



Role of visual information and learning in habitat selection by a generalist parasitoid foraging for concealed hosts

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Generalist species usually have to deal with a larger variety of cues during habitat selection than do specialists, and thus, learning has been proposed as a highly profitable strategy to find the most suitable habitat. We analysed the effect of previous experience on the use of visual information by the wasp *Diachasmimorpha longicaudata*, a generalist fruit fly larval parasitoid, in the context of host habitat selection. These parasitoids search for hosts concealed in plant structures, so visual cues from the hosts' environment could play a key role in host finding. We also studied how different visual cues used by this species interact and affect the habitat choices of female wasps. We studied three forms of visual cues: colour, shape and size. All experiments were conducted under controlled laboratory conditions, using artificial models mimicking fruit. Naïve females showed no preference pattern for colour or shape, but they showed a clear preference for larger models. These results were unaffected when females were previously exposed to host larvae (no sensitization effect). Associative learning was found for colour: tested females developed a clear pattern of preferences after they were exposed to larvae associated with a particular colour. We found that colour and size have a similar effect on the female's choice, and that this effect is neither additive nor multiplicative. Our findings emphasize the importance of both visual cues during host habitat searching by *D. longicaudata* and the influence of learning on the preference patterns of a generalist parasitoid.

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Habitat selection, the phenomenon by which individuals decide to settle or to use one of the possible habitats available, is influenced by many factors (Vinson 1998). There is a strong heritable component on habitat preferences, but accumulated experience also affects habitat selection. In a wide variety of animals, natal or preadult experience has a marked effect on the decision of individuals once they become adults (see Davis & Stamps 2004). The experience that an individual gains in the adult stage can also help it to select the most profitable habitat. Learning has been shown to be involved in habitat selection in a wide range of vertebrate species, including numerous fish, birds and mammals (Mahometa & Domjan 2005).

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Learning has also been described for many invertebrate species, including snails (Dalesman et al. 2006), the nematode *Caenorhabditis elegans* (Nuttley et al. 2002; Law et al. 2004), isopods (Baker 2005) and several insect species. Among the insects, dipterans (Papaj & Prokopy 1989; Stireman 2002), lepidopterans (Cunningham et al. 2004, and references therein) and hymenopterans (Menzel 1993; Turlings et al. 1993) are the best and oldest known examples of learning. Although some examples of preadult learning have been reported, Turlings et al. (1993) proposed that adult learning contributes to a larger extent to the foraging success of insects.

The relative importance of heritable preference and learning-induced preference on habitat selection is not known (Rolstad et al. 2000), but it has been related to the unpredictability of the quality of the habitats. When the different habitats are constant in their profitability, preference for the most profitable habitat should evolve, and

thus the need for learning is minimized. Also, when the profitability of the different habitats changes very fast and is completely unpredictable, learning cues of a given habitat that is profitable today may not be useful tomorrow. Stephens (1993) proposed that the influence of learning on habitat selection should be enhanced when the profitability of the available habitats does not change too fast within the life span of the individuals (low within-generation variability), but tends to change markedly from one generation to the next.

Habitat selection can be envisaged as a hierarchical spatial process, from choice of home range to choice of dietary item, in which different environmental cues may guide the organisms to choose the best habitat at each level (Rolstad et al. 2000, and references within). Host foraging by insect parasitoids may be viewed as a particular case of habitat selection. This process is often discussed in terms of hierarchy and sequence: parasitoid females must locate first the habitat, then the host plant, and finally, the host itself (Vinson 1984; Olson et al. 2003). During host habitat searching, chemical and visual cues may be used by parasitoids to limit their search to those microhabitats in which the probability of finding hosts is higher. When the hosts are buried in plant structures, and thus produce only subtle signals, cues coming from the habitat of the host become increasingly important (van Alphen & Vet 1986; van Alphen et al. 1991; Henneman 1998). Chemical cues associated with host habitat can attract parasitoids even in the absence of the host itself, but visual cues are also important, mostly in the final stages of the process (Godfray 1994). Colour, pattern, shape and size could be potentially used by parasitoids to locate hosts or host habitats (Michaud & Mackauer 1994; Hoffmeister et al. 1999; Fischer et al. 2003; Lobdell et al. 2005). Colour seems to be the most important stimulus at long distances, given the rather poor resolution of insects' eyes (Brown et al. 1998), whereas information on pattern and shape would be accessible only at a closer range (Wäckers & Lewis 1999).

During host habitat searching, the response of parasitoids to visual cues can be modified by experience (Vet & Dicke 1992). Some parasitoids show an increase in their general responsiveness to stimuli after a first encounter with hosts (a phenomenon termed sensitization or priming), while others are able to associate stimuli experienced in the presence of hosts (host-related cues) with the presence of a subsequent host (associative learning). Even if visual cues from the host are limited, associative learning of visual stimuli from the host environment could enable parasitoids to exploit visual information during their search (Turlings et al. 1993). Despite this, the information about this type of learning in hymenopteran parasitoids is strikingly limited (Wäckers & Lewis 1994), and mainly focused on colour learning (Wardle 1990; Wäckers & Lewis 1994, 1999; Shafir 1996; Brown et al. 1998; Oliai & King 2000). Learning of shapes has been studied to a lesser extent (Wardle & Borden 1990; Wäckers & Lewis 1999).

It has been proposed that learning is especially important for generalist species (Geervliet et al. 1998; Stireman

2002). Empirical evidence supports this idea (Steidle 1998; Dukas & Duan 2000). This mechanism would enable foraging parasitoids both to cope with the variability of either direct cues from the hosts or indirect cues produced by the host's food plant (Wäckers & Lewis 1994; Steidle 1998) and to adjust their response to changes in the abundance and quality of potential hosts (Vet & Dicke 1992; Stireman 2002). Learning should be further enhanced according to the degree of inter- and intra-generational variability in the habitats' profitability (Stephens 1993).

The relationship between the ability to learn host-related cues (either chemical or visual) and the breadth of possible host's habitats has been addressed in many insects. Most studies deal with generalist species (Menzel & Bitterman 1983; Wardle & Borden 1989; Wardle 1990; Iizuka & Takasu 1998; Vet et al. 1998; Oliai & King 2000; Sato & Takasu 2000; Keasar et al. 2001; Stireman 2002; Kaiser et al. 2003; Weiss & Papaj 2003; Wund 2005). Studies on specialist species tend to agree with the general rule, and establish that these species rely more on innate preference (Parmesan et al. 1995; Geervliet et al. 1998; McGregor & Henderson 1998), although some exceptions have been found (Poolman Simons et al. 1992; Lecomte & Thibout 1993; Mumm et al. 2005). Examples of the absence of learning ability in a generalist parasitoid are limited. Papaj (1986) argued that monophagous parasitoids of polyphagous hosts also profit from learning. Several studies have provided evidence supporting this idea (de Jong & Kaiser 1991; Zanen & Cardé 1991; Wäckers & Lewis 1994; Wäckers & Lewis 1999; Olson et al. 2003).

Diachasmimorpha longicaudata (Hymenoptera: Braconidae) is a larval-prepupal parasitoid of tephritid fruit flies (Greany et al. 1976; Cancino Díaz & Yoc 1993). It is a generalist parasitoid of at least 34 fruit flies species (Wharton & Gilstrap 1983; Vijayasegaran 1984; Chinajariyawong et al. 2000), all of which remain concealed in the fruit until they are ready to pupate. Once the fruit fly larvae leave the fruit, they are not attacked by this parasitoid (Lawrence 1981). Thus, because *D. longicaudata* parasitoids have to locate the infested fruit to find hosts, visual cues coming from the fruit have been postulated to be involved in this process (Leyva et al. 1991; Vargas et al. 1991; Messing & Jang 1992). Given the wide range of hosts species of *D. longicaudata* and that many of them are also polyphagous pests, learning of visual cues should greatly contribute to the host foraging success of this parasitoid. The different types of host habitats (i.e. different fruit species) are likely to change from one generation of parasitoids to the next, but are usually present for a significant part of the life span of individual parasitoid females, which should enhance the importance of learning under the model proposed by Stephens (1993). Nevertheless, the importance of previous experience on the use of visual information by *D. longicaudata* females has been ignored. The aim of the present study was to analyse how the previous experience of the females could modify the use of visual information by *D. longicaudata*, and how different types of visual cues interact with each other.

METHODS

Insect Rearing

Parasitoids and fruit fly larvae were reared at Laboratorio de Insectos, Instituto de Genética (INTA, Castelar). We used *Ceratitidis capitata* (Diptera: Tephritidae) larvae as hosts for the parasitoids. The colony of *D. longicaudata* was initiated with individuals coming from CIRPON, San Miguel de Tucumán (Ovruski et al. 2003).

Experimental Conditions

We conducted all experiments in a 14-m² room with white walls and ceiling, illuminated with daylight fluorescent tubes. These tubes were placed in such a way as to guarantee that the intensity of light was as even as possible in every point of the room (ca. 2500 luxes at a height of 1.30 m). The temperature was set at 25°C (±1°C) and the relative humidity at 65% (±5%).

We examined the behaviour of females facing artificial models of varying colours, shapes and sizes in an artificial arena consisting of screen cages (30 × 30 × 30 cm) with white tulle walls.

General Methodology

After emergence and until testing, females were kept with males in glass flasks, and provided with water and honey. During testing, we gently transferred one female at a time to the centre of the cage, and released her on the floor. In all experiments, only two variants of a given cue were offered simultaneously (e.g. red versus yellow, or circle versus oval). We considered that a female had chosen a given cue if she landed on the cue within 15 min after being released into the cage and she engaged in host-searching behaviours (antennating or probing; Lawrence 1981) on the chosen model for at least 30 s. For each female (replicate), we recorded three variables: (1) latency (time elapsed from the moment that she was released until she landed on one of the models); (2) the model that she chose; and (3) permanence (the time that she spent on the chosen model). Once the female left the model, we removed her from the cage and released a new female. With each new female, either the cage was rotated clockwise 90° (experiment 1) or the options were side-switched (left to right and vice versa, experiments 2, 3, 4 and 5) to avoid positional effects. After five females had been observed, the models were replaced and a new pair (i.e. a new combinations of cues) was tested. We randomly chose every new combination.

Experiment 1: Visual Cues for Naïve Females

All tested females were 5–7 days old and had no previous experience. We analysed three visual cues separately: colour, size and shape (Table 1). During the assay, four models (representing two different cues) were placed inside the cage, each one 10 cm apart from the nearest corner, 10 cm from the top, and 10 cm apart

from each other. Models belonging to the same cue were placed in opposite corners of the cage. Table 2 shows a detailed description of the spectral characteristics of the colours used in the assays. We tested 40 females with each pair of cues.

Experiment 2: Influence of Sensitization on Preference

We analysed the effect of a previous contact with larvae on the female's preference for colour and shape. Five- to 7-day-old females were exposed for 6 h to third-instar larvae of *C. capitata* enclosed in an oviposition unit (OU) (Table 1). The oviposition unit consisted of a small petri dish (5.5 cm in diameter, 1.3 cm depth) containing the larvae. For colour preference assays, we wrapped the oviposition units in white cloth, which allowed the parasitoids to successfully parasitize the larvae. For shape assays, the oviposition units consisted of a four-sided plastic box (8 × 6 cm, 1.1 cm depth) coated with yellow cloth. For both types of visual cues, larvae were exposed in an oviposition unit with a colour or shape that the females would not encounter during the test.

On the following day, we tested the response of these females to two models that were simultaneously offered inside the cage (Table 1). To test for colour preference, two oviposition units (without larvae) coated in cloth of different colour were offered (Tables 1, 2). In the case of shape preference, the choices consisted of hand-made 1.5-cm-deep models of three shapes (Table 1), coated in yellow cloth. We tested 40 females with each combination of cues.

Experiment 3: Influence of Previous Experience on a Fixed Cue

To test the influence of experience on preference for colour and shape, females were offered host larvae in conjunction with a given cue. We then tested whether the females preferred this cue to another with which they had had no previous contact. All colours and shapes used in this experiment were the same as those used in the sensitization experiment (Tables 1, 2). For each combination of cues (10 for colour and 3 for shape), we trained one group of females using one of the cues and another group of females using the alternative cue.

During the training sessions, females were offered an oviposition unit (containing third-instar larvae of *C. capitata*) of a given colour or shape for 6 h. This procedure was repeated for three consecutive days. For colour, the oviposition units were small petri dishes wrapped in coloured cloth (Table 1). For shape, the oviposition units consisted of hand-made 1.5-cm-deep models coated in yellow cloth (Table 1). The day after the training sessions, we individually offered the females two oviposition units inside the cage, one of the same colour or shape that they experienced during training and an alternative one. We tested 80 females per combination of cues.

Table 1. Description of the methodology used in the five experiments

Experiment	Analysed cue	Previous experience	Offered models	Combinations	Replicates	Variables
1: Naïve females	Colour	No	4.5-cm polystyrene spheres: black, green, orange, red, yellow*	10	40	Latency, permanence, visited model
	Shape	No	Yellow cardboard: circle (6-cm diam.), oval 1 (9 × 4 cm), oval 2 (12 × 3 cm)†	3	40	Latency, permanence, visited model
	Size	No	Yellow polystyrene spheres (diam.): 3 cm, 6 cm, 9 cm	3	40	Latency, permanence, visited model
2: Sensitization	Colour	White OU for 6 h	Coloured host-deprived OU: black, green, orange, red, yellow	10	40	Latency, visited model
	Shape	Square OU for 6 h	Shaped host-deprived OU: circle, oval 1, oval 2‡	3	40	Latency, visited model
3: Experience on a fixed cue	Colour	Coloured OU (black, green, orange, red, or yellow) for 6 h during 3 days	Coloured host-deprived OU: black, green, orange, red, yellow	10	80	Latency, visited model
	Shape	Shaped OU (circle, oval 1, oval 2) for 6 h during 3 days	Shaped host-deprived OU: circle, oval 1, oval 2‡	3	80	Latency, visited model
4a: Brightness control	Colour	Light or dark grey OU for 6 h during 3 days	Grey host-deprived OU: dark grey, light grey	1	80	Visited model
4b: Nonassoc. learn. control	Colour	Host-deprived, coloured OU (black, green, orange, red, yellow) for 6 h during 3 days	Coloured host-deprived OU: black, green, orange, red, yellow	10	40	Visited model
5: Interaction between cues	Colour and size	Yellow OU for 6 h during 3 days	Large (10-cm diam.) and small (5.5-cm diam.), red or yellow host-deprived OU	4§	80	Visited model

OU = oviposition unit (see [Methods](#) for description).

*Renoir's Acrylic Colours, Emilio Lopez S.A., Argentina.

†The three types of cardboards shapes had the same area.

‡The models had the same dimensions as those used in experiment 1.

§Plus a control test: large versus small yellow oviposition units.

Table 2. Spectral characteristics used to quantify colour

Type of model	Colour	<i>L</i>	<i>a</i>	<i>b</i>
Coloured spheres	Black	14.40	-0.14	0.40
	Green	31.21	-34.10	31.24
	Orange	42.33	40.90	50.01
	Red	25.52	37.92	20.87
	Yellow	73.64	14.06	51.25
Pieces of fabric	Black	14.31	0.16	-3.10
	Green	42.03	-35.25	26.56
	Orange	51.79	40.12	37.60
	Red	36.79	50.86	22.39
	Yellow	73.63	6.33	49.52
	Dark grey	25.12	1.32	-3.55
	Light grey	75.22	2.10	3.06

Parameters were measured for the coloured spheres used in experiment 1 and for the pieces of fabric used in experiments 2–5. Measurements were determined using a Spectrocolorimeter BYK Gardner Colour View model 9000. *L* = an index of overall brightness; *a* = a measure of hue on a scale from red to green; *b* = a measure of hue from blue to yellow (Messing & Jang 1992).

Experiment 4: Controls

(a) Influence of light intensity on colour preference

To rule out that females were detecting brightness rather than the hue of the oviposition unit, we then analysed the response of trained females to different shades of grey. Two shades of grey, bracketing the range of reflectance of the coloured cloths used in the previous assays, were compared (Table 2). Training and testing sessions were performed following the same procedure as that of experiment 3 (Table 1). We tested 80 females per combination.

(b) Nonassociative learning control

To rule out that females had chosen a given colour in experiment 3 just because they were exposed to this colour for 3 days (and not because they associated the colour to the presence of larvae), we performed an additional control test. For each combination of colours, we randomly chose one of the colours and exposed naïve females to an oviposition unit following the same

procedure as that of experiment 3, but using an oviposition unit deprived of larvae. Thus, we performed a total of 10 assays (Table 1). We tested 40 females per combination.

Experiment 5: Interaction between Visual Cues

We studied the interaction between colour and size. We used only one randomly chosen pair of colours. To induce colour preference, we trained females on a yellow oviposition unit, following the same procedure as that described above (experiment 3). Then, trained females were randomly assigned to one of the four experimental arenas that resulted from combining the colour that they had been trained with (yellow), an alternative colour (red) and two sizes of oviposition units: a small petri dish (5.5 cm diameter, 1.3 cm depth) and a large petri dish (10 cm diameter, 1.3 cm depth) (Table 1). The procedure used during the behavioural tests was the same as that described earlier (Table 1). We tested 80 females for each pair of cues.

Data Analysis

We analysed the differences in latency and permanence between options by means of a one-way ANOVA. Whenever the homoscedasticity assumption was violated, we applied a square-root transformation (Zar 1996). The frequency of visits to the two models of each combination was compared by a *G* test of goodness of fit to the equal proportion hypothesis, with the Yates correction for continuity (recommended whenever the degree of freedom equals 1; Zar 1996). We compared the proportions of visits received by the preferred model in experiment 5 using a chi-square test (Zar 1996).

RESULTS

Experiment 1

Naïve females showed no preference pattern for colours or shapes. The differences between colours in number of visits received, latency and permanence were not statistically significant (except for a shorter latency for yellow, in the green–yellow pair; Table 3). The same applied to models differing in shape (Table 3). For size, there were differences in the number of visits received between spheres of the most extreme diameters (i.e. 3 and 9 cm in diameter), but no differences were found in latency or permanence. Females' responses to a sphere of intermediate diameter did not differ from their responses to a sphere of either the smallest or the largest diameter (Table 3).

Experiment 2

In this experiment we studied sensitization only for shape and colour since, as opposed to size, no innate preference for them was found in experiment 1. The results showed that females exposed during one 6-h period to third-instar larvae of *C. capitata* did not show

any higher tendency to visit one colour than the other, for all the possible combinations of colours (Table 4). The same result was obtained when females were trained in a square oviposition unit, then offered two oviposition units of different shape (Table 4). Latency times never showed differences between models (Table 4). Only latency and number of visits were analysed, since permanence times were too long (>10 min).

Experiment 3

We studied the effect of previous experience on a fixed cue only for shape and colour, given that females showed an innate response to models of different sizes. For the same reason explained in experiment 2, only latency and the number of visits were analysed. Females made more visits to the colour that they had been exposed to during training than they did to the alternative colour (Table 5). This response was registered in almost all the pairs of colours tested, except in the yellow–orange and red–black combinations. Females showed no preference for the shape of oviposition unit in which they had been trained for any choice combination (Table 6). The mean latency never differed between cues.

Experiment 4

Here we registered only the model chosen by the females, because latency did not differ statistically between cues in experiment 3 (which was the focus of these control assays). In the brightness control test, the females that were exposed for 3 days to a dark or light grey oviposition unit did not show a preference for the shade of grey in which they had been trained, and they visited either unit as frequently (Table 7). In the nonassociative learning control, after being exposed to larvae-deprived, coloured oviposition units for 3 days, the females showed no preferences for the colour to which they had been exposed in any of the combinations offered (Table 7).

Experiment 5

We studied the interaction between those visual cues for which we found a preference pattern in the females, either innate (size) or learned (colour). The only variable registered was the option selected by the female, because, in previous assays, there was no difference in latency and permanence between the offered options. Based on experiment 3, yellow was considered the preferred colour (females were trained on it) and red was considered as the nonpreferred option. Notwithstanding that experiment 1 had already shown that large oviposition units are preferred to small ones, we again performed here a control comparing the response of females facing a small and a large yellow oviposition unit.

When females were offered two oviposition units differing only in colour, they made more visits to the one that presented the colour in which they had been trained, regardless of the size (yellow large versus red

Table 3. Number of visits, mean latency and mean permanence times (in min) for each cue for naïve females

Type of cue	Paired cues	Number of visits received	$G_{c,1}$	Latency		Permanence	
				Mean (SE)	P	Mean (SE)	P
Colour	Red	19	0.03	5.68 (1.00)	0.43	1.72 (0.33)	0.07
	Green	21		7.05 (1.30)		3.18 (0.90)	
	Red	24	1.23	5.76 (1.22)	0.93	1.98 (0.48)	0.50
	Yellow	16	1.23	5.91 (1.05)	0.95	2.56 (0.60)	0.64
	Red	24		7.25 (1.12)		2.75 (0.50)	
	Orange	16	0.63	7.36 (1.53)	0.27	3.33 (1.40)	0.74
	Red	23		8.58 (1.18)		3.22 (0.80)	
	Black	17	1.23	10.53 (1.25)	0.03	2.85 (0.68)	0.38
	Green	16		9.43 (1.60)		3.30 (1.37)	
	Yellow	24	0.63	5.32 (0.93)	0.93	5.07 (1.28)	0.22
	Green	17		6.82 (1.33)		2.22 (0.68)	
	Orange	23	2.04	6.96 (1.02)	0.50	3.85 (1.00)	0.35
	Green	25		10.05 (1.02)		3.93 (0.93)	
	Black	15	1.23	8.77 (1.67)	0.77	5.52 (1.33)	0.55
	Yellow	16		6.48 (1.15)		3.73 (1.07)	
	Orange	24	2.04	6.95 (0.98)	0.14	3.02 (0.65)	0.87
	Yellow	25		7.52 (1.20)		3.47 (0.72)	
	Black	15	1.23	10.30 (1.28)	0.88	3.70 (1.46)	0.39
	Orange	24		9.36 (1.15)		3.36 (0.76)	
Black	16		9.08 (1.46)		4.65 (1.40)		
Shape	Circle	24	1.23	4.72 (0.81)	0.09	5.73 (0.95)	0.15
	Oval 1	16	0.63	2.68 (0.75)	0.79	3.52 (1.13)	0.39
	Circle	23		4.42 (0.98)		3.77 (0.81)	
	Oval 2	17	0.63	4.33 (1.04)	0.93	3.07 (0.74)	0.40
	Oval 1	17		2.72 (0.61)		1.68 (0.28)	
	Oval 2	23		2.78 (0.58)		2.25 (0.62)	
Size	3 cm	25	2.04	2.35 (0.82)	0.83	2.57 (0.49)	0.37
	6 cm	15	16.85*	2.58 (0.74)	0.21†	3.32 (0.58)	0.80
	3 cm	7		5.46 (2.54)		3.10 (0.61)	
	9 cm	33	1.23	2.56 (0.64)	0.28	3.42 (0.55)	0.18
	6 cm	16		3.23 (0.99)		3.93 (1.05)	
	9 cm	24		4.82 (0.97)		2.58 (0.41)	

*Number of visits differed significantly between cues ($P < 0.05$).

†Homoscedasticity assumption was violated and a square-root transformation was applied.

large: $G_{c,1} = 12.33$, $P < 0.001$; yellow small versus red small: $G_{c,1} = 9.29$, $P < 0.001$; Fig. 1a, b). When the large oviposition unit was wrapped in red cloth and the small one in yellow, the females visited either of them as frequently; no differences between oviposition units were detected ($G_{c,1} = 0.11$, $P > 0.5$; Fig. 1c). When the large oviposition unit was wrapped in yellow cloth and the small in red, the females preferred the large yellow oviposition unit ($G_{c,1} = 17.78$, $P < 0.001$; Fig. 1d). Females that were offered a large yellow and a small yellow oviposition unit (not shown in Fig. 1) showed a marked preference for the large oviposition unit (large yellow = 64; small yellow = 16; $G_{c,1} = 29.47$; $P < 0.001$), corroborating the size preference found the experiment 1.

No statistical differences were found between the proportion of visits received by the large yellow oviposition unit in the arena where a large yellow and small red oviposition unit had been offered and the proportion of visits to the yellow oviposition unit in either the large yellow–large red and small yellow–small red arenas (chi-square for differences between proportions: $\chi^2_3 = 3.30$; $P = 0.607$).

DISCUSSION

Naïve *D. longicaudata* females showed no preference pattern for the colours used in this study. We stress here that our analysis involved only females displaying clear signs of host searching (Lawrence 1981; Bautista & Harris 1997; Montoya et al. 2003). Also, Leyva et al. (1991) found no colour preference in naïve *D. longicaudata* females foraging under more natural conditions. In contrast, Messing & Jang (1992) and Cornelius et al. (1999) reported that *D. longicaudata* prefers yellow targets. However, these studies did not focus only on foraging females, and no behavioural observations were performed. Furthermore, the same pattern was indistinctly observed both in males and females, probably representing a general response of *D. longicaudata* to yellow. But, regarding host-foraging behaviour, our results strongly suggest that naïve females have no colour preference.

We found that naïve *D. longicaudata* females showed a marked preference for larger spheres, in agreement with other studies (Leyva et al. 1991; Sivinski 1991). This result was rather unexpected because larger fruit,

Table 4. Number of visits and mean latency (in min) to each colour or shaped model presented to females that had one oviposition experience in a white oviposition unit or in a square oviposition unit (effect of sensitization)

Type of cue	Treatment	Number of visits received	$G_{c,1}$	Mean latency (SE)	P
Colour	Red	18	0.23	1.77 (0.30)	0.14
	Green	22		1.17 (0.26)	
	Red	19	0.03	1.41 (0.29)	0.70
	Yellow	21		1.25 (0.28)	
	Red	18	0.23	1.54 (0.21)	0.35
	Orange	22		1.30 (0.16)	
	Red	19	0.03	1.84 (0.28)	0.74
	Black	21		1.98 (0.29)	
	Green	18	0.23	2.03 (0.38)	0.16
	Yellow	22		1.41 (0.33)	
	Green	20	0.00	1.69 (0.33)	0.37
	Orange	20		2.10 (0.31)	
	Green	20	0.00	1.81 (0.33)	0.65
	Black	20		1.61 (0.31)	
	Yellow	20	0.00	1.20 (0.24)	0.47
	Orange	20		1.44 (0.23)	
	Yellow	21	0.03	1.44 (0.27)	0.26
	Black	19		1.05 (0.20)	
Orange	22	0.23	1.12 (0.16)	0.53	
Black	18		1.27 (0.22)		
Shape	Circle	22	0.23	1.99 (0.25)	0.69
	Oval 1	18		2.15 (0.29)	
	Circle	15	2.04	2.51 (0.32)	0.36
	Oval 2	25		2.13 (0.26)	
	Oval 1	21	0.03	2.00 (0.22)	0.84
	Oval 2	19		1.94 (0.18)	

Number of visits received by each model did not differ for any pair of cues.

which has more pulp and rind, could be a better refuge for the host larvae (Hoffmeister et al. 1999, and references therein). Several field surveys have also found that larger infested fruit have lower parasitism rates (Hernández-Ortiz et al. 1994; Aguiar-Menezes & Menezes 1997; Carrejo & González 1999; López et al. 1999; Sivinski et al. 2000; Aluja et al. 2003; Ovruski et al. 2004, 2005). Thus, because parasitoid efficacy is higher in small fruit, one should expect females to tend to visit smaller spheres. On the other hand, a large fruit may have the capacity to carry more host larvae and thus become more profitable for a female parasitoid than would a smaller fruit.

The size of host fruit in which larvae are attacked is strongly correlated with the ovipositor length of opiine braconid parasitoids (Sivinski et al. 2001). For example, larval-pupal parasitoids *Utetes anastrephae* (Viereck), *Opius hirtus* (Fisher) and *O. bellus* Gahan, which have a short ovipositor, forage only on a narrow range of smaller fruit (Sivinski et al. 1997; López et al. 1999; Ovruski et al. 2004). In contrast, *D. longicaudata*, which has a long ovipositor, forages on a wider range of host plant species, including large fruit, such as mango and citrus (López et al. 1999; Sivinski et al. 2000; S. M. Ovruski, unpublished data). Studies carried out under natural conditions with a fruit fly parasitoid guild of five opiine species, including *D. longicaudata*, suggest that these parasitoid species occupy niches that are differentiated by host fruit (Sivinski

et al. 1997, 2000, 2001). It appears that *D. longicaudata* has an advantage in exotic large fruit, a niche left vacant by the rest of the parasitoid species in that guild (Eben et al. 2000; Sivinski et al. 2001). Our results showing a preference for larger models fit well with these findings.

Female *D. longicaudata* showed no preferences for differently shaped models, either after one oviposition bout (sensitization assays) or after a training session of 3 days. Other parasitoid species can discriminate between shaped targets (Wardle & Borden 1990; Wäckers & Lewis 1999), however, these are species (*Exeristes roborator* and *Microplitis croceipes*, Braconidae) whose hosts are found in plant structures (stems, fruit, leaves, flowers) that vary considerably in shape. Variability in shape among these plant structures is normally higher than the variability in fruit shape, so it is reasonable that shape represents an important cue to those parasitoid species, but not to parasitoids of endophytic fruit-boring hosts, such as *D. longicaudata*.

Colour learning was important during host habitat selection for foraging *D. longicaudata* females. Our finding that sensitization seemed to be unimportant for female colour preferences, but females were able to associate the colour of the substrate with the presence of host larvae has three main implications. First, trained females were able to discriminate between colours, suggesting that females do have the optical requirements to discriminate colours; thus, the lack of preference by naïve and sensitized females probably results from a lack of motivation and not from a lack of perception. Second, females were able to establish an association between colour and hosts, a mechanism known as associative learning (Turlings et al. 1993). Finally, the strong response of females to colours after the training sessions supports our hypothesis that learning is important for a generalist parasitoid such as *D. longicaudata*. For this species, the potential host habitats are variable from generation to generation but rather constant within a generation, so females have the chance to find hosts associated with one habitat in consecutive searching bouts. Thus, by using previous experiences, they can narrow their search in future foraging events.

For the red–black and yellow–orange combinations, trained females showed no preference pattern. Given the strong response to the training found for other colour combinations, we postulate that the females are unable to discriminate between these two pairs of options. Probably, like many other hymenopterans (Peitsch et al. 1992), *D. longicaudata* lacks the visual receptors corresponding to red wavelengths. If this is the case, red should look like black, and hence females could not differentiate between these two colours. The ability to discriminate colours (independently of intensity) for a given wavelength range depends on the presence of at least two sensitive photoreceptors (L. Chittka, personal communication). This means that if red receptors are lacking, the ability to discriminate colours reliably and independently of intensity ends at about 550 nm. Thus, lack of red receptors could also explain the inability to discriminate yellow and orange by the visual system of *D. longicaudata*.

In the nonassociative learning control, females showed no preference between the colour that they were exposed to during training and the alternative colour. This control

Table 5. Number of visits and mean latency time (in min) for each cue, for females trained in oviposition units of different colours

Colour of training	Paired cues	Number of visits received	$G_{c,1}$	Mean latency (SE)	P
Red	Red	51	5.58*	1.33 (0.14)	0.59
	Green	29		1.22 (0.15)	
	Red	57	14.03***	1.36 (0.14)	0.09†
	Yellow	23		0.91 (0.09)	
	Red	55	10.76**	1.19 (0.11)	0.89
	Orange	25		1.21 (0.18)	
	Red	45	1.02	0.90 (0.08)	0.66†
Green	Black	35		1.01 (0.17)	
	Green	61	22.05***	1.67 (0.15)	0.25
	Red	19		2.07 (0.37)	
	Green	51	5.58*	1.13 (0.11)	0.17
	Yellow	29		1.43 (0.21)	
	Green	54	9.29**	1.48 (0.13)	0.96
	Orange	26		1.47 (0.21)	
Yellow	Green	62	24.38***	1.02 (0.11)	0.15
	Black	18		1.39 (0.28)	
	Yellow	53	7.95**	1.40 (0.16)	0.49
	Red	27		1.21 (0.21)	
	Yellow	54	9.29**	1.24 (0.14)	0.33
	Green	26		1.00 (0.19)	
	Yellow	41	0.01	1.73 (0.24)	0.61
Orange	Orange	39		1.90 (0.24)	
	Yellow	56	12.33***	1.68 (0.20)	0.10
	Black	24		2.32 (0.33)	
	Orange	52	6.71**	0.94 (0.11)	0.10†
	Red	28		0.66 (0.07)	
	Orange	52	6.71**	1.08 (0.13)	0.99†
	Green	28		1.34 (0.27)	
Black	Orange	34	1.52	1.41 (0.22)	0.29
	Yellow	46		1.14 (0.15)	
	Orange	55	10.76**	1.72 (0.18)	0.74
	Black	25		1.62 (0.20)	
	Black	43	0.13	1.09 (0.14)	0.34
	Red	37		1.32 (0.20)	
	Black	56	12.33***	1.36 (0.13)	0.10
Black	Green	24		1.80 (0.27)	
	Black	54	9.29**	1.21 (0.14)	0.40
	Yellow	26		1.01 (0.18)	
	Black	59	17.78***	1.49 (0.16)	0.73
	Orange	21		1.39 (0.23)	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

†Homoscedasticity assumption was violated and a square-root transformation was applied.

Table 6. Number of visits and mean latency time (in min) for each cue, for females trained in oviposition units of different shapes

Shape of training	Paired cues	Number of visits received	$G_{c,1}$	Mean latency (SE)	P
Circle	Circle	34	1.52	1.86 (0.19)	0.67
	Oval 1	46		1.75 (0.16)	
	Circle	44	0.61	2.00 (0.18)	0.95
	Oval 2	36		1.98 (0.21)	
Oval 1	Oval 1	43	0.32	2.20 (0.17)	0.29
	Circle	37		1.93 (0.19)	
	Oval 1	37	0.32	1.56 (0.16)	0.22
	Oval 2	43		1.87 (0.19)	
Oval 2	Oval 2	44	0.61	1.78 (0.19)	0.42
	Circle	36		1.97 (0.15)	
	Oval 2	36	0.61	1.78 (0.14)	0.57
	Oval 1	44		1.66 (0.16)	

Number of visits received by each model did not differ for any pair of cues.

allowed us to be certain that females were associating a host-related cue with the colour of the substrate. Without this control it would have been impossible to rule out that females were responding to the training by a different process. Females could have fixed the colour that they had experienced during training as a well-known place to search, and used this information in the choice test. Also, females could have shown some kind of neophobia and refused to search in an oviposition unit with a colour that they had never been in contact with. Thus, the results of the experiments exposing females to empty coloured oviposition units strongly support the associative learning mechanism proposed above.

The brightness control experiments showed that *D. longicaudata* females were responding to the hue of the colours and not to differences in light intensity. This result agrees with other studies that performed similar controls (Wardle 1990; Shafir 1996). Other researchers

Table 7. Number of visits to each cue by females trained in oviposition units of different shades of grey (brightness control), or by females that were exposed to empty oviposition units wrapped in coloured fabrics (nonassociative learning control)

Type of control	Control cue	Paired cues	Number of visits received	$G_{c,1}$
Brightness control	Dark grey	Dark grey	38	0.11
		Light grey	42	
	Light grey	Light grey	34	
		Dark grey	46	
Nonassociative learning control	Green	Green	21	0.03
		Red	19	
	Green	Green	22	0.23
		Yellow	18	
	Green	Green	22	0.23
		Orange	18	
	Green	Green	19	0.03
		Black	21	
	Yellow	Yellow	21	0.03
		Red	19	
	Orange	Orange	22	0.23
		Red	18	
Black	Black	18	0.23	
	Yellow	22		
Black	Black	18	0.23	
	Orange	22		

Number of visits received by each model did not differ for any pair of cues.

have inferred from their own results that colour learning is also based on hue learning (Messing & Jang 1992; Oliai & King 2000), although they did not include control assays. Brightness could be more important than hue during host searching in some insects (Wardle 1990).

How do females integrate visual information? Our results suggest that females respond to the training for

colour both in small and in large oviposition units, showing that the association between colour and hosts is maintained even when large (preferred) targets are offered. That females showed no preference when large oviposition units were coated in the nonpreferred colour (and the small ones in the preferred colour) indicates that colour and size have a similar effect on the female's choice. This result depicts a nonhierarchical relationship between these cues. The results also show neither an additive nor a multiplicative effect of these cues on the preference of the females (the percentage of females visiting large yellow oviposition units was not statistically higher than the percentage of females visiting the preferred oviposition unit in experiments in which females confronted oviposition units that differed only in colour or size). It seems that the presence of at least one of the two cues is enough, since no further increase in the number of visits was registered when another attractive visual cue was added. Perhaps, an increase in the response could be achieved if another type of sensorial cue is added, given that previous studies have found that females frequently use visual cues together with other types of cues (Wäckers & Lewis 1994; Jang et al. 2000; Fischer et al. 2001).

In summary, our study has shown that: (1) some visual cues associated with the host habitat (such as size) are innately important during host searching for *D. longicaudata*; (2) other visual cues (such as colour) are also important but only after a previous experience and through an associative learning mechanism; (3) still other cues (such as shape) seem to be ignored even after females have found hosts associated with them in previous successful foraging bouts. Thus, we conclude that both inherited and learned preferences of visual cues are important for the host-foraging behaviour of this generalist parasitoid.

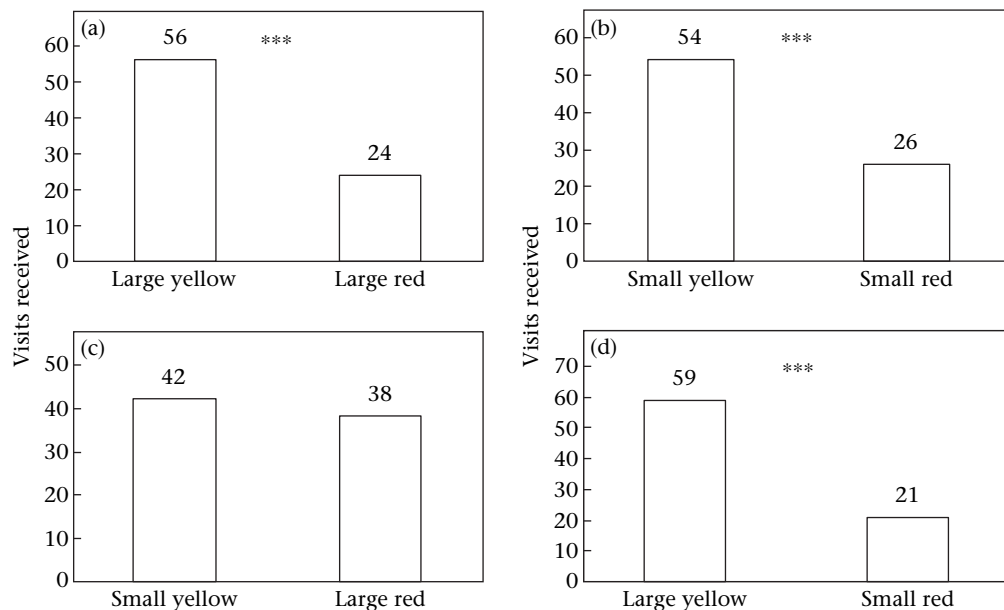


Figure 1. Effect of size and colour of paired oviposition units on the number of female *D. longicaudata* that visited each option. All females were trained on a yellow oviposition unit for three consecutive days. (a) Two large oviposition units differing in colour; (b) two small oviposition units differing in colour; (c) a small yellow oviposition unit versus a large red oviposition unit; (d) a large yellow oviposition unit versus a small red oviposition unit. *** $P < 0.001$.

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