Cuticular Hydrocarbons and Wax Esters of the Ectoparasitoid *Habrobracon hebetor*: Ontogenetic, Reproductive, and Nutritional Effects

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Hydrocarbon and wax ester components of cuticular lipids of the braconid parasitoid *Habrobracon hebetor* Say reared at 25°C on larvae of a pyralid moth have been identified by GC-MS and analyzed with respect to adult age, mating status, and diet. The hydrocarbons range in carbon number from C21 to C45 and consist of a homologous series of \( n \)-alkanes, 11-, 13-, and 15-methyl alkanes, 13,17-dimethyl alkanes, and Z-5, Z-7, and Z-9-alkenes. The wax esters found in the cuticular lipid fraction are a series of homologous compounds with the acid portion being short chain, unbranched, even carbon number acids from C8 to C20 (predominately C8 to C16). The alcohol portions of the esters are secondary alcohols with carbon number from C22 to C25 (predominately C23 and C25) with the hydroxyl function located at C6, C7, C8, and C9. Gender, age, and nutritional states were significant factors for variation in several of the individual esters, but mating status did not affect wax ester composition. Ontogenetic examinations indicated that prepupal, and early pupal cuticular lipids contain only hydrocarbons. Low levels of wax esters are detectable in late stage pupae, and somewhat greater quantities of wax esters are present on newly eclosed adults. When pharate adults emerge from the cocoon, however, their cuticular lipids consist of approximately equal amounts of hydrocarbons and wax esters, and 6d post emergence from the cocoon, wax esters are the predominant lipid component.


Keywords: chemical ecology; cuticular hydrocarbons; wax esters; mass spectrometry; gender recognition

*Habrobracon [=Bracon] hebetor* (Hymenoptera: Braconidae) is a gregarious ectoparasitoid that is an important natural control agent of many lepidopterous pests. This wasp occurs in the stored grain ecosystem (Keever et al., 1985; Antolin and Strand, 1992) where it attacks several pyralid moths, including the Indianmeal moth, *Plodia interpunctella* (Hübner), a destructive pest of stored products. It has also been extensively studied as a model organism for testing many of the theories of evolutionary biology (Waage and Greathead, 1986; Jervis and Kidd, 1996; Quicke, 1997). Little, however, is known about its chemical ecology. As part of a program on the chemical ecology of biological control agents found in the stored grain ecosystem, we have begun an examination of the surface lipids of *H. hebetor* and their possible physiological and semiochemical roles.

Cuticular hydrocarbons are the best known of insect surface lipids and are known to function as species, gender, and colony recognition cues, and as pheromones, allomones, and kairomones in a diversity of ecological situations (Howard and Blomquist, 1982; Blomquist et al., 1993; Howard, 1993). Furthermore, precedence exists in parasitic wasps for cuticular hydrocarbons serving as close
range species-, gender-, and host-recognition cues at the individual level (Howard, 1998; Howard et al., 1998; Howard and Perez-Lachaud, 2002). Although less well known, complex mixtures of wax esters are also frequently found as cuticular lipids. They are usually, however, considered to serve solely for the purpose of abrasion resistance and maintenance of insect water balances (Buckner, 1993; Nelson and Blomquist, 1995) although at least two instances are known where wax esters are postulated to serve as sexual recognition cues (Grula et al., 1980; Warthen and Uebel, 1980). In this report, we characterize the surface lipids of *H. hebetor*, establish their reliance on several environmental factors, establish the ontogenetic relationships among the hydrocarbons and wax esters, and discuss their possible physiological and semiochemical roles.

**MATERIALS AND METHODS**

**Insects**

A laboratory strain of *P. interpunctella* used for this study was reared on a diet of cracked wheat, wheat shorts, wheat germ, brewer’s yeast, honey, glycerin, and water. A strain (Miller) of *H. hebetor*, collected from wheat infested with *P. interpunctella* in Dickinson Co., Kansas, in October 1998 was cultured at 25°C and 50–55% RH on wandering larvae of *P. interpunctella*. To obtain developing *H. hebetor*, wasps were cultured on *P. interpunctella* larvae held in 15 × 100 mm plastic Petri dishes. After wasp larvae have completed feeding, they leave the parasitized hosts and spin a cocoon. By inverting the Petri dishes, development within the cocoon can be easily observed and individual stages from prepupae to newly eclosed adults can be isolated for analysis.

**Biomass Determinations**

Fresh weights, moisture contents, and dry weights of newly emerged ♀’s and ♂’s were obtained by weighing individual adults on a Mettler UMT-2 microbalance followed by heating for 5 h in a forced air oven at 60°C and re-weighing.

**Gravimetric Analyses**

Total hexane extractable lipids in groups of 5 adult wasps were determined gravimetrically. Adults were briefly anesthesized with CO₂ and placed in a glass GC vial with a teflon-lined crimp top. The septum was pierced, and then 150 μl of hexane was injected and allowed to soak the insect for 1 min, after which the hexane was removed with a syringe and placed in a tared foil planchet. Two additional extractions were conducted and the combined extracts air dried for 15 min and then oven dried at 50°C for 1 h. Planchets were re-weighed on the UMT-2 microbalance. Four replicates of newly emerged and 5-d-old female and male wasps were prepared.

**Chemical Analyses**

For identification and analyses of cuticular hydrocarbons and wax esters, groups of 5 adult wasps were extracted by immersing the insects in 3 successive 250-μl volumes of hexane for 1 min each. Three hexane extracts from each sample were combined and concentrated under a stream of N₂, and the crude extract was examined by gas chromatography-mass spectrometry (Fig. 1A). Lipids were isolated by chromatography on a 3-cm “mini-column” of BioSil A (Bio-Rad Laboratories, Richmond, CA) as described earlier (Howard et al., 1978) except that after hydrocarbons were eluted with hexane, wax esters were eluted with hexane:ether (90:10).

Electron impact mass spectral analyses were conducted by using a Hewlett-Packard 5790A gas chromatograph (GC) (Hewlett-Packard, Inc., San Fernando, CA) with a DB-5 bonded phase capillary column (20 m long, 0.25 mm inside diameter) (J and W Scientific, Folsom, CA) connected to a Hewlett-Packard 5970 mass selective detector (MSD) and a Hewlett-Packard 9133 data system. Ultrapure helium was the carrier gas, with a column head pressure of 0.75 kg/cm². Mass spectra were obtained at 70 eV. Analyses were done using temperature programming, with an initial temperature of 100°C, a final temperature of 320°C, a program rate of 5°C/min, and a 20-min final hold...
period. The splitless injector was set at 275°C and the GC/MSD interface was at 280°C. Retention times of each hydrocarbon component and equivalent chain length values (ECL) were obtained by comparison with known \( n \)-alkane standards (Howard et al., 1978). Individual components detected in the total ion scanning mode were identified from their characteristic EI-MS fragmentation patterns (Jackson and Blomquist, 1976; Nelson, 1978).

Double-bond locations in alkenes were obtained by preparing dithiomethyl ethers and examining their electron impact mass spectra (EI-MS) (Francis and Veland, 1981). Stereochemistry of the parent alkene was established from Fourier trans-
form infrared analyses. Fourier transform infrared vapor-phase spectra on the underivatized alkenes were obtained on a Hewlett-Packard 5890 GC with a 5965B FT-IR detector and a 7958A data system. A DB-5 bonded phase capillary column (20 m long, 0.25 mm inside diameter) using chromatographic conditions identical to those described above was used.

Wax esters were also identified from their characteristic EI-MS fragmentation patterns (Nelson and Blomquist, 1995; Finidori-Logli et al., 1996) and by EI-MS analysis of the secondary alcohols produced by alcoholic KOH hydrolysis and their subsequent oxidation to ketones (Blomquist et al., 1972). The presence or absence of unsaturation in either the acid or alcohol moiety was determined by conducting a microhydrogenation of the wax esters (Schwartz et al., 1972) and examining the products by mass spectrometry.

**Hydrocarbon and Wax Ester Ontogeny**

Developmental stages of female and male *H. hebetor* were isolated from cultures of *P. interpunctella* larvae parasitized by *H. hebetor* and held in plastic Petri dishes at 25°C. Individual prepupae, 1-d-old pupae, 4–6-d-old pupae, and newly eclosed adults of each sex still in the cocoon were removed from cocoons, placed in 50-μl inserts in GC-crimp-top vials with teflon liners and immediately placed into a –80°C freezer. Newly emerged adults <1 d old (i.e., eclosed adults that had emerged from their cocoons) were obtained by isolating pupae in 13 × 100 mm glass tubes and observing daily for adult emergence. Newly emerged adults were transferred to 50-μl inserts as above and stored at –80°C. Groups of adults newly emerged from the cocoons (segregated by gender) were held at 25°C for 6 d with access to honey, briefly knocked down with CO₂, placed in the GC inserts, and frozen as above. Three replicate individuals of each sex and stage were prepared. Wasps were removed from the freeze, returned to room temperature, and suspended for 1 min in 4 μl of iso-octane containing 57 ng C₂₀/μl. An aliquot of 2 μl (0.5 insect equivalent) was then removed and analyzed by gas chromatograph-mass spectrometry using the same parameters as described above.

**Analysis of Mating Status, Age, Nutritional Effects**

In these tests, we examined the effect of mating status (mated or virgin), adult age (0–1-day-old, 5-day-old, and 10-day-old), and diet (honey, honey + host larvae, or starvation) on subsequent hydrocarbon and wax ester profiles. Wasps were held in 3.2 × 8 cm plastic vials with snap-cap lids containing 40-mesh screens. Honey-fed wasps had a drop of honey placed on the screen in the vial lid. Host-fed wasps had access to 5 wandering 5th instar *Plodia* larvae in each vial in addition to a drop of honey on the lid. Newly emerged adults were also starved for 5 days. Longer starvation periods resulted in high mortality. All tests were started by adding 5 newly emerged (0–1-day-old) wasps to appropriate vials. Mated ♀’s were obtained by adding 2 virgin ♂’s to the groups of 5 virgin ♀’s. Three replicate groups of 5 adults were prepared for each treatment combination. After appropriate time intervals at 25°C and 50–55% RH, the vials were briefly cooled, wasps removed with forceps, placed in GC vials, and held at –80°C.

**Statistical Analyses**

Compositional analyses of hydrocarbons were conducted using the EI-MS system as described above, but analyses were run on hexane extracts that had not been separated on BioSil chromatography, as preliminary analysis indicated that no hydrocarbons were buried under the GC peaks representing the wax esters. Areas were summed and reported as ng per insect (for the hydrocarbons) or they were converted into percentage values (for both hydrocarbons and wax esters). Each run had an internal standard of 57 ng/μl of docosane for the hydrocarbons. No secondary alcohol wax ester was available for use as an internal standard, so percent values were calculated and used for statistical analysis. For multifactorial analyses of variance, arcsine square root transformations of proportional values were used for the percent-
Results

Biomass Determinations and Gravimetric Analyses

As is true for most Hymenoptera, male *H. hebetor* are smaller than female *H. hebetor*. The mean (± SE) dry weight of newly emerged males was 0.377 ± 0.007 mg. Newly emerged females weighed 0.413 ± 0.008 mg, and were significantly heavier than males (F_{1,78} = 10.93, P = 0.0014). Newly emerged virgin unfed female wasps had 2.6 ± 0.3 µg lipid per insect and newly emerged virgin unfed males had 2.5 ± 0.3 µg lipid per insect. The corresponding values for hydrocarbons for these insects were 0.4 ± 0.1 µg for females and 0.4 ± 0.1 µg for males, and therefore both sexes had approximately 2.1 µg of wax ester. At six days of age, honey-fed virgin females had 4.6 ± 0.3 µg of total lipid and honey-fed virgin males had 5.4 ± 0.3 µg of total lipid. The corresponding values for hydrocarbons were 0.7 ± 0.1 µg for females and 0.8 ± 0.1 µg for males, giving an estimate for the wax esters of 3.9 µg for females and 4.5 µg for the males.

Chemical Analyses

The cuticular hydrocarbon composition of *H. hebetor* is relatively simple, consisting of approximately 55 hydrocarbons comprising a homologous series of n-alkanes (C_{21} to C_{32}), internally branched monomethyl alkanes (X-MeC_{23} to X-MeC_{41}), 13,17-dimethyl alkanes (13,17-DiMeC_{33} to 13,17-DiMeC_{43}) and isomeric monoenes (Z-5; Z-7; Z-9-C_{23:1} to Z-5; Z-7; Z-9-C_{29:1}) (Table 1). The position of the

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*ECL, equivalent chain length; CN, carbon number.
Ions in brackets are for dimethyl disulfide derivatives of alkenes.
methyl branch in the monomethyl alkanes moved toward the center of the molecule as the chain length increased (11-Me for C_{23} to C_{30}, 13-Me for C_{31} to C_{39} and 15-Me for C_{41}). No gender specific hydrocarbons were found on either the males or females.

The wax ester fraction of the cuticular lipids of *H. hebetor* consists of 29 partially resolved chromatographic peaks that contain a homologous series of compounds consisting of short-to-medium chain length fatty acids (C_{8} to C_{20}) esterified to long-chain secondary alcohols (C_{22} to C_{25}) with the hydroxyl located at C_{6}, C_{7}, C_{8}, and C_{9} (Fig. 1B, numbered peaks). Neither acids or alcohols contained further unsaturation as evidenced by the recovery of only starting material from the micro-hydrogenation reaction. The mass spectra of these esters did not correspond with the spectra obtained from esters composed of medium chain fatty acids and primary alcohols (Attygalle et al., 1987): notably they lacked a molecular ion. They did agree, however, with the mass spectra presented by Finidori-Logli et al. (1996) for secondary alcohol wax esters from the eulophid wasp *Diglyphus isaea*.

A typical mass spectrum for these wax esters is shown in Figure 2A (taken from EI-MS scans across peak 4, Figure 1B). Here the acid is eight carbons in length and the secondary alcohol is 7-tricosanol. The ion at m/z 145 arises from a transfer of two hydrogens to the acid moiety and the base peak at m/z 127 arises from a loss of water from the m/z 145 fragment ion. The ion at m/z 322 arises from elimination of two hydrogens from the alcohol portion of the molecule. Note that no molecular ion is present. Figure 2B shows the structures of the four isomeric wax esters present in peaks 3 to 5. Structures 2 and 3 of Figure 2B (7-OH and 8-OH) are the major isomers. Several of the other GC peaks shown in Figure 1B contain mixtures with different chain length fatty acids esterified to different positional isomers of an alcohol of constant chain length.

The chain lengths and structures of these various fatty acids and secondary alcohols were determined by base hydrolysis of the total wax ester mixture and separate analysis of the acids and alcohols. The individual alcohols gave readily interpretable mass spectra with diagnostic alpha-cleavage ions (Fig. 3). Confirmation of these structures was obtained by oxidizing the free alcohols to ketones and examining their mass spectra. Again, confirmatory diagnostic alpha-cleavage ions and the m/z 58 McLafferty re-arrangement ion were found in every case (Fig. 4). Confirmation of the nature of the fatty acids was obtained by preparation of methyl esters and comparison of their mass spectra to known standards. In every case the fatty acids were unbranched, devoid of any other functionality, and co-eluted with known standards.

**Hydrocarbon and Wax Ester Ontogeny**

Cuticular lipids were examined as a function of ontogeny. Life stages examined included prepupa (of unknown gender), early and late stage female and male pupae, newly eclosed female and male pharate adults still in the cocoon, female and male adults newly emerged from the cocoon, and female and male adults 6 days post cocoon emergence. Common ontogenetic trends were obtained for both females and males. Prepupal, and early pupal cuticular lipids contain only hydrocarbons (Figs. 5A,B, 6A,B). Low levels of wax esters are detectable in late stage pupae (Figs. 5C, 6C), and somewhat greater quantities of wax esters are present on newly eclosed adults (Figs. 5D, 6D). By the time that the pharate adults emerge from the cocoon, however, their cuticular lipids consist of approximately equal abundances of hydrocarbons and wax esters (Figs. 5E, 6E), and 6-d post-emergence from the cocoon adults have the wax esters as their predominant cuticular lipid (Figs. 5F, 6F). Relative abundances of all components can be roughly estimated by comparison to the internal standard peak in each TIC trace representing 114 ng of C_{20}. Note, however, that because the mass selective detector response is not linear with mass, relative peak heights of hydrocarbons and wax esters are not directly comparable.

**Analysis of Mating Status, Age, Nutritional Effects**

A factorial analysis of variance indicated that the absolute amount of cuticular hydrocarbon on
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Fig. 2. A: EI-MS of 7-tricosanoyloctanoate from peak 4 of Figure 1B. B: Isomeric wax esters obtained from peaks 3–5 of Figure 1B. Structure 1 has the hydroxyl at C6 of a C23 alcohol, structure 2 at C7, structure 3 at C8, and structure 4 at C9.

*H. hebetor* varied as a function of gender, age, mating status, and nutritional status (Table 2). Because of biological constraints (such as males do not host feed, starved individuals do not live as long as fed individuals, etc.), the design could not be balanced, hence no interaction terms were measurable and some of the factors are possibly confounded. To partly overcome this, two-way ANOVA's were conducted on subsets of the data. No significant differences as a function of gender were found for total hydrocarbon on newly emerged vs. 5-day-old virgin, non-fed males and females (*F*1,7 = 4.73, *P* = 0.0662). However, age was a significant factor (*F*1,7 = 51.65, *P* = 0.0002) with males increasing their total hydrocarbon almost threefold from day 0 to day 5 and females increasing their total hydrocarbon approximately twofold during the same interval. Analysis of the effects of gender and age on
Fig. 3. EI-MS of a mixture of 7-pentacosanol and 8-pentacosanol obtained by base hydrolysis of wax esters from *H. hebetor*.

Fig. 4. EI-MS of a mixture of 7-pentacosanone and 8-pentacosanone obtained by CrO₃ oxidation of the alcohols obtained from base hydrolysis of the wax esters of *H. hebetor*. 

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Fig. 5. Total ion traces of lipid extracts from prepupal, pupal and adult stages of female *H. hebetor*. A: Prepupae; B: Early pupae; C: Late pupae; D: Newly eclosed adult still in cocoon; E: Adult newly emerged from cocoon; F: Six-day-old emerged adult. Peak labeled IS represents 114 ng of C20, an internal standard. The peaks marked with * are from the Dufour gland of the adult female. Peaks marked WE are the dominant wax esters in a given TIC. Changes in total amounts of hydrocarbons and wax esters can be compared to the IS peak in each of the six traces.
Fig. 6. Total ion traces of total lipid extracts from prepupal, pupal and adult stages of male *H. hebetor*. A: Prepupae; B: Early pupae; C: Late pupae; D: Newly eclosed adult still in cocoon; E: Adult newly emerged from cocoon; F: Six-day-old emerged adult. Peak labeled IS represents 114 ng of C20, an internal standard. Peaks marked WE are the dominant wax esters in a given TIC. Changes in total amounts of hydrocarbons and wax esters can be compared to the IS peak in each of the six traces.
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A factorial analysis of variance on the proportional composition of the wax esters suggested that the mixtures varied as a function of gender, age, and nutritional status (Table 4). Two-way ANOVA’s were carried out on the same sample pairings as for the hydrocarbon analyses. Unfed virgin males and females, either newly emerged or 5-day-old, showed 7 out of 29 wax ester peaks that varied significantly with gender (*P* ≤ 0.05) (peaks 5, 6, 7, 13, 14, 21, and 22), whereas only two out of 29 peaks varied with age (peaks 1 and 13). A comparison of the proportions of wax esters on virgin, honey-fed males and females of 5 or 10 days of age indicated that 7 out of 29 wax ester peaks varied significantly with gender (peaks 4, 5, 7, 14, 21, 22, and 26), whereas none of the wax ester peaks varied significantly as a function of age. Nine out of 29 of the wax esters of 5-day-old virgin females that were either starved, honey-fed, or fed honey and host hemolymph showed significant differences as a function of nutritional status (peaks 9, 16, 17, 18, 19, 21, 22, 25, and 26). As with the hydrocarbons analysis above, none of the wax esters showed any relationship to mating status.

**DISCUSSION**

Studies on the cuticular lipids of braconids are sparse. Espelie and Brown (1990) examined lipids of newly emerged *Ascogaster quadridentata* Wesmael, a parasitoid of the codling moth, *Cydia pomonella* L. Surface lipids of this species contained only *n*-alkanes (C<sub>20</sub>–C<sub>31</sub>) and *n*-alkenes (C<sub>23:1</sub>–C<sub>31:1</sub>) (of unknown double bond location or stereochemistry). They also reported that males and females appeared to differ in relative abundance for some components. Swedenborg and Jones (1992) examined surface lipids of female *Macrocentrus grandii* Goidanich but only identified some of the components (a series of Z,Z-9,13-dienes), which function in the sex pheromone complex of this species. The nature of other cuticular lipids in this species remains unknown. The only other study of braconid cuticular lipids is that by Syvertsen et al. (1995) of *Cardiochiles nigriceps* (Viereck). This species contains *n*-alkanes (C<sub>23</sub>–C<sub>31</sub>), *n*-alkenes with internally-placed double bonds of Z-stereochemistry (C<sub>23:1</sub>–C<sub>35:1</sub>), and *n*-alkadienes (C<sub>25:2</sub>–C<sub>35:2</sub>) with Z,Z-stereochemistry and with one of the double bonds in the 5-, 6-, or 7-position and the other near the center of the hydrocarbon chain (po-
The alkadienes were only isolated from females and were shown to be involved in courtship behavior. In addition, at least two monoenes (Z-6- and Z-7-C_{27:1}) were present in a greater than 10-fold amount in males than in females. In none of these three braconid species were any other cuticular lipids reported, nor was any indication given that their cuticular hydrocarbons might differ with various environmental or ontogenetic factors.

In addition to these three braconids, cuticular lipids have been examined in a number of other hymenopteran parasitoids. These include five species in the family Bethylidae (Howard 1992, 1998; Howard and Baker 1994).

### TABLE 3. Mean Percentage Composition of H. hebetor Hydrocarbons as a Function of Gender, Age, Mating, and Feeding Status*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gender</th>
<th>Age, days</th>
<th>Mating status</th>
<th>Feeding status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td>0–1</td>
<td>5</td>
</tr>
<tr>
<td>C_{21}</td>
<td>0.47a</td>
<td>0.23b</td>
<td>0.26b</td>
<td>0.28a</td>
</tr>
<tr>
<td>C_{22}</td>
<td>0.14a</td>
<td>0.09b</td>
<td>0.09a</td>
<td>0.10a</td>
</tr>
<tr>
<td>Z-9-C_{23:1}</td>
<td>0.33a</td>
<td>0.88b</td>
<td>0.61a</td>
<td>0.67a</td>
</tr>
<tr>
<td>Z-7-C_{23:1}</td>
<td>1.10a</td>
<td>1.52a</td>
<td>1.35a</td>
<td>1.32a</td>
</tr>
<tr>
<td>Z-5-C_{23:1}</td>
<td>2.06a</td>
<td>1.08b</td>
<td>1.70a</td>
<td>1.44a</td>
</tr>
<tr>
<td>C_{24}</td>
<td>11.05a</td>
<td>10.60a</td>
<td>10.06a</td>
<td>8.83a</td>
</tr>
<tr>
<td>Z-9-C_{25:1}</td>
<td>0.15a</td>
<td>0.12a</td>
<td>0.12a</td>
<td>0.12a</td>
</tr>
<tr>
<td>Z-7-C_{25:1}</td>
<td>0.93a</td>
<td>0.92a</td>
<td>0.75a</td>
<td>0.88a</td>
</tr>
<tr>
<td>Z-5-C_{25:1}</td>
<td>1.34a</td>
<td>1.28a</td>
<td>1.26a</td>
<td>1.09a</td>
</tr>
</tbody>
</table>

*Means with different letters for a given compound and factor are significantly different at $P \leq 0.05$. All comparisons were made using multifactorial ANOVA on arcsine square root transformations of proportional data. Separations of means on the transformed data was conducted using the sequential Bonferroni test (Rice, 1989). Each factor mean represents pooled samples from each level of the other 3 factors since not all treatment combinations were biologically possible.
Hydrocarbon and Wax Ester Components of *H. hebetor*  

Howard and Infante, 1996; Howard and Perez-Lachaud, 2002), three species in the family Pteromalidae (Espelie et al., 1990; Howard and Liang, 1993; Howard, 2001), two species in the family Eucharitidae (Vander Meer et al., 1989; Howard et al., 2001), one species in the family Perilampidae (Espelie and Brown, 1990), and one in the family Eulophidae (Finidori-Logli et al., 1996). In every case, except for the eulophid, hydrocarbons were the only cuticular lipids reported. Considerable differences in the chemistry of the hydrocarbons and their relative abundances were demonstrated in this diverse group of families.

Wax esters as cuticular lipids are not commonly found in Hymenoptera. However, the cuticular lipids of females of the eulophid *Diglyphus isaea* Walker are dominated by secondary alcohol wax esters of medium chain fatty acids (C₉ and C₁₀) and long-chain alcohols (C₂₁–C₂₅) with the hydroxyl group at C-11, with only a few hydrocarbons (mainly n-alkanes) being present (Finidori-Logli et al., 1996). In contrast, male cuticular lipids of *D. isaea* are dominated by alkanes (n-alkanes [16%], monomethyl alkanes [19%], dimethyl alkanes [46%, and trimethyl alkanes [4%]) with only a very low amount of the wax esters. In *H. hebetor*, a sexual dimorphism of cuticular lipids also exists, but it is much more subtle since both sexes contain the same qualitative mixtures of hydrocarbons and secondary wax esters. The wax esters of *H. hebetor*, while clearly related to those of *D. isaea*, differ in two ways: the acids range over a greater carbon number (C₈ to C₂₀), and the secondary alcohols exist as positional isomers for each car-
bon number (with hydroxyl groups at C-6, C-7, C-8, and C-9) and they have chain lengths of C_{22} to C_{25}. The only other example of cuticular lipids containing wax esters with secondary alcohols is the work of Blomquist et al. (1972) on grasshoppers in the genus *Melanoplus*. The wax esters from these insects are more similar to the *H. hebetor* esters than to the *D. isaea* esters, with acid moieties ranging from C_{12} to C_{20} and secondary alcohols with carbon numbers of C_{21} to C_{27}, each alcohol consisting of two to four isomers with the hydroxyl group being located toward the center of the molecule.

Life stages of *H. hebetor*, like those of many insect species, experience different microhabitats. Adaptation to the environmental stresses, primarily desiccation, within these habitats may be reflected in qualitative and quantitative changes in surface lipid composition during development. During feeding, the ectoparasitic wasp larvae are, in essence, connected to a continuous source of water in host hemolymph and would be expected, therefore, to have a limited need of cuticular lipids for protection from water loss. Preliminary experiments indicated that this is apparently the case, with very low levels of larval cuticular hydrocarbons (mainly n-alkanes), and no detectable wax esters (data not shown). After cessation of feeding, the larvae drop off the remains of their host to pupate, spinning a silk cocoon for protection during metamorphosis. Within the microhabitat of the cocoon, the cuticular lipids undergo a dramatic shift during prepupal, pupal, and pharate adult development. Surface lipids in prepupae are similar to those of larvae, and while the amount of hydrocarbon increases in young pupae, wax esters are not detected until pupae are in their final developmental stages. Young adults remain in the cocoon for approximately 24 h after eclosion, and during this time they continue to lay down surface lipid and to increase the amount of wax ester relative to hydrocarbon. Several days after emergence from the cocoons, both males and females have established the adult specific cuticular lipid compositions of about 15% hydrocarbon and 85% wax ester.

Conservation of water is the most important non-semiochemical function for cuticular lipids in insects. Hydrocarbons, wax esters, and fatty acids are the dominant chemical classes that make up the total lipid fraction (Lockey 1985, 1988; de Renobales et al., 1991; Nelson and Blomquist 1995) and consistent correlations between lipid composition and abundance and cuticular permeability to water loss have been noted (Hadley, 1978; Toolson and Hadley, 1979; Toolson, 1982, 1984; Rourke and Gibbs, 1999). Cuticular permeability to water remains essentially constant for most insects as temperature is raised, but sharply rises as a critical or transition temperature is reached, with the given temperature being characteristic for each insect (Chapman, 1998). This transition is thought to occur because the previously “solid” lipid layer begins to “melt” thus allowing water to pass more freely across the cuticle (Rourke and Gibbs, 1999). Gibbs and Pomonis (1995) used model systems to examine the effects of chain length, methyl branching, and unsaturation on the phase behavior of typical insect cuticular hydrocarbons and Patel et al. (2001) conducted similar studies with wax esters. For hydrocarbons, melting temperatures for n-alkanes of 21 to 40 carbons increased by 1 to 3°C for each additional carbon. The effect of methyl branches depended on where the methyl branch was located, but in general as the methyl branch moved more internally, the melting point temperature decreased by up to 30°C. Addition of a second methyl branch resulted in increases in the melting point. Insertion of a double bond into the alkane chain resulted in decreases in the melting point by 50°C or more (Gibbs and Pomonis, 1995). Patel et al. (2001) found that wax esters of primary alcohols, which are the typical wax esters of terrestrial arthropods, occur in a solid state under physiological conditions. Wax esters of secondary alcohols obtained from melanopline grasshoppers melted more than 60°C below primary esters of the same molecular weight and lowered the melting point of the grasshopper total surface lipids to environmental values.

The total cuticular lipids of *H. hebetor* are a complex mixture of hydrocarbons and secondary wax
esters, with some of the components tending to be higher melting and potentially solid at physiological temperatures (the long chain n-alkanes and mono- and dimethyl alkanes), whereas other cuticular components (the alkenes and sec-wax esters) are likely to be liquid at physiological temperatures. We have not determined the melting properties of the *H. hebetor* lipids but strongly suspect that since the wax esters are such a major component of the total mixture that the lipids would be rather fluid under normal ecological conditions. These parasitoids are commonly associated with pyralid moths in the stored grain ecosystem, an environment that can be both rather hot, dry, and dusty. The cuticular lipids from adults newly emerged from their cocoons represent about 0.65% of their dry weight biomass (0.63% for ♀s, 0.66% for ♂s) and at 6 d post emergence from the cocoons, the female cuticular lipids represented 1.11% of dry biomass and the males 1.43%. These values suggest that the wasps have evolved with greater quantities of lower melting lipids to achieve water conservation. In addition, these wasps, like many insects, spend a great deal of time grooming themselves. Thus, an additional possible function of this particular mixture of lipids might be to facilitate the removal of dust particles and potential pathogenic organisms, such as fungal spores during the grooming process. Experiments to evaluate these hypotheses remain to be conducted.

Few studies have examined the effects of environmental and nutritional factors on cuticular lipids of parasitoids, although other groups of insects have had limited studies done in this area (Lockey, 1988; Howard, 1993; Schal et al., 1994; Howard and Akre, 1995; Howard et al., 1995). Howard (1998) examined the ontogenetic, reproductive, and nutritional effects on the cuticular hydrocarbons of the stored product bethylid *Cephalonomia tarsalis*. Males showed little change in their hydrocarbon profiles with any of these factors, but females showed striking changes in both absolute abundance and composition of their cuticular hydrocarbons between emergence from the cocoon and 3 weeks of age, the greatest change occurring at 1 week. The major factor effecting this dramatic increase in total hydrocarbon at day 7 (approximately 200 vs. 800 ng) is the host feeding status of the female. Those females who were allowed to host feed did not show an increase in hydrocarbons, whereas non-host fed females did so.

Howard (1998) hypothesized that the observed large increase in total hydrocarbon of female *C. tarsalis* not allowed to host feed might have occurred because the female reabsorbed the developing eggs, thus causing the internal hydrocarbons that would normally be deposited in the ovaries (Schal et al., 1994) to be shunted to the cuticle of the females. Does *H. hebetor* show this physiological response? As Table 2 indicates, virgin females appear to have significantly more hydrocarbon than do mated females, and honey-fed and host-fed females have significantly less hydrocarbon than do starved females. Nevertheless, it can not be unequivocally said that the effect of mating status and feeding status is not confounded by age effects. However, using a subset of the total data, a comparison of 5-day-old virgin females that had either been starved, honey-fed, or fed both honey and host hemolymph indicated no effect of nutritional status on total hydrocarbon. Similarly, a comparison of 5-day-old mated or virgin females subjected to the same dietary regimes indicated that neither mating status or nutritional status affected their total hydrocarbons. This strongly suggests that *H. hebetor* may not respond to deprivation of host-feeding in the same manner as does *C. tarsalis*.

Our studies have shown that wax esters are the predominant lipid components in both male and female adults of *H. hebetor*. Although we compared the relative amounts of individual peaks as a function of gender, age, mating status, and nutritional status, the multicomponent nature of the peaks makes it difficult to draw conclusions about peak area changes resulting from these biotic factors. Certainly the wax esters on females of the eulophid *D. isaea* are critically involved in gender recognition and courtship behavior (Finidori-Logli et al., 1996), and it is possible that they serve a similar function in *H. hebetor*. We do know that wax esters become an increasing proportion of the total surface lipids during the adult aging process.
Whether this reflects semiochemical functions or physiological function/adaptations is unknown.

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