Innate and Learned Cues: Scale Cover Selection by *Aphytis melinus* (Hymenoptera: Aphelinidae)

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*Aphytis melinus* recognizes and accepts covers of its host, *California red scale*, *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae), before assessing the size or quality of the scale body beneath. We evaluated the role of a non-volatile kairomone, O-caffeoyltyrosine, and prior experience with hosts on the recognition (antennal drumming) and acceptance (ovipositor probing) of scale covers differing in age and, therefore, size. We tested several hypotheses concerning the role of experience with hosts on host recognition and acceptance. The first predicts that experience with a particular host size leads to increased selection of that host size in the future. The second predicts that the “quality” of the experience with hosts sets a threshold of quality for future acceptance. We manipulated the quality of wasp experience with hosts by exposing some wasps to high-quality hosts (large scale insects under large covers) and other wasps to low-quality hosts (small scale insects under small covers and small scale insects under large covers). Control (naive) wasps were held without experience with hosts. Wasps were then offered five size classes of covers directly after removal from the scale insects (unmanipulated) or after removing the kairomone from the covers (kairomone-free). For covers with natural levels of kairomone, no effect of experience with hosts on host recognition was observed. When the kairomone was removed, however, any experience with hosts increased recognition compared to naive wasps. Moreover, experienced wasps preferentially recognized covers of the size to which they had experience. Results for host acceptance differed from those for host recognition. Wasps given experience with large hosts accepted more covers with kairomone than wasps in other treatments, but the size preference did not vary among treatments. When the kairomone was removed, however, wasps given experience with large hosts under large covers preferred larger covers. Host recognition and acceptance are controlled by different behavioral mechanisms. Prior experience with hosts

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does not alter host recognition but does affect the rate of acceptance. The quality of the experience does not affect the acceptance of covers containing natural levels of kairomone but does affect host acceptance when the kairomone is removed. Experience with hosts alters the motivation of wasps to accept covers, and the direction of this effect is determined by the size (quality) of body the wasps were given during the experience.

**KEY WORDS:** Aphyis melinus; Aonidiella aurantii; experience; learning.

**INTRODUCTION**

Parasitoids optimize their fitness by choosing the highest quality of hosts available. Any deviation from this will be severely penalized, by reductions in either offspring fitness or the number of offspring produced, and so will be selected against (McNamara and Houston, 1986). The most accurate measurements of host quality are host size and chemical composition, but for many host–parasitoid system these measurements are not feasible, either because the host is concealed or because the time expended making the measurements would prove more expensive than the potential fitness gain (Vet et al., 1991). The parasitoid must then fall back to using specific stimuli that indirectly indicate host quality (see Vinson, 1985).

The responses of parasitoids to such stimuli range from being completely innate to completely learned. Innate responses arise from stimuli that are not only reliable but also consistent over an evolutionary time scale (Vet et al., 1995). Parasitoids benefit from using innate cues by minimizing the time required to investigate a host before accepting or rejecting it. A cost of innate responses is that suitable hosts that do not produce the cues will not be recognized. Learned responses arise from stimuli that are consistent and reliable only within the foraging life span of the parasitoid. The cost of relying on learned cues lies in the time necessary to acquire the experience to develop the responses. Learned responses are most adaptive in situations where the host or conditions in which the host is growing are variable (Stephens, 1993; Roitberg et al., 1993). Many responses lie between the two extremes of being completely innate and completely learned. Some innate components have a degree of plasticity, and, therefore, are open to modification (Papaj, 1993). Additionally, many parasitoids use more than one stimulus to assess host quality, which may result in the utilization of a combination of both innate and learned responses (Lewis et al., 1990).

A difficulty in studying the relative importance of innate versus learned cues is that they cooccur and cannot easily be separated. Furthermore, because innate cues are more reliable and consistent, they may overshadow responses to learned cues (Lewis et al., 1990). Comparison of host selection behavior by
parasitoids on different hosts may help to explain and characterize responses. However, different host species differ in numerous ways, making it difficult to isolate and evaluate any individual cue.

The system involving the parasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) with its host California red scale *Aonidiella aurantii* (Maskell, Homoptera: Diaspididae) is ideal for such a study for several reasons. First, the interaction between the wasp and the host has been extensively described, and host size is the primary attribute of host quality (Luck and Podoler, 1985; Opp and Luck, 1986; Yu *et al.*, 1990). Second, an important chemical cue involved in host recognition and acceptance has been identified as *O*-caffeoyltirosine (*OCT*) (Hare *et al.*, 1993; Millar and Hare, 1993). Third, we can remove *OCT* with minimal damage to host material (Morgan and Hare, 1997), allowing us to characterize subordinate cues used in host recognition and acceptance in the absence of the primary cue. Finally, the system not only provides us with a tool for studying the mechanisms of innate versus learned responses, but it may have applied value in understanding the response of parasitoids to hosts varying in quality.

We assessed the importance and quality of wasp experience with hosts on the selection of scale covers differing in age (and therefore size and *OCT* concentration) and evaluated several hypotheses. The null hypothesis (*H₀*) is that experience with hosts does not affect host selection. The first alternative hypothesis (*H₁*) predicts that the experience with a particular host leads to an increase in the selection of that host in future encounters (Vet *et al.*, 1990; Vet and Papaj, 1992). This suggests that experience with hosts should increase selection of the host, irrespective of the quality of that experience. The second alternative hypothesis, which is based on our unpublished observations, is that the quality of experience with hosts sets thresholds of quality acceptance in future encounters. This process is similar to that by which some phytophagous insects become imprinted upon leaves of a particular shape, flowers of a particular color, or fruits of a particular size (reviewed by Papaj and Prokopy, 1989). From this, we would expect wasps to select hosts of equal or higher quality compared to the hosts with which they had experience. This hypothesis can be broken down further by testing whether it is the size of the cover (*H₂*) or the size of the body (*H₃*) that is most important in determining the threshold of quality for host acceptance.

**MATERIALS AND METHODS**

**Biology and Behavior of *Aphytis melinus***

*A. melinus* is a facultatively gregarious ectoparasitoid of the larval stages of Diaspididae. The wasp can parasitize a wide size range of immature hosts.
Only growing immature stages are parasitized. The molt stages and scale insects below 0.15 mm\(^2\) in body area are rejected. For scale insects from 0.15 to 0.39 mm\(^2\) in area, mainly unfertilized (males) eggs are laid on hosts, while fertilized (female) eggs are laid on hosts larger than 0.39 mm\(^2\). On the largest hosts, frequently more than one egg is laid. A greater proportion of females, larger offspring, and more offspring result from large hosts; it is therefore adaptive for wasps to select the largest available hosts (Luck and Podoler, 1985; Opp and Luck, 1986; Yu et al., 1990).

The wasp's host is concealed by a cover that protects the host from desiccation (Foldi, 1990). The wasp depends entirely on characteristics of the scale cover to recognize the host. Host recognition in *A. melinus* involves a series of behaviors that must be completed in sequence before an egg is laid. These behaviors have been carefully described by Luck et al. (1982) and are summarized here. *A. melinus* forages by walking upon the plant substrate, and the first contact with the scale may be at random. The first phase is a "drumming and turning" phase, which is characterized by the wasp climbing on to the scale cover, positioning herself centrally, lowering her antennae, then palpitating ("drumming") the scale cover as she walks forward until she encounters the scale margin. At that point, she backs up, rotates right or left approximately 90°, then repeats the behavior. Several episodes normally occur, as if the scale cover is being measured. Wasps typically drum several scales within a patch before selecting one for ovipositor probing and frequently may leave a scale cover after drumming it and return to it and engage in several more episodes of drumming before finally accepting it. If the wasp accepts the scale cover, then the second behavioral phase commences when she then inserts her ovipositor through the scale cover. After contacting the scale insect body beneath the cover with her ovipositor, the wasp probes the body of the scale insect. Finally, if the scale body is accepted, then the wasp retracts her ovipositor from the scale body, but not from beneath the cover, and deposits an egg on the scale body but beneath the cover (Luck et al., 1982). This series of behaviors must be completed in sequence. The sequence often is broken in several places and restarted before oviposition occurs, but no steps can be skipped. By offering scale covers without bodies, we can assess scale cover recognition and acceptance (antennal drumming and probing through the cover) separately from investigation of the body for physiological suitability for growth and survival of progeny.

*A. melinus* uses *OCT* to recognize California red scale (Quednau and Hübsch, 1964; Luck and Uygün, 1986; Hare et al., 1993). This compound is added to the cover by the scale insect during scale cover enlargement. *OCT* is transient and is implicated in scale cover formation (Hare et al., 1993). *OCT* is not found on covers of mature and molting scale insects, thus the presence of *OCT* informs *A. melinus* of the presence of a scale insect that is in a stage of
development available for parasitism. *A. melinus* may also use the kairomone quantitatively to assess the size of the body beneath the cover because larger growing scale insects produce more OCT (Hare *et al.*, 1993).

**Insect Rearing Procedures**

We reared *A. melinus* on oleander scale, *Aspidiotus nerii* (Bouché), themselves reared on "Eureka" lemon fruit (Tashiro, 1996). All colonies were kept at 26°C and 40–60% RH, on a 16:8 L:D photoperiod (Opp and Luck, 1986). Because wasp behavior is affected by early adult experience with hosts (Hare, 1996), we isolated all wasps used in the study at the pupal stage from their hosts. It is easy to separate the wasp pupae from the remains of the host insect, meconia, and the plant because *A. melinus* is an ectoparasitoid. We collected the newly emerged wasps daily and placed them in a container with a drop of honey to provide nutrition. The wasps were held for 24 h to allow mating and maturation of eggs (Rosen and DeBach, 1979).

**Experience Treatments**

The wasps were provided with one of four pre-bioassay treatments (Fig. 1): (1) wasps given experience with large scale insects under large covers, LC-LB wasps, were provided with hosts large enough to support a single male

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**Diagram**

**WASP TREATMENTS**

- NAIVE
- LC-LB
- SC-SB
- LC-SB

**SCALE COVER TREATMENT**

- UNMANIPULATED COVERS
- KAIROMONE-FREE COVERS

10 WASPS PER REPPLICATE
5 REPLICATES PER WASP TREATMENT

Fig. 1. Diagram of wasp treatments and scale cover treatments tested. See text for details.
offspring, (2) wasps given experience with small scale insects under small scale covers, SC-SB wasps, were provided with hosts only large enough to support a single male offspring, (3) wasps given experience with small scale insects under large covers, LC-SB wasps, were provided with bodies only large enough to support a single male offspring, but concealed under the cover from a scale insect capable of supporting female/multiple offspring, and (4) host-naive wasps were held for an equal amount of time, but without any experience with hosts.

We prepared lemons on which scale insects were growing for the three experience treatments. We used third-instar scale insects (>0.8-mm scale cover radius) on lemons for LC-LB wasps. We removed all scale insects except for 60 that were above the size limit but still immature. We then placed the lemon in a container with the wasps for 24 h. For SC-SB wasps, we used second-instar scale insects (<0.4-mm scale cover radius) on lemons. We removed all scale insects except for 60 that were in the second instar and below the size limit. We placed the lemon in the container with the wasps for 24 h. The third experience treatment provided wasps experience with large scale covers (>0.8 mm) over small bodies (<0.4 mm; LC-SB). We prepared a lemon with second-instar California red scale, as above. We then removed the covers, leaving the bodies on the lemon. We collected covers from living third-instar scale insects and set them on the bodies of the small scale insects using a drop of water. Wasps were offered the experience with hosts as above. For the maintenance of "host-naive" wasps, those with no prior experience with hosts, we simply held wasps in a container for 48 h with a drop of honey.

We used 15 mated female wasps for each treatment. Two wasp treatments were set up each day. After the 24-h wasp treatment period, we removed the lemons with scale and held the wasps in containers with honey for a further 24 h prior to bioassay. We held wasps for this extra time to replenish their reserves of mature eggs (e.g., Heimpel et al., 1997).

**Bioassay Procedures**

We studied host selection behavior using a simple bioassay derived from Luck et al. (1982). We constructed an arena using the microscope slide as the base on which scale covers were placed. A second slide with a hole in it (2.0 cm diameter) was placed on top of the first to form a well. An intact microscope slide was placed over the well to complete the arena. We introduced a single female wasp into the arena and observed her activity over a 5-min interval. We recorded the number of bouts of antennal drumming after scale cover contact and the number of cover acceptances (ovipositor insertions) on each scale cover over this interval. The wasp was removed after the time had elapsed. For each set of scale covers, 10 wasps were tested. Using this method, we carried out two treatment replicates each day. Each of the eight treatment combinations
(four wasp treatments by two cover treatments) was replicated five times with new scale covers.

We assessed scale cover size selection by offering wasps five scale cover ages ranging from the smallest acceptable host size to scale covers concealing unmated but mature hosts. Five scale covers from each of five age classes of scale insects were removed from the bodies of the insects (0.38-, 0.52-, 0.66-, 0.08-, and 0.94-mm radius). One set of scale covers was prepared for each cover treatment (Fig. 1). For the unmanipulated cover treatment (i.e., with OCT), we placed the scale covers directly onto a microscope slide arranged in a 5 × 5 Latin square design with a 2-mm separation between each cover. For the kairomone-free cover treatment (scales with OCT removed), we dipped scale covers in 1 N NaOH for 5 sec, then washed them in three changes of distilled water before mounting on a microscope slide as above (Morgan and Hare, 1997).

Specific Hypotheses and Statistical Procedures

We addressed the following questions within each cover treatment: (1) Do naive wasps and wasps experienced with hosts differ in their response to scale covers (H₀ vs H₁)? (2) Does the quality of experience with hosts affect the response of wasps to scale covers ([H₀, H₁] vs H₂, H₃)? and (3) If the quality of experience with hosts does affect the response of wasps to scale covers, is the experience associated with cover characteristics or scale body characteristics (H₂ vs H₃) (Table 1)?

We tested for differences in the mean size class of covers selected for drumming and ovipositor probing from among the five size classes of scale

<table>
<thead>
<tr>
<th>Wasp treatment</th>
<th>Naive</th>
<th>LC-LB</th>
<th>SC-SB</th>
<th>LC-SB</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₀</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Experience does not affect response</td>
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<tr>
<td>H₁</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Any experience enhances response</td>
</tr>
<tr>
<td>H₂</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Experience with large covers enhances response</td>
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<tr>
<td>H₃</td>
<td>-</td>
<td>+</td>
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<td>-</td>
<td>Experience with large bodies enhances response</td>
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</table>

*The responses tested are distribution of drumming and probing events on scale covers differing in size for each treatment and gross frequency of drumming and probing events for each treatment. Symbols indicate similar size selection or frequencies (=), smaller size selections or lower frequencies (−), and larger size selections or higher frequencies (+). Naive, wasps held without host experience; LC-LB, wasps given experience with large scale insect bodies under large covers; SC-SB, wasps given experience with small scale insect bodies under small covers; LC-SB, wasps given experience with small scale insect bodies under large covers.
covers offered. For these analyses, we calculated the mean scale cover size selected for drumming and probing for each replicate group of scale covers within each experience treatment (10 wasps) and tested for significant difference in mean scale cover size selected as a function of experience treatment by analysis of variance [SAS procedure GLM (SAS Institute, 1988)]. Where results were not statistically significant, we also calculated the Type II error rate (β) using the %power macro of SAS (Latour, 1992). We also analyzed the data using a G test to ascertain if scale size selection differed significantly from random selection. Any differences in distributions would indicate a change in cover size preference due to experience with hosts and/or kairomone removal. We also examined the total number of drums and probes by wasps in each treatment irrespective of scale cover size selected. Any differences would indicate a change in the recognition of covers (for drumming events) and/or a change in motivation to accept covers (for probing events) due to experience.

For the frequency analyses, the independent variable (drum or probe frequency) was summed for each bioassay treatment replicate (the total number of drums or probes made by 10 wasps for each set of scale covers). The count data were square root transformed and analyzed by analysis of variance [GLM procedure (SAS Institute, 1988)] as a function of wasp treatment, cover treatment, and their interaction. To ensure that any differences observed in probing were not an artifact of different drumming frequencies, we also analyzed the proportion of drums that resulted in probes among treatments, cover size classes, and their interaction by analysis of variance as above after arcsine square root transformation (Sokal and Rohlf, 1981).

RESULTS

Host Recognition

Wasps that were offered unmanipulated scale covers drummed on scale covers in the 0.66-mm radius size class more than on other classes (Fig. 2). This size preference was not affected by prior experience with hosts (F_{3,16} = 2.39, P > 0.10, β = 0.52). All distributions of drums on unmanipulated scale covers differed significantly from the null hypothesis of random scale cover size selection (G = 9.05, df = 4, P < 0.001). The number of drumming events by each wasp on unmanipulated covers also did not vary with the, type of experience with hosts (F_{3,16} = 0.42, P = 0.740, β = 0.88; Table II).

The distribution of drumming events by naive wasps on kairomone-free scale covers did not differ significantly from the null hypothesis of random scale cover size selection (G = 2.33, df = 4, P = 0.091). The distribution of drumming events by wasps in the three experience treatments (LC-LB, SC-SB, and LC-SB) all differed significantly from random cover size selection (G >
Fig. 2. Drumming frequencies on different sizes of scale covers. White bars indicate unmanipulated covers, black bars indicate kairomone-free covers. Naive, wasps without prior host experience. LC-LB, wasps given experience with large hosts under large covers; SC-SB, wasps given experience with small hosts under small covers; LC-SB, wasps given experience with small hosts under large covers.

7.32, df = 4, P < 0.009; Fig. 2). In particular, SC-SB wasps drummed on smaller kairomone-free scale covers than their counterparts on unmanipulated scale covers ($F_{1,8} = 23.79, P = 0.0012$; Fig. 2). Because this did not occur for LC-SB wasps ($F_{1,8} = 3.23, P = 0.1099, \beta = 0.64$), the change in host size preference caused by the different experience with hosts appears to be determined by the size of the scale cover, not the size of the body beneath.

Naive wasps drummed less on kairomone-free scale covers than on unmanipulated scale covers ($F_{1,8} = 139.49, P < 10^{-4}$). Removing OCT reduces the recognition of scale covers by naive wasps. All wasps given experience with hosts maintained a drumming frequency on kairomone-free covers not significantly different from that on unmanipulated scale covers ($F_{2,25} = 0.22, P = 0.80, \beta = 0.92$; Table II), thus removing OCT did not significantly alter the ability of wasps given experience with hosts to recognize scale covers.

Table II. Frequency of Drumming Events on All Scale Covers by Wasps Given One of Four Treatments (Mean ± SE)*

<table>
<thead>
<tr>
<th>Cover treatment</th>
<th>Wasp treatment</th>
<th>Hypothesis supported</th>
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<tbody>
<tr>
<td></td>
<td>Naive</td>
<td>LC-LB</td>
</tr>
<tr>
<td>Unmanipulated</td>
<td>3.82 ± 0.45*</td>
<td>3.46 ± 0.24*</td>
</tr>
<tr>
<td>Kairomone-free</td>
<td>1.00 ± 0.09*</td>
<td>4.10 ± 0.34*</td>
</tr>
</tbody>
</table>

*Naive, no prior host experience; LC-LB, large hosts under large covers; SC-SB, small hosts under small covers; LC-SB, small hosts under large covers. Means followed by the same letter are not significantly different (ANOVA; $P > 0.05$).
**SCALE COVER RADIUS (mm)**

Fig. 3. Probing frequencies on different sizes of scale covers. White bars indicate unmanipulated covers; black bars indicate kairomone-free covers. Naive, wasps without prior host experience; LC-LB, wasps given experience with large hosts under large covers; SC-SB, wasps given experience with small hosts under small covers; LC-SB, wasps given experience with small hosts under large covers.

**Host Acceptance**

The distribution of probing events on the five size classes of unmanipulated scale covers were similar to the drumming distributions (cf. Figs. 2 and 3). The 0.66-mm radius covers were selected more than the other covers, and experience with hosts did not alter the distribution ($F_{3,16} = 2.53, P = 0.94, \beta = 0.49$). All probing distributions on unmanipulated covers differed.

The frequency of probing events on unmanipulated covers differed significantly between wasp treatments ($F_{3,16} = 17.07, P < 10^{-4}$; Table III). LC-LB wasps probed significantly more covers than wasps in the other three treatments. Wasps probe more after experience with large hosts, and the experience is associated with body characteristics rather than cover characteristics.

<table>
<thead>
<tr>
<th>Table III. Frequency of Probing Events on All Scale Covers by Wasps Given One of Four Treatments (Mean ± SE)*</th>
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<tr>
<td><strong>Cover treatment</strong></td>
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<tr>
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<tr>
<td>Unmanipulated</td>
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<td>Kaironome-free</td>
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</table>

*Naive, no prior host experience; LC-LB, large hosts under large covers; SC-SB, small hosts under small covers; LC-SB, small hosts under large covers. Means followed by the same letter are not significantly different (ANOVA; $P > 0.05$).
After OCT was removed from scale covers, host-naive wasps exhibited no significant size preferences \((G = 0.63, df = 4, P = 0.648; \text{Fig. 3})\). A size preference was maintained with LC-LB wasps \((G = 17.91, df = 4, P < 10^{-4})\), but the preferred size class increased from 0.66 to 0.80 mm with kairomone-free covers \((F_{1,8} = 10.92, P = 0.011)\). SC-SB wasps and LC-SB wasps also showed no significant cover size preference \((G = 1.86, df = 4, P = 0.157, \text{for SC-SB wasps and } G = 1.77, df = 4, P = 0.175, \text{for LC-SB wasps; Fig. 3})\).

Removing OCT from scale covers reduced the number of probes made by naive wasps \((F_{1,8} = 15.42, P = 0.004; \text{Table III})\). SC-SB wasps and LC-SB wasps also had reduced probe frequencies \((F_{1,8} = 34.6, P < 0.001, \text{for SC-SB wasps and } F_{1,8} = 36.9, P < 0.001, \text{for LC-SB wasps})\), but LC-LB wasps maintained a probe frequency not significantly different from that found on unmanipulated covers \((F_{1,8} = 2.34, P = 0.16, \beta = 0.73)\). The resulting pattern between kairomone-free treatments was similar to that found with unmanipulated covers; wasps probe more after experience with large hosts, and the experience is associated with body characteristics.

**Proportion of Drums Resulting in Probes**

To test if the variation in probing frequencies was solely a function of variation in drumming frequencies, we analyzed probing as a proportion of drumming events. Under this null hypothesis, the proportion of drums resulting in probes should be uniform across all treatments and scale size classes. On unmanipulated scale covers, the proportion of drums that resulted in probes differed significantly among cover size classes \((F_{4,80} = 5.71, P < 0.0001)\) and among treatments \((F_{3,80} = 3.941, P = 0.006)\) but not due to their interaction \((F_{12,80} = 0.66, P = 0.62; \text{Fig. 4a})\). Scale covers in the 0.66-mm radius class were most likely to be probed by wasps regardless of treatment, and wasps in the LC-LB treatment were more likely to probe any scale cover after drumming than wasps in the other treatments.

Removing the kairomone from the test covers removed the effect of scale cover size on the proportion of drums that resulted in probes \((F_{4,80} = 0.742, P = 0.57; \text{Fig. 4B})\), but the effect of wasp experience treatment remained \((F_{3,80} = 9.805, P < 0.0001)\). The interaction between scale cover size class and wasp experience treatment also remained nonsignificant overall \((F_{12,80} = 1.143, P = 0.34)\). For individual comparisons within wasp treatments, removing the kairomone from the test covers did not change the percentage of drums resulting in probes for naive wasps \((F_{1,40} = 0.60, P = 0.666)\) or for LC-LB wasps \((F_{1,40} = 0.032, P = 0.99)\), but it did reduce the percentage of drums resulting in probes for LC-SB wasps \((F_{1,40} = 5.89, P < 0.001)\) and for SC-SB wasps \((F_{1,40} = 10.29, P = 0.002)\).
Fig. 4. Mean proportion (± binomial SE) of drumming events resulting in probing on scale covers of different sizes. (A) Unmanipulated scale covers; (B) kairomone-free scale covers. Naive, wasps without prior host experience; LC-LB, wasps given experience with large hosts under large covers; SC-SB, wasps given experience with small hosts under small covers; LC-SB, wasps given experience with small hosts under large covers.

= 8.511, P < 0.0001). In no case were there significant interactions between wasp treatment and scale cover size class (all P's < 0.08 and all but one <0.27). Thus, the differences in the frequency of probes between treatments were not solely a function of the differences in drumming frequencies but also in the proportion of drums that led to probes. Although there were consistent differences in the percentage of drums that resulted in probes due to differences in scale cover size on unmanipulated scale covers, the percentage of drums resulting in probes was additionally enhanced by prior experience with large
hosts. In the absence of OCT, this acceptance rate did not vary with scale cover size and was actually reduced after experience with small bodies (irrespective of cover size) compared to the acceptance rate of naive wasps.

**DISCUSSION**

The importance of experience with hosts varies between host recognition and host acceptance, and also whether or not OCT, the primary chemical cue, is present. Although chemical removal of OCT may seem to create an artificial condition, actually it reflects the conditions that *A. melinus* may face in the field because of the high variation in OCT found in covers of field-reared scales (Hare and Luck, 1994). In addition, some of the scale species on which *A. melinus* can be reared do not produce OCT (e.g., Hare, 1996).

In the presence of OCT, prior experience with hosts and covers does not increase host recognition. Nor does experience with hosts affect the size distribution of scale covers that are recognized (H$_0$). The effect of experience with hosts on host selection depends upon the quality of the experience, however. Only prior experience with high-quality hosts (i.e., those with large bodies) increased the overall level of host acceptance (H$_1$). Prior experience with low-quality hosts did not change host selection levels compared to naive wasps. In neither case did experience with hosts significantly affect the size distribution of hosts selected (H$_0$).

In the absence of OCT, any prior experience with scales and covers increases the rate of host recognition (H$_1$). Moreover, the quality of that experience is also important in that the size of the covers (but not bodies) with which wasps had experience sets a preference for recognition subsequently; wasps preferentially recognized covers whose size was similar to that of the covers with which they had experience regardless of quality (H$_1$). Experience with hosts affected cover acceptance in the absence of OCT only when wasps had experience with high-quality hosts. Then they increased their overall level of cover acceptance and showed a preference for larger covers. The effects of prior experience with hosts were more apparent when OCT was removed, and wasps learned a cue correlated with cover size to recognize subsequent covers. These results reaffirm the importance of OCT in host recognition and acceptance (Morgan and Hare, 1997). They also demonstrate that *A. melinus* can learn a secondary cue for host recognition, although the value of such a cue was apparent only when OCT was absent. Results also reaffirm that *A. melinus* has no innate scale cover size preference (Morgan and Hare, 1997).

The fact that *A. melinus* exhibits a learned response to scale cover size is consistent with the theory that the learned cues should be reliable within the foraging life of the parasitoid but are not consistent from generation to generation (Vet *et al.*, 1995). Previously, Hare and Luck (1994) reported that scale cover
area only qualitatively predicted scale body area. Cohorts of scales were reared monthly on leaves and bark of two cultivars of lemon and two cultivars of orange, and the ratio of cover area to body area varied from 1.90 to 3.44. In general, covers were larger per unit of body area when scales were (1) reared on lemon cultivars, (2) reared on leaves of any cultivar, and (3) reared during the cooler months (Hare and Luck, 1994). Despite the variation in the relationship between cover size and body size among cohorts, cover size, and body size were always highly significantly and linearly related within cohorts (Hare and Luck, 1994). Thus, it is reasonable that the response of *A. melinus* to scale cover size should be learned from the cohort of hosts that the wasps experienced.

Although *A. melinus* responded innately and quantitatively to *OCT* (Hare *et al.*, 1993, Morgan and Hare, 1997), the wide variation in *OCT* levels in field-reared scales of similar physiological age (Hare and Luck, 1994) suggested that *OCT* may serve more importantly as a qualitative cue for host species recognition and identification of a suitable growing stage within a species. California red scale, which is native to Southeast Asia, is unlikely to be the evolutionary host of *A. melinus*, which itself was first collected from northwestern India (Rosen and DeBach, 1979; R. F. Luck, personal communication). However, the presumed evolutionary host is *Aonidiella aurantii* (Newstead), a congener of *A. aurantii*, and *OCT* has been found on its covers (Hare, unpublished). *OCT* appears to be an essential component in the production of scale covers for these two species, and so is a reliable indicator of a growing scale insect. Thus, *OCT* appears to meet the criterion for innately recognized cues of being reliably and consistently associated with host presence over an evolutionary time (Vet *et al.*, 1995).

Our overall conclusion is that *A. melinus* most likely will select medium-to large third-instar California red scale, which are of highest quality for the production of fecund female offspring, as the result of two behavioral mechanisms. First, these are the scales with highest levels of *OCT*, the innate chemical cue. Second, it is for these scales that wasps are most likely to develop a size preference and maintain a high rate of acceptance through prior host experience. This conclusion is relevant toward understanding the variation in the effectiveness of *A. melinus* as a biological control agent for California red scale.

California red scale reared on bark of citrus trees suffer less parasitism from *A. melinus* than scale reared on leaves (Reeve and Murdoch 1986; Murdoch *et al.*, 1989) even though *A. melinus* searches the bark for scale (Murdoch *et al.*, 1989). Although bark-reared scale are smaller than leaf-reared scale (Walde *et al.*, 1989; Hare *et al.*, 1990), the reduced host size accounts for only one-tenth of the reduction in parasitization rates of scale on bark compared to scale on leaves (Walde *et al.*, 1989). The suggestion was that bark-reared scale may be less attractive or recognizable to *A. melinus* than scales reared on leaves or fruit (Walde *et al.*, 1989). Previously, Hare and Luck (1994) found support for that suggestion from the fact that bark-reared scale had far lower (2- to over 10-fold
less, depending upon season and cultivar) OCT levels than scales reared on leaves and fruit. Thus, we would expect bark-reared scales indeed to be innately less recognizable than leaf- or fruit-reared scales. In addition, the results from the present study strongly suggest that any prior host experience is most likely to reinforce the behavior of wasps to reject these scales in favor of larger scales under larger covers.

One model for foraging behavior of parasitoids postulates that different stimuli are ranked by their importance, or strength, of their response potential but that experience can increase or decrease the response potential of a particular stimulus (Vet et al., 1990). For naive A. melinus, OCT has a high response potential, while scale cover size has a lower potential. With experience, OCT maintains its importance, but the response potential of scale cover size increases. The degree of increase depends upon the quality of the experience. Nevertheless, for this narrowly oligophagous parasitoid, the response potential of scale cover size always remains below that of OCT.

A. melinus will recognize and successfully develop on some scale species that do not produce OCT, such as oleander scale. This is the preferred host for rearing A. melinus in commercial insectaries because of its large size and parthenogenetic reproduction (DeBach and White, 1960). Nevertheless, even when reared on oleander scale, A. melinus will reject oleander scale covers in favor of California red scale covers 75% of the time (Hare, 1996; Hare et al., 1997). Thus, we would predict that, in an environment where potential hosts with and without OCT are present, wasps will concentrate on patches of scale insects with OCT; any prior experience with other hosts may have little impact on host selection. Perhaps the only times that the learning abilities of A. melinus for scale cover size come into play is when wasps are confronted with host whose covers do not contain OCT. Such hosts may be either species that do not produce OCT or species such as field-reared California red scale, in which whatever OCT that is produced has been rapidly incorporated into the scale's cover, thus minimizing the host's innate chemical conspicuousness.

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