

## **Learning in the Generalist Tachinid Parasitoid *Exorista Mella* Walker (Diptera: Tachinidae)**

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*Many parasitoids have been shown to learn visual and/or olfactory cues associated with hosts. In contrast to the Hymenoptera, learning in dipteran parasitoids is relatively unstudied. This study explores the ability of a polyphagous tachinid, *Exorista mella*, to learn to associate visual and olfactory cues with hosts. In an experiment involving colored host models, flies trained on models of one color were subsequently attracted more strongly to models of the color that they had not experienced. The unsuitability of these models as hosts suggested that the flies may have engaged in avoidance learning. Flies demonstrated the ability to learn to associate colored disks with hosts. A separate experiment demonstrated that flies responded to volatile plant compounds but failed to find evidence for odor learning. Learning of host-associated cues by *E. mella* may allow this generalist parasitoid to take advantage of locally abundant host populations and maintain host-searching efficiency in an unpredictable environment.*

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**KEY WORDS:** associative learning; parasitoid; Tachinidae; visual cues; host location.

### **INTRODUCTION**

The ability to learn is now known to be widespread in insects (Papaj and Lewis, 1993). The value of learning is most easily understood in species with generalized life histories (Stephens 1993). Such insects are often faced with

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a variable environment and must possess the flexibility to find profitable habitats, locate high-quality resources (and avoid noxious ones), and respond to fluctuations in the abundance of potential resources. The capacity to learn enables insects to exploit a variety of resources or habitats while still maintaining searching efficiency and achieving efficient rates of oviposition, often in the face of competition from more specialized species. In parasitoids, which must locate inconspicuous hosts that are under strong selection to avoid detection, the ability to learn may be particularly valuable (Vet *et al.*, 1991, 1995), especially in generalist parasitoids (Vet and Dicke, 1992; Poolman Simons *et al.*, 1992; Geervliet *et al.*, 1998). In contrast, insect species that face a relatively constant and predictable environment (or a completely unpredictable one) with respect to hosts or other resources may not be expected to learn, as the ability to learn may entail costs in terms of both physical processing machinery and an increased likelihood of making mistakes (Stephens, 1993; MacGregor and Henderson, 1998).

Indeed, learning has been well documented in hymenopteran parasitoids (Turlings *et al.*, 1993). In their 1993 review of parasitoid learning, Turlings and colleagues stated that almost 20 species of parasitic wasps had been demonstrated to engage in some form of learning. Since then, the occurrence of learning has been established in many more species (e.g., Powell *et al.*, 1998; Dutton *et al.*, 2000; Dukas and Duan, 2000; Olai and King, 2000). Most of the studies demonstrating learning in wasps have focused on olfactory cues such as those derived from plants (Vet and Groenewold, 1990) and host feces (e.g., Turlings *et al.*, 1989). A small number of studies have also demonstrated learning of visual cues in parasitic wasps (e.g., Arthur, 1967; Wardle, 1990; Wäckers, 1999).

Evidence of learning in other parasitoid taxa is essentially nonexistent. Flies in the family Tachinidae are second only to parasitic Hymenoptera in their diversity and ecological importance as parasitoids (Godfray, 1994). Tachinids are exceptional among parasitoids in their broad host ranges (Belshaw, 1994), with some species attacking hosts in dozens of insect families and multiple orders [e.g., *Compsilura concinnata* (Arnaud, 1978; Herting, 1980, Boettner *et al.*, 2000)]. In addition, the ability to learn color and odor cues associatively has been documented in several close relatives of tachinids including calliphorids (McGuire, 1984; Fukushi, 1985, 1989; Campbell and Strausfeld, 2001) and muscids (McGuire, 1984; Conlon and Bell, 1991). Given these generalist life histories and the capacity for learning in related taxa, tachinids should be expected to exhibit well-developed learning abilities. Although a number of studies have examined mechanisms of host location and acceptance in tachinids (e.g., Roth *et al.*, 1978, 1982; Roland *et al.*, 1995; Mondor and Roland 1997, 1998; Tanaka, 1999a), only one (Monteith, 1963) has purported to demonstrate learning.

Tachinid flies use both olfactory and visual cues to locate hosts (Martin *et al.*, 1991; Monteith, 1955, 1956; Stireman, 2002) and may be expected to be able to learn cues associated with both of these sensory modalities. However, the relatively large eyes, the well-developed visual systems characteristic of higher Diptera (Dethier, 1963; Strausfeld, 1970; Bushbeck and Strausfeld, 1997; Campbell and Strausfeld, 2001), and the demonstrated use of visual cues by tachinids (Monteith, 1956; Weseloh, 1980; Tanaka, 1999a,b) suggest that learning of visual cues may be especially well developed in this group.

Here, I employed three laboratory experiments to examine the ability of the generalist tachinid parasitoid *Exorista mella* (Walker) to learn visual and olfactory cues associated with hosts. The results of these experiments are used to evaluate whether *E. mella* is able to learn host-associated cues, what types of cues are learned, and how the learning abilities demonstrated may function in nature.

## MATERIALS AND METHODS

### Study Organisms

*Exorista mella* is a polyphagous tachinid parasitoid that uses a wide variety of externally feeding Macrolepidoptera as hosts across much of North America (Sabrosky and Arnaud, 1965). It has been recorded to attack more than 50 species of caterpillars belonging to 10 families, though most records have tended to come from hairy species of Arctiidae and Lasiocampidae and other families (Arnaud, 1978). Females firmly glue hard-shelled eggs onto the surface of the host with a soft extensible ovipositor. These eggs must undergo a period of embryonic development [48–72 h (Adam, 1968)] before the larva hatches and burrows into the host. Females generally produce from 100 to 300 eggs over their lifetimes, with approximately 5–10 available for deposition at any one time (Adam and Watson, 1971; Stireman, unpublished data). Multiple individuals may develop within a single host, but the final adult mass of flies decreases with increasing numbers of larvae per host (Adam and Watson, 1971; Stireman and Singer, unpublished data).

Flies used in this study were between second- and fifth-generation offspring of individuals reared from natural populations of *Grammia geneura* (Lepidoptera: Arctiidae) caterpillars collected from mesquite savannas in southeastern Arizona. These black “woolly-bear” caterpillars are highly polyphagous, they dwell primarily on the ground, and they are able to ambulate extensively and rapidly (Singer, 2001; Singer and Stireman, 2001). A culture of *G. geneura* was reared in the laboratory to ensure continuous availability of flies, although additional *E. mella* individuals reared from

field-collected caterpillars were added on a regular basis to sustain the laboratory culture. Flies were kept in two glass terraria (32 × 41 × 26 cm high, 43 × 62 × 33 cm high) with screened openings situated adjacent to a partly shaded south-facing window, providing a natural light/dark regime. Each terrarium was provided with separate petri dishes containing water, sugar, and a yeast extract/sugar mixture. Terraria were kept at room temperature, about 22–25°C, however, the temperature in the cages was often much higher due to incident sunlight. Males and females were kept together to ensure mating. All females used in these experiments were at least 1 week old to ensure that they had the opportunity to mate and develop fertile eggs [mean preoviposition period, 3.2 ± 1.1 days (Adam and Watson, 1971)]. All caterpillars were reared individually in 6-oz plastic cups on an artificial diet in a large growth chamber [see Singer (2001) for detailed rearing methods].

### Experiment 1. Colored Caterpillar Models

An initial experiment was conducted to examine if and how the responses of flies to moving colored host models changed with experience. Moving host models were used due to initial observations that only moving caterpillars and models elicited strong responses from flies (Stireman, 2002). Training and testing sessions were conducted under a clear plastic cylindrical (27.3 × 22.5-cm diameter) foraging arena. This arena was placed atop an elevated white laminate board. Caterpillar models were composed of vacuum-dried final-instar *G. geneura* caterpillars painted with either yellow (Sun Yellow) or blue (Pacific Blue) spray-paint (Krylon brand), with their ventral surface glued to small metal paper clip. The colors yellow and blue were chosen because they are widely separated in wavelength along the visible color spectrum and because a related fly species (*Lucilia cuprina*: Calliphoridae) has been demonstrated to learn these colors readily (Fukushi, 1989). These paints differed in hue, intensity, and possibly other characteristics as well. All references to “color” in this study refer to these combined attributes. For each training session, a host-experienced *E. mella* female (i.e., a female that had been exposed to caterpillars on at least two separate occasions but did not necessarily oviposit on them) was released into the chamber and allowed to acclimate for 2 min before the caterpillar model was introduced. When the session began, a magnet held under the board was used to pull the model around a circular track 15 cm in diameter drawn lightly onto the board at the rate of approximately 5 cm/s. Each trial lasted 90 s. Previous observations indicated that this was a sufficient amount of time in which to observe a response by the flies. Flies were randomly assigned to either yellow or blue caterpillar models, and each experienced only a single training session in which they were initially exposed to the painted corpses.

One hour after the training session, flies were exposed in the same manner to both blue and yellow caterpillar models presented singly in random order. The time to first contact of the each of the models was recorded using a stopwatch. Two of 29 flies tested on models were excluded due to the lack of response to any treatment within the 90-s trials.

### Experiment 2. Colored Paper Disks

To test whether *Exorista mella* is able to learn to associate visual cues with hosts, female flies were subjected to training sessions with *G. geneura* caterpillars associated with green and orange paper disks. The training sessions were conducted by placing flies in clear plastic hexagonal chambers (15 × 26 × 17.5 cm) with two petri dishes (9.5-cm diameter), one containing a green paper disk and one an orange paper disk. The paper disks were cut from construction paper to the same size as the interior of the petri dishes. The colors orange and green were used because they lie well within the range of color though to be perceived by related species of flies (Dethier, 1963) and an innate response to these colors in studies of related flies was lacking (Fukushi, 1989).

In each training session, four female flies that had previous experience with hosts (at least two separate 20-min exposures) were placed in individual chambers separated by Styrofoam barriers to prevent visual interaction. Flies were randomly assigned to treatments—either the green disk or the orange disk contained a live *G. geneura* caterpillar. The position of the petri dishes in each chamber was systematically varied so that learning of a location would not occur. Each time a fly was observed to oviposit on a caterpillar, the caterpillar was removed and replaced with a fresh caterpillar on the same colored disk, and the positions of the petri dishes were switched. Most caterpillars did not leave the petri dishes, but when they did they were immediately placed back in the original dish. Each fly was trained for at least 1 h and up to 2 h or until three caterpillars had been parasitized. Flies were subjected to two such training sessions on consecutive days.

One hour after the end of the second training session, the behavior of each fly was examined in a no-choice test. This test was conducted in the same type of chamber as the training sessions. Each fly was presented for 10 min with a single empty petri dish containing a randomly chosen colored disk and subsequently presented with a petri dish with the alternative colored disk. The time taken from the moment the fly entered the chamber to the first contact with the petri dish within the maximum 10-min period was recorded using a stopwatch. As soon as the fly made contact with a petri dish the trial was terminated. Of 36 flies subjected to training in nine sessions, 16 were excluded from most analyses because they did not oviposit on any

caterpillar during training and/or did not respond to any treatment during testing.

### Experiment 3. Response to Odors in an Olfactometer

The response of *Exorista mella* to odor cues that it had previously encountered in association with hosts was assessed in a no-choice olfactometer assay. Females were given experience in hexagonal chambers (described above) with live caterpillars that had been lightly brushed with a paint brush dipped in dilute solutions of two distinct volatile organic compounds, either carvone ( $C_{10}H_{14}O$ ; a monoterpene) or coumarin ( $C_9H_6O_2$ ; a phenylpropanoid). These two highly volatile compounds are both commonly found in a variety of plant species. The solutions were subjected to a dilution series in mineral oil (carvone) or distilled water (coumarin) until I could barely detect the scent of the compounds, resulting in solutions of 0.025 and 0.0083%, respectively. Although this method of standardizing the solutions was subjective, it was deemed more appropriate than using equal concentrations due to differences in volatility of the compounds. Application of the solutions occurred immediately before the caterpillars were placed in the training chambers. Due to the effort required to train flies, a maximum of six was trained per session. As in the colored disk experiments, caterpillars were replaced after receiving at least one fly egg. Flies were subject to a single training session, which lasted 2 h or until flies had laid eggs on at least two hosts bearing the training odor (at least 1 h). Approximately 1 h after the training session the response of flies to these odors along with a control were examined in an olfactometer. Of 40 flies subjected to training sessions, 7 did not deposit eggs on hosts and were excluded from testing.

The olfactometer chamber consisted of a 1000-ml side-arm filtering flask fitted with a rubber stopper (see Stireman, 2002). Laboratory air was passed through tubing into a cylindrical glass chamber holding an odor source and then passed through additional tubing into the flask 4 cm above the filter paper-covered bottom. Treatments consisted of (1) a plain cotton ball (control), (2) 10  $\mu$ l of a carvone solution soaked into a cotton ball, and (3) 10  $\mu$ l of a coumarin solution in a cotton ball. These volumes approximated the volume of solution initially brushed on the caterpillars. Each fly was subjected to all treatments in random order. For each trial, flies were placed in the olfactometer chamber and allowed to explore it for 1 min before the testing commenced. Laboratory air was passed through the odor chamber and into the olfactometer chamber at a rate of about 28 ml/s. The latency and duration of all resting, grooming, walking, and flying behaviors were recorded continuously using The Observer (Noldus, 1993). After each odor treatment the olfactometer chamber and odor chamber were washed thoroughly with

soap and water, the filter paper was replaced, and air was flushed through the system for 5 min. In addition, a set of 14 flies that had no experience with either odor was subjected to the same set of treatments to allow informal post hoc comparisons to be made with trained flies to provide insight into the effects of training on the flies' responses to odors.

### Analysis

Because each of the experiments described above involves repeated measurements of the same individuals, all analyses were conducted in a paired fashion. Repeated-measures, ANOVA models were used in all experiments to allow the effects of all factors and their interactions to be assessed. Simple contrasts were specified *a priori* in additional within-group repeated-measures ANOVAs in SPSS version 10.0 (SPSS Inc., 1999) to compare within-treatment group responses to testing sessions (with Bonferroni corrected  $\alpha$  for multiple tests). Data resulting from most experimental treatments were nonnormally distributed due to the truncation of trials and the large variance in fly behavior. Examination of the observed residuals plotted against expected did not reveal any systematic pattern of heteroscedasticity. Fixed-effect ANOVA statistical analyses, such as those used here, are relatively insensitive to violations of normality assumptions (Scheffe, 1959). Where possible, data were log-transformed to meet assumptions of normality as judged by the highly conservative Shapiro–Wilk test for normality, with an  $\alpha$  of 0.01.

In the first experiment, the time to contact (latency) the colored caterpillar models was used as a response variable to test whether the flies responded more rapidly to model colors that they had experienced previously. Analyses compared the latency (log-transformed) to contact the training model versus the alternative model, with effects of training color and its interaction with training effect included in the statistical model. Thus the effect of training was initially analyzed for all flies, and the effect of training color was subsequently included as a separate factor. Flies that did not contact either treatment source in this and all other experiments were excluded from ANOVAs. Initial responses to colored hosts were evaluated using a Kruskal–Wallis test.

In the colored disk experiment the same set of explanatory factors (training effect, training color, interaction) was analyzed with a repeated-measures ANOVA model. As in the colored model experiment, the time to contact the trained color versus the alternative color was compared for each fly. Repeated-measures ANOVA and simple *a priori* contrasts were used to compare the responses of flies to colored disks within groups. A McNemar's test (Zar, 1996) was used to evaluate whether the number of flies that made

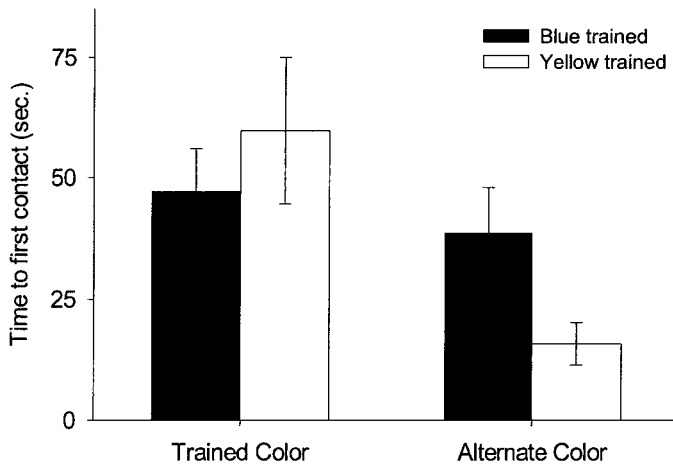
contact with the colored paper disks depended upon whether they were trained on that particular color.

The latency and total duration of walking behavior were used as response variables in repeated-measures ANOVAs of the odor learning experiment. These measures were used because once *E. mella* females detect the presence of hosts, they almost always approach them by walking rather than flying (under laboratory conditions). In addition, other tachinid species have been observed to respond to odors associated with hosts with rapid ambulation (Roth *et al.*, 1982). A Bonferroni-corrected  $\alpha$  of 0.017 was applied to multiple simple specified contrasts of responses to different odor treatments. Statistical analyses were performed using StatView 4.02 (Abacus Concepts Inc., 1993) and SPSS version 10.0 (SPSS Inc., 1999)

## RESULTS

### Experiment 1. Colored Caterpillar Models

In the initial training sessions, no significant difference in response to yellow or blue models was observed (Kruskal–Wallis test:  $n = 27$ ;  $\chi^2 = 0.784$ ,  $df = 1$ ,  $P = 0.38$ ). After training, *E. mella* flies exhibited a significantly different response to models on which they had been trained versus those that they had not experienced (Fig. 1) (repeated-measures ANOVA:

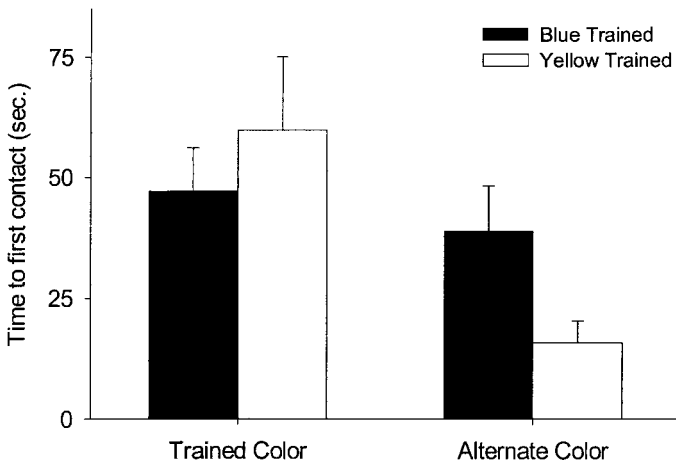


**Fig. 1.** The means ( $\pm$ SE) of the time to contact colored host models that were experienced during training sessions and models that were not experienced (alternate color) for flies trained on yellow ( $N = 14$ ) and blue ( $N = 13$ ) models.

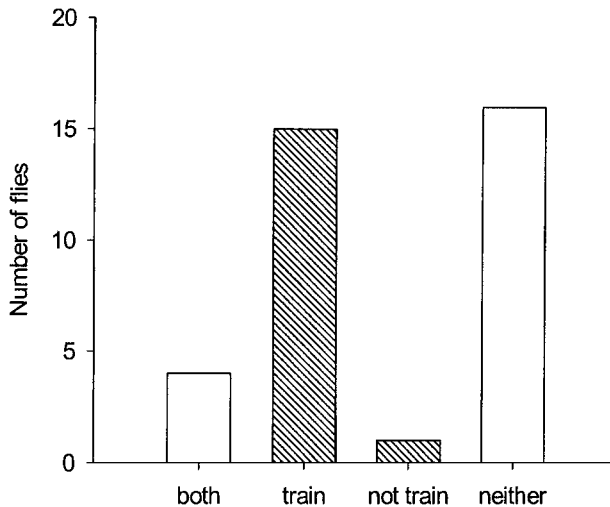
treatment,  $F_{1,26} = 3.60$ ,  $P = 0.042$ ; treatment  $\times$  color,  $F_{1,27} = 4.166$ ,  $P = 0.027$ ). However, this response was the opposite of what had been expected and was dependent upon training color. Flies trained on yellow host models contacted blue host models more rapidly than the yellow ones they were trained with ( $F_{1,14} = 12.28$ ,  $P = 0.004$ ) and those they experienced in subsequent tests (repeated-measures contrast:  $F_{1,14} = 8.075$ ,  $P = 0.013$ ), while those trained on blue models exhibited no difference in response to models of either color ( $P > 0.5$ ). Despite this difference in the responses of blue- and yellow-trained flies to subsequent models, the effect of training color as a whole was not found to be significant ( $F_{1,27} = 0.029$ ,  $P = 0.867$ ).

### Experiment 2. Colored Paper Disks

In tests with colored disks, flies that responded to the disks approached the color on which they had been trained more quickly than the alternative (repeated measures ANOVA: treatment,  $F_{1,18} = 16.779$ ,  $P = 0.001$ ) (Fig. 2). The larger proportion of orange-trained flies (0.67 vs 0.44) that responded to test treatments may indicate that this color is more easily associated with hosts, but among flies that responded to test treatments I found no significant difference in ability to learn to associate either color with hosts [i.e., no effect



**Fig. 2.** The means ( $\pm$ SE) of the time to contact colored paper disks that flies had experienced previously in association with hosts. Flies were trained with hosts associated with either green ( $N = 8$ ) or orange ( $N = 12$ ) paper disks. Sixteen flies that did not contact either colored disk during testing are excluded. The difference between the trained and the untrained color is significant for green-trained flies ( $P = 0.009$ ) and marginally so for orange-trained flies ( $P = 0.028$ ; see text).

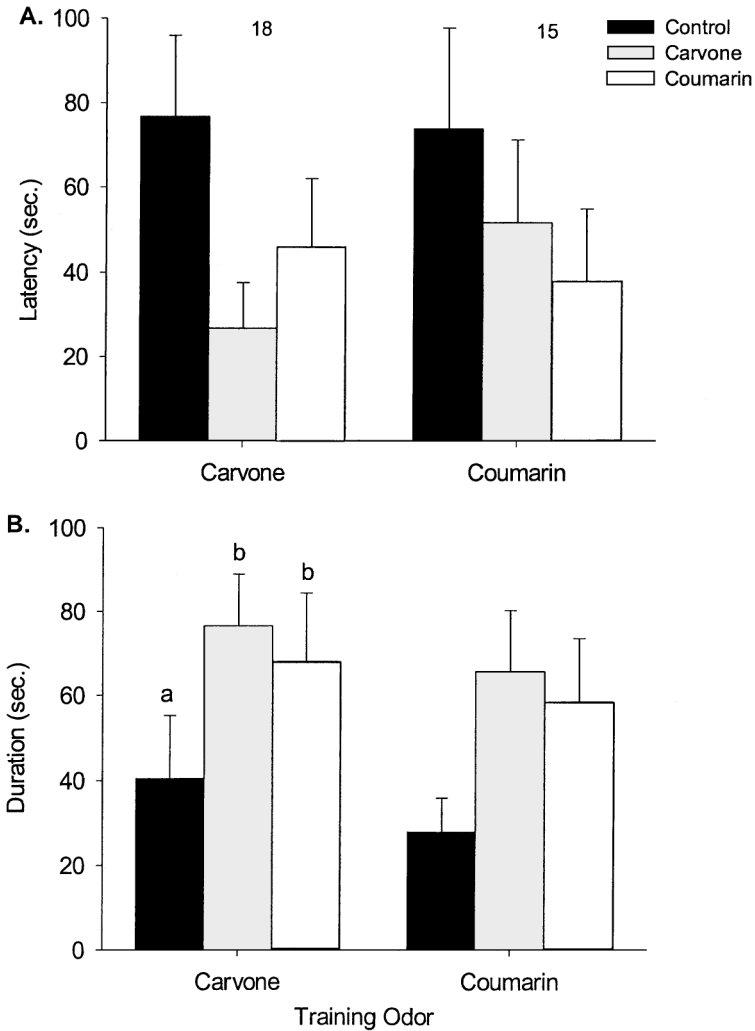


**Fig. 3.** The total number of flies that contacted either both colored disks (both), the colored disk they were trained with (train), the alternate colored disk (not train), or neither colored disk (neither) during testing. See text for statistics.

of color ( $F_{1,18} = 3.263$ ,  $P = 0.088$ ) or color  $\times$  treatment ( $F_{1,18} = 1.074$ ,  $P = 0.314$ ]. Simple planned contrasts indicate a significant response to colors associated with caterpillars for green-trained flies ( $F_{1,7} = 9.244$ ,  $P = 0.019$ ) and marginally for orange-trained flies as well ( $F_{1,11} = 6.645$ ,  $P = 0.026$ ). These results underestimate the effect of training because flies that failed to make contact with a colored disk in a particular trial were assigned a latency equal to the total duration of the trial (600 s). The total number of flies that made contact with the colored disk on which they were trained within the test duration was significantly higher than the number that made contact with the alternative colored disk (Fig. 3) (McNemar's test:  $\chi^2 = 10.56$ ,  $df = 1$ ,  $P < 0.005$ ).

### Experiment 3. Response to Odors in an Olfactometer

Analysis of the odor-learning experiments revealed that flies began walking more quickly and walked for a longer duration when presented with an odor than when exposed to the no-odor treatment (Fig. 4) (repeated-measures ANOVA: latency,  $F_{2,30} = 4.04$ ,  $P = 0.028$ ; duration,  $F_{2,30} = 8.375$ ,  $P = 0.001$ ). However, there was no significant difference as a whole in their response to the odor they had been trained with versus the alternate odor (Fig. 4). In addition, no significant effect of training odor or



**Fig. 4.** Latency (A) and total duration (B) of the time spent walking in an olfactometer in response to three odor treatments ( $\pm$ SE) by flies trained with either carvone or coumarin. Numbers above the columns indicate the number of flies tested in each treatment; letters refer to significant differences. Panels without letters indicate that within-group contrasts were not significant ( $P > 0.017$ ) despite overall significant effects.

odor  $\times$  treatment interactions on walking duration or latency were indicated by repeated-measures ANOVA (odor,  $F_{2,29} = 0.450$ ,  $P = 0.507$ , and  $F_{1,30} = 0.023$ ,  $P = 0.881$ ; interaction,  $F_{2,30} = 0.223$ ,  $P = 0.802$ , and  $F_{2,29} = 0.023$ ,  $P = 0.978$ , for duration and latency, respectively). Contrasts

within treatment groups (with Bonferroni-corrected  $\alpha = 0.017$ ) indicated that flies trained with carvone exhibited a significantly longer walking duration in the presence of either carvone or coumarin relative to control air ( $P < 0.01$ ), whereas those trained with coumarin did not respond significantly to either odor. Carvone-trained flies also exhibited a trend toward a shorter latency of walking behavior in response to carvone than controls ( $P = 0.059$ ). Coumarin-trained flies showed no significant difference in latency or duration of walking among treatments, but the low  $P$  values (e.g., duration: carvone vs control,  $P = 0.032$ ; coumarin vs control,  $P = 0.058$ ) suggest that this may be a function of the relatively low power of these contrasts (0.542 and 0.538, respectively). The separate experiment, in which flies with no prior experience with either carvone or coumarin were tested in the same manner, revealed no significant difference in response to odor sources and control air in walking latency or duration, although the sample size ( $N = 14$ ) and power (0.27) were quite low (repeated-measures ANOVA:  $F_{2,12} = 1.83$ ,  $P = 0.181$ , and  $F_{2,12} = 0.786$ ,  $P = 0.467$ , respectively).

## DISCUSSION

### Response to Colored Models

The results of the colored model experiment do not provide evidence of either sensitization (*sensu* Papaj and Prokopy, 1989) or positive associative learning as was expected, but they do demonstrate an interesting change in behavior with experience. When *E. mella* females approach caterpillars, they typically drum their tarsi on the host, presumably gathering chemosensory information regarding the suitability of the host. It is likely that the paint provided negative information in these experiments since no flies attempted to oviposit on these models, and in a similar experiment flies readily attacked dead unpainted caterpillars presented in the same manner (Stireman, 2002). The more rapid approach to the alternative model color for those flies trained on yellow models suggest that *E. mella* may engage in avoidance learning. Habituation might result from experience with unsuitable hosts (especially those that are distasteful and likely toxic), causing a decrease in the attractiveness of those hosts upon subsequent encounters and perhaps an increase in attractiveness of hosts that appear different. Avoidance learning in response to electric shock has been demonstrated in other Diptera (McGuire *et al.*, 1990), though the form of avoidance learning suggested here may be more similar to the "food aversion learning" which has been demonstrated in generalist herbivorous and predatory insects (Bernays, 1993).

The stronger response of the yellow-trained flies to blue models than the blue-trained flies to yellow models may reflect a difference in the ability of flies to learn these colors. Yellow is innately attractive to and relatively easily learned by the calliphorid *Lucila cuprina* (Fukushi, 1989). It is interesting to note that many aposematically colored insects advertise their unpalatability or potent armament with bright yellow coloration (e.g., wasps, bees, butterflies, caterpillars). It has been shown that at least some animals can learn to avoid unpalatable prey bearing such coloration better than those bearing other colors (e.g., Gittleman and Harvey, 1980; Sword, 1999), and aposematic species may be exploiting these preexisting biases in learning ability. In the present context, the ability of *E. mella* females to learn to avoid yellow hosts more easily than blue could be adaptive in that many chemically defecated caterpillars, which are likely to be unsuitable as hosts for *E. mella*, are bright yellow (e.g., some Arctiidae, Sphingidae, Saturniidae), and few are blue or otherwise darkly colored (e.g., *Chlosyne* spp.; Nymphalidae).

### Conditioning with Colored Disks

The attraction of *Exorista mella* to colored disks that they had previously experienced in association with caterpillars demonstrates discrimination learning and is consistent with associative learning of visual cues. It is not certain what characteristic(s) of the disks (e.g., wavelength or intensity) the flies used to differentiate the two colors. Related taxa are known to learn wavelength irrespective of brightness (Fukushi, 1985), and it is likely that *E. mella* did not respond solely to the slight difference in brightness between the two colored disks used. In the field, this ability to associate colored objects with hosts may aid *E. mella* in locating microhabitats such as certain plants or plant parts that are likely to contain hosts. A few hymenopteran parasitoids are known to use visual microhabitat cues to locate hosts (e.g., Glas and Vet, 1983) and this may be related to the ability of certain taxa to learn colors or other visual cues associated with hosts in the laboratory (e.g., Arthur, 1966, 1967; Wardle, 1990; Olai and King, 2000). The ability to learn visual cues associated with host microhabitats in the tachinid *Exorista mella* and in hymenopteran parasitoids may represent convergent solutions to the problem of locating suitable hosts faced by both types of parasitoids.

Alternatively, *E. mella* may use color learning primarily to reinforce visual cues directly associated with hosts. Given the large host range of *E. mella*, refinement of visual cues associated directly with hosts could increase the efficiency of parasitization of locally and/or temporarily abundant hosts. Although the present experiment does not test this, the ability of *E. mella* to associate colored disks with hosts may be a by-product of selection for rapid learning of the color and/or form of hosts themselves.

Many of the individual flies used in this experiment failed to respond to either colored disk after training. There are a number of possible factors that may have interfered with learning, including variation in the reward experienced, age, hunger, and egg load (Turlings *et al.*, 1993; Takasu and Lewis, 1993; Pérez-Maluf and Kaiser, 1998). Further studies are needed to examine how these and other factors may influence host-associated learning in this species.

### Odor Conditioning

The response of flies to carvone and coumarin in the olfactometer confirms previous findings (Stireman, 2002) that *E. mella* responds to volatile plant compounds with increased walking behavior. Despite slight trends toward a lower latency and an increased duration of walking by flies in response to odors they had experienced, the experiment did not demonstrate any clear effects of experience on the response of *E. mella* females to either of the two volatile compounds (i.e., flies did not respond significantly more to the odor they had been trained on than the alternate odor). The significance of the small differences between carvone- and coumarin-trained flies in their response to odor treatments (as indicated by the within-group contrasts) is unknown. It may be that associative learning occurred during this experiment but that *E. mella* is incapable of distinguishing the two volatile chemicals or that the flies engaged in sensitization, which increased their sensitivity to both volatile chemicals after training. The first scenario is unlikely due to the very different molecular properties and distinctive odors of the two compounds. The latter scenario cannot be properly evaluated because the response of the flies to the volatile compounds was not measured before flies were given experience. The apparently weaker response of untrained flies to the compounds may support this interpretation, but this may be an artifact of the small sample size of the untrained group. It is also possible that the responses of flies in this experiment could have been to the solvent (distilled water and mineral oil) rather than the volatile plant compounds, because these solvents were not used in the controls. Given the small volumes, relative lack of odor, and low volatility (mineral oil), this explanation seems unlikely.

The inability of this experiment to demonstrate associative odor learning cannot be taken as evidence that *E. mella* lacks the ability to associate odors with the presence of hosts. There are many possible reasons why this experiment may have failed to demonstrate odor learning even if it is present in this species. The number of separate training experiences with hosts may have been too small (Turlings *et al.*, 1993), the odor solutions may have

affected the acceptability of training caterpillars, and the response variable may have been too crude to detect behavioral preferences for the trained odor. In addition, the power of several of these tests was relatively low (less than 0.7 in several cases), suggesting that any negative results with respect to learning must be interpreted with caution.

## CONCLUSIONS

In summary, the results of this study indicate that *E. mella* is able to associate visual cues with the presence or suitability of hosts. In addition, the response of flies to volatile chemicals is consistent with a priming effect of the odors associated with hosts, but an innate response to these compounds cannot be ruled out. The general finding that *E. mella* uses information gained through experience to alter its response to environmental stimuli associated with hosts represents a convergent strategy with parasitic wasps to overcome the difficulties associated with the parasitoid lifestyle. The short-term nature of these experiments, however, makes it difficult to extrapolate how long these learned responses may be retained in the field. Though learning appears to be widespread in other parasitoids, these results provide only the second reported case of learning in a tachinid fly. Interestingly, the other reported instance of learning in a tachinid parasitoid also involved visual cues (Monteith, 1963). In this study, the tachinid *Drino bohemica* learned to associate the movement of a tray in the bottom of a cage with the presence of hosts.

The wide host range of *E. mella*, its use of polyphagous hosts, and the concomitant unpredictability of host resources should select strongly for a well-developed capacity to learn cues associated with those hosts. The current study represents merely a first step in understanding learning in this parasitoid and how it may be shaped by characteristics of its hosts. A wide array of further experiments is suggested by these initial results. Along with Monteith's earlier work (1963), this study suggests that behavioral flexibility could be a widespread characteristic of tachinids that may profoundly affect their ecological interactions with hosts in natural and managed systems. Recent theoretical work has indicated that learning in polyphagous parasitoids can stabilize host-parasitoid population dynamics and contribute to the coexistence of species (Hastings and Godfray, 1999). Learning of host-associated cues by *E. mella* may allow this generalist parasitoid rapidly to take advantage of abrupt increases in the local abundance of various host species, as well as to occupy a wide diversity of habitats and an extensive geographic range. In addition, host-associated learning may allow this species to persist with more efficient specialist parasitoids and contribute to the stabilization of host population dynamics.

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