instar host odours (CS2), recently moulted host odours (CSrm) and parasitised hosts odours (CSp), the exponentiated confidence interval did include the 1, indicating that orientation is similar to the orientation to CS2 host odours, which was the reference level (Table 3). Recently parasitised host odours induced a similar orientation response as CS3 host odours because the exponentiated confidence interval did not include the value 1 (Table 3). In general, the BIC of the sub-optimal model was very high, indicating that host orientation response is largely influenced by host odour (Table 3). From these results, CS2, CSrm and CSp were classified as poor-quality hosts according to our classification, because random orientation was found when larvae were exposed to the mentioned odours (Table 2). We selected CS2 as the poor-quality host odour for the following experiment because it is more readily available and easily accessible than CSrm and CSp hosts.

Table 2. Orientation of *Mallophora ruficauda* larvae to host odours of different quality.

	Larvae condition	Cues used	Success	Total
Poor-quality host	1	CS2	58	108
		CSrm	40	85
		CS3	119	179
		CSrp	61	96
		CSp	43	93
Experience and level of intraspecific competition	1	CS2	58	108
	100		44	80
	200		86	142
	300		48	81
	400		56	88
	500		51	78
	600		55	80
Intraspecific competition and orientation to poor-quality hosts	600	CS2	55	80
		CSrm	64	105
		CS3	94	136
		CSp	69	108

CS3, third-instar hosts; CS2, second instar hosts; CSrm, hosts recently moulted to the third instar; CSrp, third-instar hosts that were recently parasitised by another larva, CSp, third-instar hosts that were parasitised for a long time by another larva (see Material and methods section for details). Larvae rearing conditions: individually, 1; in groups, 100–600 larvae per flask.

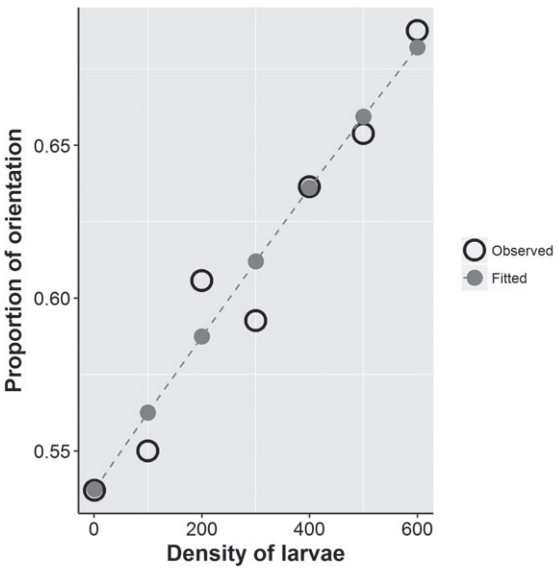

Fig. 1. Proportion of *Mallophora ruficauda* larvae that oriented to poor-quality host odours under different levels of pre-parasitism intraspecific competition. The observed (open circles) values were fitted (closed circles) by means of generalised linear mixed models. A proportion near 0.5 indicates that larvae oriented randomly during the experiment.

Experience and level of intraspecific competition

The orientation response of larvae to odours of CS2 hosts was dependent on the intensity of intraspecific competition. A significant linear relationship between orientation to CS2 host odours and the intensity of intraspecific competition was found, as the exponentiated confidence interval did not include the 1 (Fig. 1; Table 3). The BIC of the competition model was very high, indicating that orientation response to CS2 odours is largely influenced by the intensity of intraspecific competition (Table 3). As the exponentiated slope coefficient is 1.000 874, we estimate a 0.1% increase in the odds of orienting to CS2 host odour per one larva increase in the density of intraspecific competition (Table 3). The predicted orientation response to CS2 host odours depicted in Fig. 2, for example, shows that at a density of 2000 larvae, a 90% positive orientation should be observed.

Table 3. Estimated regression coefficients and standard errors of variables/factors that could affect orientation to poor-quality hosts.

	Variable/factor	Estimate	SE	Exp (CI)	*Z*-value	*P*-value	BIC
Poor-quality host (sub-optimal model)	CS3	0.741	0.173	1.152–2527	4.286	1.82e–05**	782.87
	CS2	0.157	0.208	0.778–1.770	0.756	0.450	
	CSrm	−0.174	0.236	0.524–1.331	−0.736	0.462	
	CSrp	0.562	0.227	1.128–2.767	2.469	0.013**	
	CSp	−0.202	0.226	0.520–1.268	−0.893	0.372	
Experience and level of intraspecific competition (competition model)	Density	0.000874	0.00041	1.00007–1.00168	2.116	0.0343*	901.50
Intraspecific competition and orientation to poor-quality hosts (generalisation model)	CS3	0.870	0.205	1.621–3.646	4.246	2.18e–05**	577.59
	CS2	−0.016	0.314	0.533–1.839	−0.049	0.961	
	CSrm	−0.396	0.290	0.380–1.185	−1.366	0.172	
	CSp	−0.257	0.284	0.441–1.350	−0.906	0.365	

*$P < 0.05$, **$P < 0.01$.

Variable/factors: CS3, third-instar hosts; CS2, second-instar hosts; CSrm, hosts recently moulted to the third instar; CSrp, third-instar hosts that were recently parasitised by another larva; CSp, third-instar hosts that were parasitised for a long time by another larva; density, density at which larvae were raised before experiments, from 100 to 600 larvae per flask with increases of 100 larvae between each level of density; BIC, Bayesian information criterion estimates the relationship between variables – high values, i.e. >10, indicate very strong relationships; Exp (CI), exponentiated confidence interval.

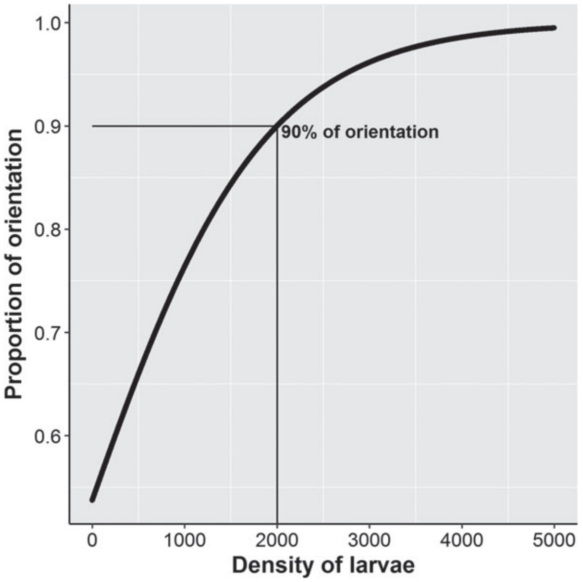

Fig. 2. Prediction of the proportion of orientation to poor-quality host odours among larvae of *Mallophora ruficauda* according to our proposed model. When the intensity of pre-parasitism competition is as high as 2000 larvae (100 ml)$^{-1}$ soil, we would expect 90% of larvae to orient towards poor-quality host odours.

Intraspecific competition and orientation to poor-quality hosts

The orientation response of larvae to all poor-quality hosts was influenced by the level of intraspecific competition. When larvae raised under high levels of intraspecific competition (600 larvae per flask) were exposed to second-instar host odours (CS2), recently moulted host odours (CSrm) and parasitised hosts odours (CSp), orientation was similar to that of CS3 odour, as all the confidence intervals for every factor contained 1 (Table 3). The BIC obtained from the generalisation model shows that intensity of intraspecific competition influences orientation to poor-quality host odours in general, rendering competition a highly influential factor (Table 3).

Discussion

In this work we studied how larva of *M. ruficauda* evaluates quality of hosts in different states and tested whether the exposure to increasing density of conspecifics prior to orienting to a host affects the decisions made by larvae regarding orientation towards and acceptance of poor-quality hosts. Our results show that *M. ruficauda* can at least determine the quality of hosts as suitable or not suitable for parasitism. We also found that exposure of larvae to conspecifics prior to locating hosts modifies the orienting decisions and lowers the selectivity thresholds in a linear fashion.

First we studied the orientation decisions of solitary-raised larvae to odours of hosts of different quality and found that larvae only orient to odours of healthy third-instar hosts or recently parasitised hosts. Solitary-raised larvae in a good physiological state are highly selective and should only orient to odours of the bests hosts, as they should have enough reserves to search for a better host in cases where the host encountered is of low quality. In this context, second-instar hosts, recently moulted hosts and parasitised hosts are, in effect, low-quality hosts. The fact that hosts recently moulted to the third instar are sensed as low-quality hosts reinforces the idea that after moulting, hosts take several days before producing the attractive cue. In fact, hosts' chemical cues can change over time and turn into a novel chemical identity allowing parasitoids to determine the time since parasitism occurred (Lebreton *et al.*, 2010). Our results from this section show that *M. ruficauda* can detect changes in host odours several days after physiological changes have occurred, such as moulting or parasitism. However, if a third-instar host is detected as a second instar even after several days have passed since moulting, then the larva is missing an opportunity to parasitise an optimal host.

We then tested the orientation to second-instar host odours of larvae that had been reared under increasing intensities of intraspecific competition. We selected second-instar host odours as our cue for testing the effect of intraspecific competition on orientation because it is easily accessible. We found, for the first time in a host-seeking larva, that orientation to second-instar host odours increases with increasing levels of intraspecific competition in a linear fashion. This result shows that the environment in which larvae are raised can greatly influence the orientation decisions they make. In this sense, either developmental or contextual plasticity may be acting in this species. Recall that developmental plasticity indicates the extent to which an individual's behaviour at the current time is affected by past experiences such as exposure to conspecifics (Stamps, 2016). On the other hand, contextual plasticity indicates the extent to which an individual's behaviour at the current time is affected by actual experiences (Stamps, 2016). However, in our experiments, larvae were exposed to conspecifics sharing the same natal habitat until they were used in the assays, so we cannot distinguish between these two possibilities.

The fact that many larvae in a confined place are looking for a host provokes encounters with each other that result in different kinds of interaction. Very little is known about interactions that occur in the soil between larvae, but what we know is that they do not engage in fights in the way that other parasitoids do. However, when resources are scarce, cannibalism has been noted (J. Crespo, pers. comm.). Cannibalism may occur as a different way of interacting. Larvae may recognise each other through chemical odours or they may simply encounter each other by chance. Previous work suggests that larvae cannot detect each other through chemicals so it is possible that they simply find each other by chance in the soil. So, increasing density should increase the number of mechanical contacts between larvae. Encounters with other competing larvae would be an indicator that being selective is not the best strategy because, if one larva does not orient to a specific host, then the next larva will. In this context, the conditions in which a larva is raised influence the quality of the host to which it will orient and ultimately accept, determining its fitness. The decision of orienting to a host during the second instar is of paramount importance in this species because once the parasitoid larva attaches to a host, it does not detach unless the host dies (Castelo, 2003). However, the effects on the development of *M. ruficauda* of accepting an under-developed host are still unknown.

Natal experience on habitat selection can affect a disperser's estimate of search time, encounter rate with high-quality habitats or habitat quality, modifying the selectivity during search or the level of preference for natal-type habitats of the disperser (Stamps & Davis, 2006). In this work, we have considered the experience in the natal habitat and how it influences larval choice among different quality hosts. Stamps and Davis (2006) proposed that natal experience affecting the disperser's estimate of the encounter rate with different quality habitats could alter its chances of accepting low- or medium-quality habitat if the animal uses a sequential search, as is the case for *M. ruficauda*. Nevertheless, Stamps and Davis (2006) stated that changes in selectivity in this context should mainly occur when the high-quality natal habitats are perceived. In our experiments, we raised larvae in low-quality conditions regarding the presence of hosts. However, choosiness during search was influenced by the density of conspecifics, in that larval selectivity decreased when density increased. This result contradicts the assumption that selectivity should only change when high-quality natal habitat is perceived. In contrast to the predictions of Stamps *et al.* (2005), in this species when natal habitat is perceived to be of low quality, selectivity also changes if intraspecific competition is intense.

We also predict in our model the proportion of larvae that should orient to poor-quality hosts with increasing density of conspecifics in the natal habitat. For instance, a 90% orientation is expected when the density of larvae is as high as 2000 larvae $(100\,\text{ml})^{-1}$ substrate. This density could well be encountered in the field, as females of this species often lay many egg-clutches in the same plant. It is common to find 10–15 egg-clutches in a plant (Castelo, 2003). As larval dispersion depends greatly on wind conditions, larvae may be born on days with low wind speeds so a very high density of larvae can accumulate in the soil. The results of this study can serve as a framework to test these predictions in other experiments.

Finally, we tested our prediction that intraspecific competition would have similar effects on the orientation to all poor-quality hosts. When we tested the orientation of larvae to recently moulted third-instar hosts and third-instar parasitised hosts, we found that larvae under high density of conspecifics orient to poor-quality host odours in the same way as they do to second-instar host odours.

Our experiments show that *M. ruficauda* larvae exhibit many behaviours similar to hymenopteran parasitoids. Just as with hymenopterans, 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 respond in adaptive ways.

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