

Ontogenetic, Reproductive, and Nutritional Effects on the Cuticular Hydrocarbons of the Host-Specific Ectoparasitoid *Cephalonomia tarsalis* (Hymenoptera: Bethyridae)

RALPH W. HOWARD

U.S. Grain Marketing Research Laboratory, USDA-ARS, 1515 College Avenue, Manhattan, KS 66502

Ann. Entomol. Soc. Am. 91(1): 101-112 (1998)

ABSTRACT Cuticular hydrocarbons have been identified from adult males and females of the bethylid wasp *Cephalonomia tarsalis* (Ashmead). The 2 sexes possess the same cuticular hydrocarbons, but in different amounts and proportions. Hydrocarbon components identified include n-alkanes (C_{23} - C_{37}), 5-methyl alkanes (5-Me C_{25} -5Me C_{29}), 5,X-dimethyl alkanes (5,17- and 5,19-diMe C_{29} and 5,17- and 5,19-diMe C_{33}), and a homologous series of Z-monones with double bonds at Δ^{11} , Δ^9 , and Δ^7 ($C_{25,1}$ - $C_{37,1}$). The n-alkanes and alkenes are the predominant components in all cases, the 5-methyl alkanes being only minor components. The dimethyl components are present in only trace amounts. Age, mating status, and, for females, host-feeding status, were examined for their effect on hydrocarbon quantities and proportions. Males showed little change in their hydrocarbon profiles, but females showed strong age, host feeding, and age \times host feeding effects on total hydrocarbon quantities and on individual hydrocarbon components. Mating status did not affect female hydrocarbons. The silk cocoons from which the parasites emerged yielded the same cuticular hydrocarbon components as were found on the newly emerged male and female wasps, but n-alkanes were now the major components. Hydrocarbon profiles of female cocoons differed from male cocoon hydrocarbons primarily in the relative abundances of the monene isomers. Bioassays of male response to hexane extracts of female and male cocoons indicated that the male could differentiate between the two. The primary gender recognition cue is not the cocoon hydrocarbons, but rather a female-produced sex pheromone, the hydrocarbons serving as a secondary recognition cue.

KEY WORDS *Cephalonomia tarsalis*, pheromone, gender recognition, ontogeny, courtship, behavior

THE PRIMITIVE ACULEATE family Bethyridae primarily parasitizes small, cryptic larvae of Coleoptera and Lepidoptera (Evans 1964). These wasps subdue their hosts by multiple stinging and lay 1 to several eggs externally. The resulting bethylid larvae develop as ectoparasitoids, dropping off the exhausted remains of their host to pupate gregariously in silk cocoons nearby. Males normally emerge before females and inseminate their sisters or even their mothers in some cases (Evans 1964).

Several bethylids are commonly associated with the stored commodity environment, including various *Cephalonomia* spp. and *Laelius* spp. These parasites are frequently host-specific and can be important biocontrol agents (Flinn and Hagstrum 1995, Flinn et al. 1996). Although basic life-history data have been gathered on many of these parasites (*Cephalonomia waterstoni* Gahan: Rilett 1949, Finlayson 1950, Howard and Flinn 1990; *C. stephanoderis* Betrem: Baker 1984, Barrera et al. 1989, Abraham et al. 1990, Baker and Barrera 1993; *C. tarsalis*

(Ashmead): Powell 1938; and *Laelius utilis* Cockerell: Mertins 1985), little is known of the biochemistry or chemical ecology of these important parasites. Howard (1992) recently reported on the cuticular hydrocarbon composition of *C. waterstoni* and *L. utilis* and their respective hosts, *Cryptolestes ferrugineus* (Stephens) and *Trogoderma variable* Ballion; and Howard and Infante (1996) described the cuticular hydrocarbon composition of *C. stephanoderis* and its host, the scolytid beetle *Hypothenemus hampei* (Ferrari). In this article I report on the cuticular hydrocarbon composition of *C. tarsalis*, a host-specific parasitoid of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.). I show that although males and females have the same cuticular hydrocarbons, they differ in absolute quantity and in relative proportions. These hydrocarbon profiles are dynamic, and respond to ontogenetic, reproductive, and nutritional effects. I also show that the silk cocoons of these insects contain gender-specific hydrocarbon profiles and that the male wasps are able to differentiate female and male cocoons by chemical cues. Finally, I show that this differentiation is primarily made by using a female-produced

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation by USDA for its use.

pheromone, but that the cuticular hydrocarbons also function as a secondary recognition cue.

Materials and Methods

Insects. Cultures of *C. tarsalis* (the parasite) and *O. surinamensis* (L.) (the host) were collected from farm-stored wheat in Kansas and have been in laboratory culture on wheat for 1 yr. Parasites to be used for experiments were collected from stock cultures as newly emerged adults and were placed in vials partially filled with whole-wheat kernels. A cotton ball soaked with 50% honey-water solution was added to each vial and was rewetted daily. Vials were held in a rearing chamber at $30 \pm 1^\circ\text{C}$ and $50 \pm 10\%$ RH with a photoperiod of 16:8 (L:D) h.

Chemical Analyses. Insects were frozen at -5°C for 24 h. Cuticular lipids were extracted by immersing the insects in 3 successive 0.25-ml portions of hexane for 1 min each time. The combined portions from each sample were concentrated under a stream of N_2 , and hydrocarbons were isolated by chromatography on a 3-cm minicolumn of Biosil A (Bio-Rad, Richmond, CA) as described earlier (Howard et al. 1978). Because the parasitoids are very small insects containing insufficient hydrocarbon for single insect analyses, samples consisted of 5 insects per replicate, and 2 replicates of each experimental treatment were analyzed.

Electron impact mass spectral analyses were conducted using a Hewlett-Packard 5790A gas chromatograph (GC) (Hewlett-Packard, San Fernando, CA) containing a DB-5 bonded phase capillary column (15 m long, 0.25 mm i.d.) (J and W Scientific, Folsom, CA) connected to a Hewlett-Packard 5970 mass selective detector and a Hewlett-Packard 9133 data system. Ultrapure helium was the carrier gas, with a column head pressure of 0.75 kg/cm^2 . Mass spectra were obtained at 70 eV. Analyses were done using temperature programming, with an initial temperature of 80°C , a final temperature of 320°C , a program rate of $5^\circ\text{C}/\text{min}$, 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 were obtained by comparison with known *n*-alkane standards (Howard et al. 1978). Individual components in the total ion scanning mode were identified from their characteristic EI-MS fragmentation patterns (Jackson and Blomquist 1976, Nelson 1978). Quantitative analyses were conducted using the selective ion scanning mode, m/z 55 and 57 being the target ions and an internal standard of $50.2 \text{ ng}/\mu\text{l}$ each of heptadecane and 1-heptadecene.

Double-bond locations in alkenes were obtained by preparing dithiomethyl ethers (DTME) and examining their electron impact mass spectra (EI-MS) (Francis and Veland 1981). Stereochemistry of the parent alkene was established from Fourier transform infrared analyses. Fourier transform infrared vapor-phase spectra on the underivatized alkenes

were obtained on a Hewlett-Packard 5890 GC with a 5965B FTIR detector and a 7958A data system. A DB-5 bonded phase capillary column (15 m long, 0.25 mm i.d.) using chromatographic conditions identical to those described above was used.

Effects of Mating Status, Host-Feeding Status, and Age-Related Changes on Female *C. tarsalis* Cuticular Hydrocarbons. The 3×2 factorial experimental design compared virgin versus mated females; host-fed versus nonhost-fed females; and females of 0–1, 7, 14, and 21 d of age. To get females 0–1 d old, cocoons were held in individual vials until emergence. Between 0800 and 0900 hours CST, all emerged wasps were collected. Between 1600 and 1700 hours of that same day, the vials were checked again and additional newly emerged wasps were collected, added to the morning wasps, and held until the following morning at which time they were 16–24 h old. For at least some of the time intervals, mating and host feeding were directly observed. Matings always paired each female with 3–5 males. When females were placed in vials with host larvae, they were removed after 10 d and placed in new vials to separate them from emerging offspring. At the appropriate age, the females were frozen and held at -20°C until they were extracted.

Effects of Mating Status and Age-Related Changes on Male *C. tarsalis* Cuticular Hydrocarbons. The 2×2 factorial design compared virgin versus mated males, and males of 0–1 and 7 d of age (males do not live >8 –10 d). Males had access to honey water. Newly emerged males to be mated were placed individually in petri dishes with virgin females. After matings were observed, males were placed in vials and held for the appropriate length of time before being placed in the -20°C freezer. Males of age 0–1 d were obtained in the same manner as described for females 0–1 d old.

Size and Weight Measurements of *C. tarsalis*. Fifty females and males were killed by freezing and weighed on a microbalance. Twenty-five each of these specimens were then measured for total body length (from the most forward part of the head capsule to the tip of the abdomen), and head capsule width (at its widest point).

Hydrocarbon Composition of Silk Cocoons. Cocoons that had visibly dark pharate adults in them were carefully opened and the wasps removed and their sex determined. The male and female silk cocoons then were separately extracted with hexane and analyzed by selective ion scanning mode as detailed above.

Gender Recognition of Cocoons by Males. Cocoons containing live *C. tarsalis* almost ready to emerge, and various *C. tarsalis* extracts, were placed on artificial cocoons to test their attractiveness to *C. tarsalis* males. The following 4 extracts were tested: (1) Total nonpolar female cuticular lipids: 100 virgin females, 0–1 d of age, were rinsed 3 times with $200 \mu\text{l}$ for 1 min each time. The pooled extracts were concentrated with N_2 to $100 \mu\text{l}$, yielding a solution of 1 female equivalent/ μl . (2) Total female cuticular

hydrocarbons: 100 virgin females, 0–1 d of age, were rinsed 3 times with 200 μl of hexane for 1 min each time. The pooled extract was concentrated with N_2 to $\approx 100 \mu\text{l}$ then placed on a 3-cm column of BioSil A in a 9-in pasteur pipet. The column was flushed with 3 ml hexane and the eluant concentrated with N_2 to 1 female equivalent/ μl . (3) Total male cuticular lipids: 86 virgin males, 0–3 d old, were rinsed 3 times with 200 μl hexane for 1 min each time. The pooled extract was concentrated with N_2 to 1 male equivalent/ μl . (4) Total polar female cuticular lipids: immediately after the hydrocarbons had been eluted from the BioSil A column of extract 2, the column was flushed with 3 ml of 10% ethyl ether in hexane, and the eluant was concentrated with N_2 to 1 female equivalent/ μl . A control extract was prepared by taking 600 μl of hexane and concentrating it under N_2 to 100 μl .

Artificial cocoons ≈ 2 mm long and 1 mm in diameter were rolled from 100% absorbent cotton that had been extracted 3 times with hexane and 3 times with acetone, then allowed to dry completely in an operating fume hood. Just before each bioassay, a single artificial cocoon was placed in a clean 15-cm glass petri dish and 2 μl (2 insect equivalents) of an extract was applied, and the treated cocoon was allowed to dry for ≈ 2 min. Live cocoons that were within 0–1 d of emergence were placed in individual vials ≈ 1 h before testing. Plastic 3.5-cm petri dishes were lined with filter paper and a sparse layer of whole-wheat kernels. A single virgin male *C. tarsalis* 0–1 wk old was placed in each dish and allowed to acclimate for ≈ 5 min. One test cocoon was then placed in the dish and resulting behaviors were filmed for 2 min using a Panasonic Digital Video camera, Model WV-CP4100 (Panasonic Broadcast and Television Systems, Secaucus, NJ) attached to a Wild M8 microscope (Wild Heerbrugg, Heerbrugg, Switzerland) and a Sony digital video cassette recorder, Model EV-S7000 (Sony, Park Ridge, NJ). The total time within 2 min that each male spent walking on and rapidly antennating the cocoon was recorded. For each bioassay, fresh filter papers were used. Males and cocoons were used only once; 129 live cocoons were initially tested for male response. After testing, all live cocoons were placed in individual numbered vials. Each vial was checked periodically for adult emergence and the sex of the emerged adult. Cocoons that failed to produce an adult within 1 h were eliminated from the experiment.

Bioassay To Test for Ability of Male *C. tarsalis* To Use Female *C. tarsalis* Cuticular Hydrocarbons as a Recognition Cue During Courtship. A bioassay setup similar to that used to test for gender recognition of cocoons was used. Males were virgins 1–7 d old, and they were presented with a choice of 4 artificial cocoons treated either with hexane (control 1), female-derived pheromone (0.5 female equivalent) (control 2), female pheromone plus 2 female equivalents of female *C. tarsalis* cuticular hydrocarbon, and female pheromone plus 2 male

equivalents of male *C. tarsalis* cuticular hydrocarbons. The female pheromone was obtained by holding 5 virgin females 0–1 d old for 3 h in a 1-ml vial fitted with a small wire basket on top that held 10 artificial cocoons which absorbed the diffusing volatile pheromone. The cocoons were removed from the basket as needed for each bioassay and were either used as is or were treated with *C. tarsalis* cuticular hydrocarbons. The order of each treatment was randomly assigned for each bioassay replicate. The male was given 10 min in the bioassay chamber; the total time spent on each artificial cocoon was recorded using the video apparatus described above. Sixteen males were individually bioassayed, each male being used for only 1 bioassay.

Statistical Analyses. Compositional analyses of hydrocarbons were conducted using the EI-MS system as described above. Area counts obtained from electronic integration in the selective ion scanning mode (m/z 55 and 57) were summed and used as nanogram per insect values or they were converted into percentage values. Each run had an internal standard of 50.2 ng/ μl each of heptadecane and 1-heptadecene. For multifactorial analysis of variance (ANOVA), nanogram values were used. Significantly different means were separated using the least significant difference (LSD) test at the 95% CL. Reported means are on percentage values. Comparisons of male responses to cocoons were done by paired *t*-tests. Male responses to the pheromone plus hydrocarbon bioassay were compared using the non-parametric Kruskal-Wallis test, because the Bartlett test indicated that the standard deviations of the means of the 4 treatments did not have equal variances ($P = 0.0008$) and various transformations of the raw data failed to produce a normalized data set. Separation of median values of the 4 treatments was obtained by using a box-and-whisker plot with the median notch option. All statistical analyses were conducted using the personal computer software program Statgraphics (1997).

Voucher Specimens. Voucher specimens of *C. tarsalis* (lot no. 71) have been deposited in the Research Collection of Insects, Kansas State University, Manhattan.

Results

The same 48 cuticular hydrocarbons were identified, albeit in different relative abundances, from male and female *C. tarsalis* (Fig. 1). The major components (>95 % of the total mixture) are n-alkanes (C_{23} – C_{37}) and n-alkenes ($\text{C}_{25:1}$ – $\text{C}_{37:1}$), the latter being a series of Δ^{11} , Δ^9 , and Δ^7 isomers as confirmed by analysis of their dithiomethyl ethers (Francis and Veland 1981). Infrared analysis of these alkenes indicated that they were of the Z configuration (absence of a strong band at 970 cm^{-1} and presence of a weak-moderate band at $1,650 \text{ cm}^{-1}$ (Nakanishi 1962). In addition, $\approx 5\%$ of 5-methyl alkanes (C_{25} , C_{27} , and C_{29}) and trace amounts

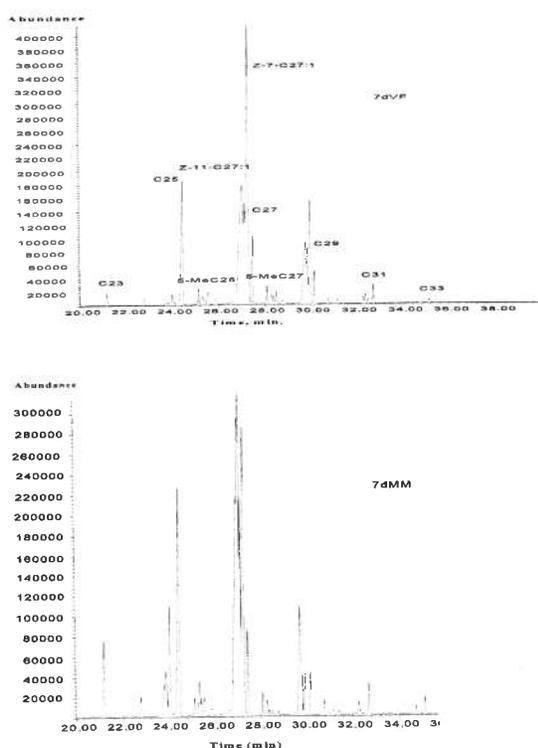


Fig. 1. Total ion trace of cuticular hydrocarbons of *C. tarsalis*. Virgin females 7 d old 7dVF; 7dMM, mated males 7 d old.

(<0.01%) of 5,17- and 5,19-dimethyl alkanes (C_{29} and C_{33}) also were present (Table 1).

The effects of 4 different parameters on the cuticular hydrocarbon profiles of *C. tarsalis* were examined—gender, age, mating status, and for females, whether they had host-fed. These comparisons were made for individual hydrocarbon components and for 5 hydrocarbon classes—total hydrocarbon, saturated hydrocarbons, n-alkanes, 5-methyl alkanes, and alkenes.

Males and females differed strongly in total hydrocarbon ($F = 21.50$; $df = 1, 33$; $P < 0.0000$), saturated hydrocarbon ($F = 22.21$; $df = 1, 33$; $P < 0.0000$), n-alkanes ($F = 20.43$; $df = 1, 33$; $P = 0.0001$), 5-methyl alkanes ($F = 27.04$; $df = 1, 33$; $P < 0.0000$), and alkenes ($F = 19.22$; $df = 1, 33$; $P = 0.0001$). Given these strong gender differences, subsequent factor comparisons were made separately for the males and females.

Over all treatments, female *C. tarsalis* have ≈ 449.8 ng of total hydrocarbon partitioned into 27.4% saturated hydrocarbon (of which 25.8% are n-alkanes, 1.6% are 5-methyl alkanes, and trace amounts are the 5, X-dimethyl alkanes) and 72.6% alkenes. Multifactorial ANOVA indicated that the mating status (virgin or mated) of the females had no significant effect on total hydrocarbon ($F = 0.00$; $df = 1, 19$; $P = 0.9824$), saturated hydrocarbon ($F =$

Table 1. Cuticular hydrocarbons of *C. tarsalis*

Compound	CN	ECL	Diagnostic EI-MS ion fragments ^a
n-C ₂₃	23	23.00	324
n-C ₂₄	24	24.00	338
Z-11-C _{25:1}	25	24.68	350 [61,201,243,444]
Z-9-C _{25:1}	25	24.76	350 [61,173,271,444]
Z-7-C _{25:1}	25	24.82	350 [61,145,299,444]
n-C ₂₅	25	25.00	352
5-MeC ₂₅	26	25.50	85,309,351
Z-11-C _{26:1}	26	25.68	364 [61,201,257,458]
Z-9-C _{26:1}	26	25.75	364 [61,173,285,458]
Z-7-C _{26:1}	26	25.83	364 [61,145,313,458]
n-C ₂₆	26	26.00	366
Z-11-C _{27:1}	27	26.69	378 [61,201,271,472]
Z-9-C _{27:1}	27	26.76	378 [61,173,299,472]
Z-7-C _{27:1}	27	26.82	378 [61,145,327,472]
n-C ₂₇	27	27.00	380
5-MeC ₂₇	28	27.51	85,337,379
Z-11-C _{28:1}	28	27.69	392 [61,201,285,486]
Z-9-C _{28:1}	28	27.74	392 [61,173,313,486]
Z-7-C _{28:1}	28	27.80	392 [61,145,341,486]
n-C ₂₈	28	28.00	394
Z-11-C _{29:1}	29	28.69	406 [61,201,299,500]
Z-9-C _{29:1}	29	28.75	406 [61,173,327,500]
Z-7-C _{29:1}	29	28.82	406 [61,145,355,500]
n-C ₂₉	29	29.00	408
5-MeC ₂₉	30	29.53	85,365,407
C _{30:1}	30	29.71	420
5,17 + 5,19-DiMeC ₂₉	31	29.82	85,197,267,379; 85,169,295,379
n-C ₃₀	30	30.00	422
Z-11-C _{31:1}	31	30.67	434 [61,201,313,514]
Z-9-C _{31:1}	31	30.75	434 [61,173,341,514]
Z-7-C _{31:1}	31	30.81	434 [61,145,369,514]
n-C ₃₁	31	31.00	436
C _{32:1}	32	31.70	448
n-C ₃₂	32	32.00	450
C _{33:1}	33	32.70	462
n-C ₃₃	33	33.00	464
C _{34:1}	34	33.70	476
5,17 + 5,19-DiMeC ₃₃	35	33.82	85,253,267,435; 85,225,295,435
n-C ₃₄	34	34.00	478
C _{35:1}	35	34.70	490
n-C ₃₅	35	35.00	492
n-C ₃₆	36	36.00	506
C _{37:1A}	37	36.68	518
C _{37:1B}	37	36.75	518
C _{37:1C}	37	36.81	518
n-C ₃₇	37	37.00	520

CN, carbon number; ECL, equivalent chain length.

^a Ions in brackets are for dithiomethylether derivatives.

1.77; $df = 1, 19$; $P = 0.1995$), n-alkanes ($F = 2.42$; $df = 1, 19$; $P = 0.1359$), 5-methyl alkanes ($F = 2.04$; $df = 1, 19$; $P = 0.1690$), or alkenes ($F = 0.14$; $df = 1, 19$; $P = 0.7162$). Female age (0–1, 7, 14, 21 d old) and host feeding status (fed, nonfed), however, did have significant effects on all 5 of these hydrocarbon classes: total hydrocarbon (age, $F = 4.67$; $df = 3, 19$; $P = 0.0131$; feeding status, $F = 23.13$; $df = 1, 19$; $P = 0.0001$), saturated hydrocarbon (age, $F = 5.56$; $df = 3, 19$; $P = 0.0065$; feeding status, $F = 16.45$; $df = 1, 19$; $P = 0.0007$), n-alkanes (age, $F = 7.23$; $df = 3, 19$; $P = 0.0020$; feeding status, $F = 14.01$; $df = 1, 19$; $P = 0.0014$), 5-methyl alkanes (age, $F = 8.21$; $df = 3, 19$; $P = 0.0010$; feeding status, $F = 48.86$; $df = 1, 19$; $P < 0.0000$), and alkenes (age, $F = 6.46$; $df = 3, 19$; $P =$

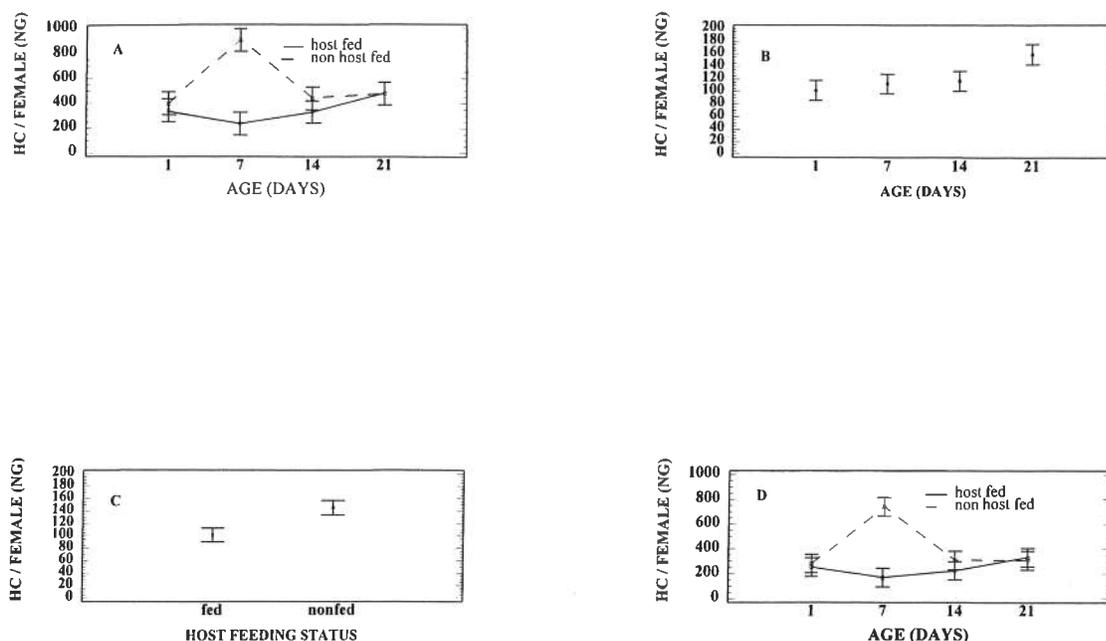


Fig. 2. Significant effects of age and host feeding status on abundance of total hydrocarbon, saturated hydrocarbon, and alkenes of female *C. tarsalis*. (A) Age \times feeding status interaction plot for total hydrocarbons. (B) Age plot for saturated hydrocarbons. (C) Feeding status plot for saturated hydrocarbons. (D) Age \times feeding status plots for alkenes. Plotted data points are least square means with standard error bars at the 95% CL. Mating status was not a significant factor ($P > 0.05$) for total hydrocarbon, saturated alkanes, n-alkanes, 5-methyl alkanes, or alkenes.

0.0034; feeding status, $F = 21.83$; $df = 1, 19$; $P = 0.0002$). Significant age \times feeding status interactions occurred for the total hydrocarbons ($F = 12.74$; $df = 3, 19$; $P = 0.0001$), 5-methyl alkanes ($F = 11.17$; $df = 3, 19$; $P = 0.0002$), and for the alkenes ($F = 15.06$; $df = 3, 19$; $P < 0.0000$). Graphical depictions of these mean differences are shown in Fig. 2. Clearly the major influence on total hydrocarbon and alkene differences is the host-feeding status, and it is felt almost entirely at day 7, where nonhost-fed females have significantly more total hydrocarbon than do host-fed females. The age effect for saturated hydrocarbons does not show up until day 21, and, over all treatments, nonhost-fed females have more total hydrocarbons than do host-fed females (Fig. 2).

Over all treatments, male *C. tarsalis* have ≈ 198.9 ng of total hydrocarbon partitioned into 31.7% saturated hydrocarbons (29.6% are n-alkanes, 2.1% are 5-methyl alkanes, and trace amounts are the 5, X-dimethyl alkanes) and 68.3% alkenes. Unlike the females, multifactorial ANOVA indicated that male mating status did have a significant effect on saturated hydrocarbons ($F = 16.58$; $df = 1, 4$; $P = 0.0152$) and n-alkanes ($F = 16.06$; $df = 1, 4$; $P = 0.0160$), but did not have a significant effect on total hydrocarbons ($F = 6.69$; $df = 1, 4$; $P = 0.0609$), 5-methyl alkanes ($F = 5.27$; $df = 1, 4$; $P = 0.0833$), or on the alkenes ($F = 4.40$; $df = 1, 4$; $P = 0.1041$). Again, unlike the females, multifactorial ANOVA indicated that age was not an important factor in regulating

hydrocarbon abundances. Only the 5-methyl alkanes were significantly affected by male age ($F = 52.17$; $df = 1, 4$; $P = 0.0019$), but the total amount of hydrocarbon involved (< 6 ng) is small and may not be biologically significant. Fig. 3 provides a graphical depiction of the mean differences in the effects of age and mating status on the abundance of male saturated hydrocarbons, 5-methyl alkanes, and alkenes.

Body size or biomass differences between females and males agree with the above hydrocarbon differences. Females have a wet biomass of 14.8 ± 1.4 μg (mean \pm SD), whereas males have 7.5 ± 0.8 μg , for a ratio (female/male) of 1.97. The corresponding ratio for the average hydrocarbon abundance of the wasps is 2.26, suggesting that despite the size differences between females and males, the quantity of hydrocarbon per microgram of wet biomass is essentially the same for the 2. Females are 2.10 ± 0.09 mm long but males are 1.65 ± 0.14 mm long, and the female's head is 0.42 ± 0.02 mm wide versus 0.37 ± 0.03 mm wide in males.

Quantitative comparisons also were made of all individual hydrocarbons present in $> 0.1\%$ abundance (35 of the 46 components). The percentage comparisons are listed in Table 2 and Table 3, but absolute nanogram values were used for the multifactorial ANOVAs. For the females, significant differences ($P < 0.05$) occurred for all 3 factors (age, mating status, and host feeding status) and for all 3

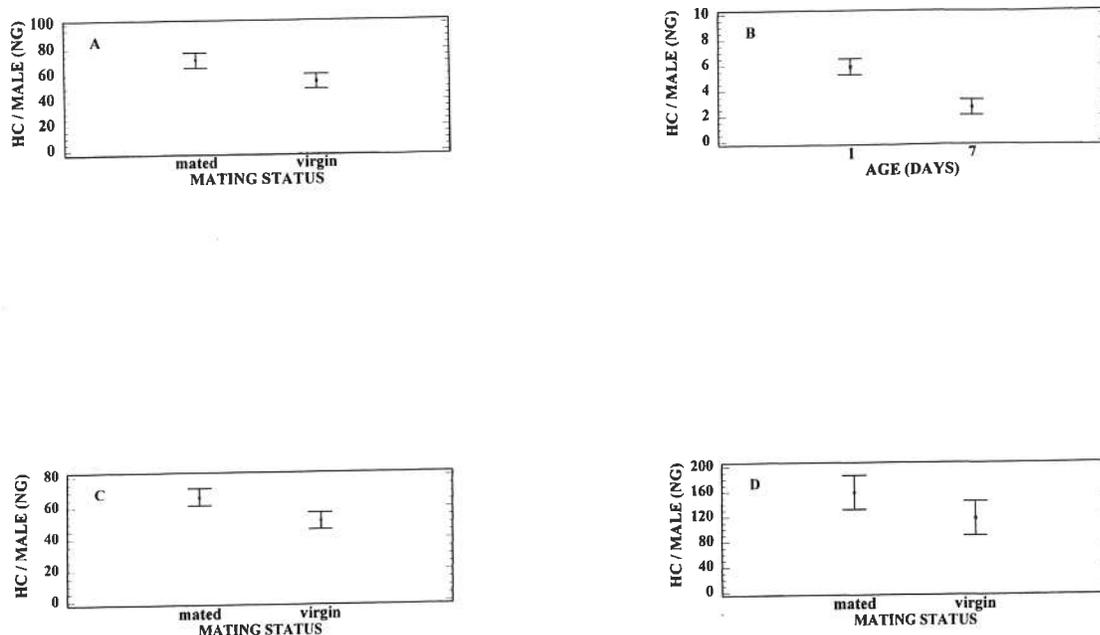


Fig. 3. Significant effects of age and mating status on abundance of saturated hydrocarbons, n-alkanes, 5-methyl alkanes, and alkenes of male *C. tarsalis*. (A) Mating status plot for saturated hydrocarbons. (B) Age plot for 5-methyl alkanes. (C) Mating status plot for n-alkanes. (D) Mating status plot for alkenes. Plotted data points are least square means with standard error bars at the 95% CL. Note that mating status was not a significant factor for alkenes ($P > 0.05$).

possible binary interactions. For the factor age, 8 of 11 n-alkanes, all of the 5-methyl alkanes, and 9 of 21 of the alkenes differed. With regard to mating status, however, only 2 of 11 n-alkanes and 3 of 21 of the alkenes differed. The biggest differences occurred with respect to host-feeding status, where 9 of 11 n-alkanes, all of the 5-methyl alkanes, and 14 of 21 of the alkenes differed. For the age \times mating status interaction, 5 of the 35 components differed; for the age \times feeding status interaction, 25 of the 35 differed; and for the mating status \times feeding interaction, 7 of the 35 components differed significantly.

For the males, fewer individual hydrocarbons differed as a result of age, mating status, or the age \times mating status interaction. With respect to age, 2 of 11 of the n-alkanes, 2 of 3 of the 5-methyl alkanes, and 6 of 21 of the alkenes significantly differed. With regard to mating status, 6 of 11 of the n-alkanes and 1 of 21 of the alkenes differed significantly, and for the age \times mating status interaction 1 of 3 of the 5-methyl alkanes and 8 of 21 of the alkenes differed.

The biggest obvious difference between females and males is the ratio of the 3 double-bond positional isomers at each carbon number. Fig. 4 depicts the differences for the major C₂₇ alkenes on days 1 and 7. The males are characterized by greater proportions of the Z- Δ^{11} isomer and the females are characterized by greater proportions of the Z- Δ^7 isomer. The Z- Δ^9 isomer is approximately the same for both males and females.

The cocoons of *C. tarsalis* contain the same hydrocarbons as the adult wasps (Fig. 5). Unlike the adults, the silk cocoons possess a greater abundance of n-alkanes than they do n-alkenes (Fig. 1 versus Fig. 5). The differences between the individual components of male and female cocoons are fewer than between the adults themselves, but several components, including the C₂₇ monoenes, appear to show the same sex-specific differences.

Virgin males were used in a bioassay to see if they could discriminate between artificial cocoons treated with hexane extract of female cocoons, hexane extract of male cocoons, or a hexane-treated blank. Responses of these males were compared with responses of the males to live males and females just ready to emerge from their silk cocoons. Males exposed to live female cocoons, in which the females emerged within 1 h of testing, spent 22.9 s (of 2 min) on the cocoon, rapidly antennating it and showing signs of intense excitement ($n = 9$) compared with 1.6 s on male cocoons with much less antennation and no apparent excitement ($n = 10$) ($t = 2.65$, $P = 0.029$). Using the artificial cocoons and insect extracts, virgin males exposed to hexane controls spent no time on the cocoons ($n = 10$). When exposed to 2 female equivalents of total lipid extract, the males spent 26.1 s on the cocoons, displaying rapid antennation and excitement compared with 0.1 s when 2 male equivalents of total lipid extract was applied to the cocoons ($n = 15$) ($t = 7.18$, $P <$

Table 2. Mean percentage composition of cuticular hydrocarbons of male *C. tarsalis* present in >0.01% abundance as a function of age and mating status ($n = 2$)

Compound	1-d mated	1-d virgin	7-d mated	7-d virgin
C ₂₃	1.71	1.53	3.05	3.50
C ₂₄	0.58	0.51	0.74	0.77
11-C _{25:1}	0.94	0.65	2.23	2.08
9-C _{25:1}	1.44	1.12	1.28	2.54
7-C _{25:1}	4.77	4.27	4.07	6.95
C ₂₅	14.48	16.72	15.45	14.65
5-MeC ₂₅	1.37	1.70	0.29	0.28
11-C _{26:1}	1.13	0.99	1.49	1.51
9-C _{26:1}	0.67	0.54	0.31	0.62
7-C _{26:1}	0.96	0.85	0.34	0.66
C ₂₆	0.70	0.88	0.52	0.40
11-C _{27:1}	25.42	22.71	34.15	30.61
9-C _{27:1}	6.75	7.31	6.58	6.38
7-C _{27:1}	19.03	19.08	6.49	11.57
C ₂₇	5.24	6.71	5.31	4.02
5-MeC ₂₇	0.89	1.02	0.56	0.65
11-C _{28:1}	0.53	0.47	0.61	0.60
9-C _{28:1}	0.21	0.17	0.06	0.10
7-C _{28:1}	0.27	0.24	0.06	0.10
C ₂₈	0.19	0.27	0.26	0.16
11-C _{29:1}	4.63	4.00	7.87	4.37
9-C _{29:1}	1.10	0.72	0.26	0.64
7-C _{29:1}	1.52	1.09	0.31	0.76
C ₂₉	2.41	2.69	3.26	2.58
5-MeC ₂₉	0.43	0.51	0.46	0.51
11-C _{30:1}	0.14	0.10	0.13	0.19
9-C _{30:1}	0.12	0.07	0.03	<0.01
7-C _{30:1}	0.18	0.14	0.16	0.19
C ₃₀	0.12	0.14	0.18	0.11
11-C _{31:1}	0.19	0.14	0.74	0.72
9-C _{31:1}	0.10	<0.01	0.10	0.19
7-C _{31:1}	<0.01	0.07	0.06	<0.01
C ₃₁	1.49	2.06	2.42	1.82
C ₃₂	<0.01	0.07	0.10	<0.01
C ₃₃	0.65	0.68	0.91	0.60

0.0000). To see if the hydrocarbons were the factor responsible for these differences, the female extracts were chromatographed over BioSil A and separated into a hydrocarbon fraction and a polar fraction. Treating the artificial cocoons with 2 female equivalents of the isolated hydrocarbons resulted in the virgin males spending 0.5 s out of 2 min on the cocoon ($n = 15$), whereas treating the artificial cocoon with 2 female equivalents of the polar fraction resulted in the males spending 12.4 s out of 2 min on the cocoons displaying rapid antennation and general excitement ($n = 14$) ($t = 4.70$, $P = 0.0004$).

To assess whether the cuticular hydrocarbons of females might serve as a secondary gender recognition cue after the male had been exposed to the polar pheromonally behaving compound, the 4-way choice bioassay with pheromone and male or female cuticular hydrocarbon extracts was conducted. The median amount of time spent by the male on the cocoons treated with female hydrocarbons was significantly different from the median amount of time spent on the cocoon treated with female pheromone and male cuticular hydrocarbons ($P < 0.0000$) (Fig. 6).

Discussion

The cuticular hydrocarbon composition of 4 bethylids are now known—*Cephalonomia stephanoderis* (Howard and Infante 1996), *C. tarsalis*, *C. waterstoni* (Howard 1992), and *Laelius utilis* (Howard 1992). Although there are certainly some similarities among these taxa, there are far more dissimilarities. Adult *C. stephanoderis*, whose host is the coffee berry borer (Coleoptera: Scolytidae), have the most complex cuticular hydrocarbon profile of the 4 bethylids. The major components of adult *C. stephanoderis* are n-alkanes (C₂₁–C₃₃), monomethyl alkanes (3-, 5-, 7-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, and 17-methyl), and Z-10-monomethyl alkenes, with the methyl branch at C₁₁–C₁₆. Minor components include a series of 3,X-, 4,X-, 5,X-, 6,X-, 7,X- and 8,X-dimethyl alkanes, with X at C₁₄–C₁₉, and a series of 3,9,13- and 3,11,15-trimethyl alkanes. Male *C. stephanoderis* are characterized by high proportions of n-alkanes and lower proportions of the monomethyl alkanes and methyl-branched alkenes. Female *C. stephanoderis* are characterized by lower proportions of n-alkanes and higher proportions of the monomethyl alkanes and methyl branched alkenes.

Adult *C. tarsalis*, whose host is the sawtoothed grain beetle (Coleoptera: Cucujidae), have a much simpler cuticular hydrocarbon composition than does *C. stephanoderis*, being composed of n-alkanes (C₂₃–C₃₇), 5-methyl alkanes (5-MeC₂₅–5-MeC₂₉), 5,17- and 5,19-dimethyl alkanes (5,X-diMeC₂₉ and 5,X-diMeC₃₃), and Z-7-, Z-9-, and Z-11-monoenes (C_{25:1}–C_{37:1}) (Table 1). The alkanes and alkenes are the predominant components of both sexes (>95%), the 5-methyl alkanes accounting for ≈5% and the dimethyl alkanes present in only trace amounts. Males differ significantly from females with respect to absolute quantity of hydrocarbon per insect in all hydrocarbon classes and in at least 11 major hydrocarbon components, including the C₂₅, C₂₇, and C₂₉ monoenes, 2 n-alkanes, and 1 5-methyl alkane.

Adult *C. waterstoni*, whose host is the rusty grain beetle (Coleoptera: Cucujidae), possess n-alkanes (C₂₃–C₂₉), 2-, 3-, and 5-methyl alkanes (X-Me-C₂₃–X-MeC₂₉), 5,15-, 5,17-, and 5,19-dimethyl alkanes (5,X-diMeC₂₃–5,X-diMeC₂₅), and a series of Z-11-monoenes (C_{25:1}–C_{29:1}). In addition, the males possess small quantities of Z-7-monoenes (C_{25:1}–C_{27:1}). The males have monoenes and n-alkanes as their major components (38 and 24%, respectively, of total hydrocarbons), whereas the females have 5-methyl alkanes and dimethyl alkanes as their major components (39.4 and 20%, respectively, of total hydrocarbon).

The parasitoid *L. utilis*, whose host is *Trogoderma variable* Ballion (Coleoptera: Dermestidae), has the simplest hydrocarbon profile of the 4 bethylids, possessing only n-alkanes (C₂₁–C₂₉), 2-, 3-, 7-, 9-, and 13-methyl alkanes (C₂₃–C₂₇), and a series of Z-9-monoenes (C_{22:1}–C_{27:1}). Males and females differ only slightly in relative abundances of these 3

Table 3. Mean percentage composition of cuticular hydrocarbons of female *C. tarsalis* present in >0.01% abundance as a function of age and host-feeding status (n = 4)

Compound	1d H ²	1d N	7d H	7d N	14d H	14d N	21d H	21d N
C ₂₃	0.97	1.21	0.42	0.69	0.40	1.68	0.61	1.93
C ₂₄	0.43	0.48	0.40	0.30	0.34	0.47	0.47	0.62
11-C _{25:1}	0.11	0.17	0.10	0.20	0.13	0.47	0.17	0.56
9-C _{25:1}	0.16	0.15	0.35	0.16	0.30	0.32	0.38	0.27
7-C _{25:1}	0.86	0.55	0.35	0.69	0.18	1.47	0.36	1.46
C ₂₅	14.68	15.83	11.90	9.52	12.06	16.29	13.10	18.21
5-MeC ₂₅	1.16	1.59	0.18	0.77	0.17	0.27	0.14	0.29
11-C _{26:1}	0.38	0.45	0.13	0.47	0.14	0.62	0.16	0.73
9-C _{26:1}	0.52	0.44	0.51	0.32	0.51	0.34	0.63	0.28
7-C _{26:1}	0.94	0.79	0.76	0.64	0.67	0.64	0.81	0.62
C ₂₆	0.80	0.87	0.87	0.33	1.50	0.40	1.48	0.55
11-C _{27:1}	17.20	18.17	6.97	21.56	5.81	22.63	7.90	22.34
9-C _{27:1}	8.68	8.20	14.72	10.08	13.69	6.51	14.67	4.59
7-C _{27:1}	28.27	26.64	25.19	29.67	18.40	23.21	18.49	18.60
C ₂₇	4.40	4.92	6.10	2.74	10.50	4.46	8.57	5.56
5-MeC ₂₇	0.79	0.87	0.50	0.89	0.45	0.69	0.42	0.76
11-C _{28:1}	0.37	0.44	0.20	0.43	0.26	0.37	0.28	0.43
9-C _{28:1}	0.56	0.47	0.69	0.45	0.97	0.21	1.00	0.18
7-C _{28:1}	0.76	0.68	0.85	0.47	1.05	0.37	1.04	0.39
C ₂₈	0.13	0.20	0.38	0.09	0.62	0.18	0.56	0.30
11-C _{29:1}	3.68	4.13	2.02	6.65	1.73	6.14	2.85	6.81
9-C _{29:1}	3.91	2.80	8.04	2.42	10.31	1.91	8.62	1.57
7-C _{29:1}	6.69	5.65	10.17	6.60	10.92	3.65	9.40	3.68
C ₂₉	1.43	1.55	3.33	1.26	4.39	2.52	3.65	3.52
5-MeC ₂₉	0.19	0.26	0.27	0.28	0.20	0.38	0.21	0.43
11-C _{30:1}	0.09	0.10	0.08	0.11	0.09	0.07	0.10	0.10
9-C _{30:1}	0.06	0.06	0.13	0.06	0.13	0.03	0.14	0.03
7-C _{30:1}	0.20	0.26	0.32	0.26	0.28	0.23	0.27	0.30
C ₃₀	0.07	0.13	0.22	0.10	0.26	0.17	0.26	0.25
11-C _{31:1}	0.16	0.26	0.27	0.45	0.10	0.53	0.10	0.67
9-C _{31:1}	0.47	0.38	1.33	0.48	1.06	0.37	0.81	0.84
7-C _{31:1}	0.18	0.18	0.47	0.23	0.35	0.17	0.55	0.19
C ₃₁	0.71	0.83	1.71	0.82	1.87	1.95	1.65	2.57
C ₃₂	<0.01	0.07	0.06	0.05	0.07	0.12	0.07	0.17
C ₃₃	0.20	0.49	0.33	0.25	0.22	0.70	0.27	0.92

Multifactorial ANOVA indicated that mating status had no significant effect ($P > 0.05$) on hydrocarbon composition. 1d H, host-fed females 1 d old; 1d N, nonhost-fed females 1 d old; analogous reasoning applies to the remaining labels.

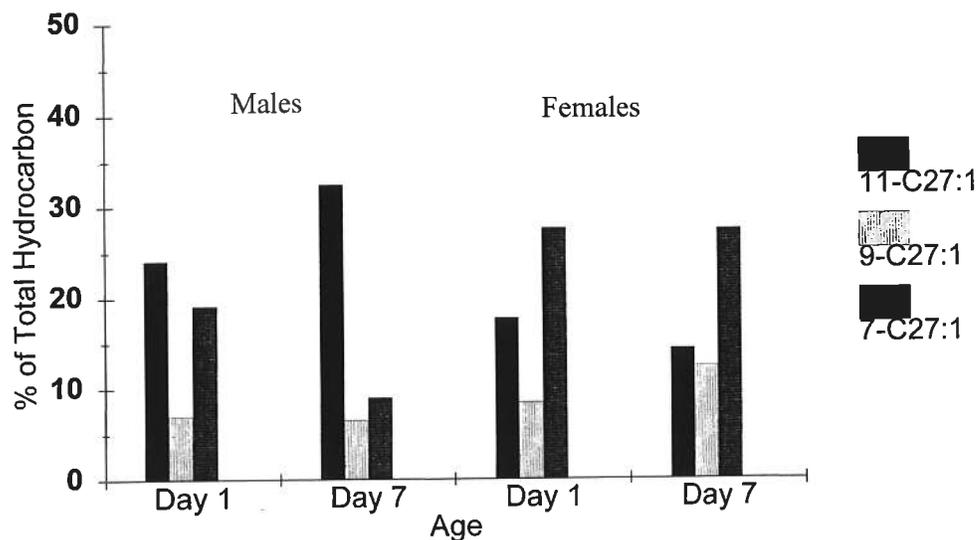


Fig. 4. Comparison of isomer ratios for C_{27:1} between male and female *C. tarsalis* at 1 and 7 d. Isomer abundances are expressed as percentage of total hydrocarbon abundance; means are pooled over mating status and host feeding status for females.

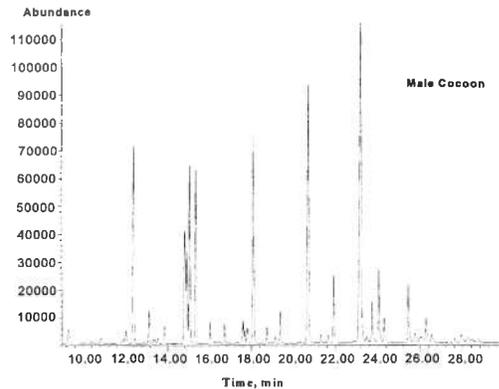
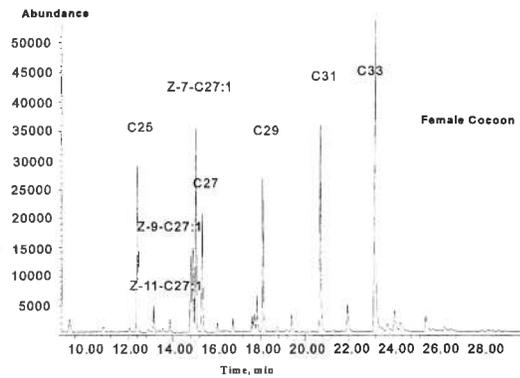


Fig. 5. Total ion trace of hydrocarbons obtained from female and male cocoons of *C. tarsalis*.

classes of hydrocarbons, males having $\approx 33\%$ n-alkanes, 4% branched alkanes, and 52% alkenes, and females having $\approx 45\%$ n-alkanes, 4% branched alkanes, and 63% alkenes.

Not only do these 4 bethylids differ markedly in their cuticular hydrocarbon profile, they all differ substantially from their hosts' cuticular hydrocarbon profile (Howard 1992, Howard et al. 1995, Howard and Infante 1996), and the hosts all differ greatly from one another in their cuticular hydrocarbon profiles. These findings represent an emerging trend in host-parasite cuticular hydrocarbon studies, and can be contrasted with the situation with highly integrated inquilines in social insect societies, where well-integrated inquilines at least often mimic very closely the cuticular hydrocarbon profiles of their host (Howard 1993, Howard and Akre 1995).

It is becoming increasingly clear that the cuticular hydrocarbon profiles of many insects are not static, but rather respond dynamically to ecological, environmental, ontogenetic, and physiological factors (Lockey 1988, Howard 1993, Schal et al. 1994, Howard and Akre 1995, Howard et al. 1995). Inas-

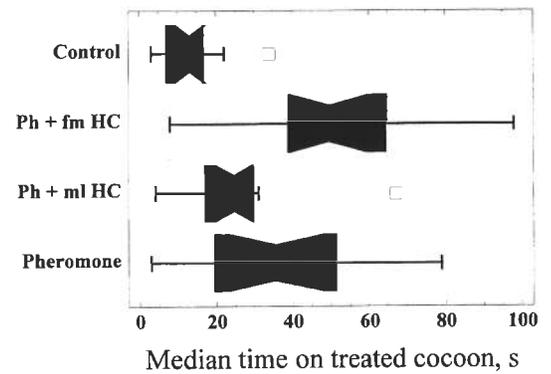


Fig. 6. Box-and-whisker comparison plot of median time spent by male *C. tarsalis* on artificial cocoons treated with female sex pheromone and male or female *C. tarsalis* cuticular hydrocarbons. Y-axis legends: control, hexane only; Ph + fm HC, female pheromone plus female cuticular hydrocarbons; pheromone, female pheromone only; Ph + ml HC, female pheromone plus male cuticular hydrocarbons.

much as male *C. tarsalis* live only ≈ 1 wk, feed sparsely, if at all, and exist only to mate with the females (Powell 1938), I predicted that they would show few changes in either abundance or composition of their cuticular hydrocarbons. In general, this was found to be true. The major change observed was a modest increase in abundance of n-alkanes and 5-methyl alkanes after the males had been mated (Fig. 3). No data exist to clarify whether these modest changes have any influence on the acceptability of the mated male to virgin females, who mate only once (Powell 1938).

Female *C. tarsalis*, however, may live several weeks (Powell 1938), they routinely feed on the hemolymph of their paralyzed hosts, and, as just noted, they mate only once. Therefore, I predicted that their cuticular hydrocarbon composition might be somewhat more dynamic, and, indeed, I found this to be the case. The large disparity in absolute quantity of total hydrocarbon between females and males (a factor of ≈ 2.26 in favor of the female) is not unexpected given the difference in body size between the 2 sexes (the females are ≈ 2.5 times the biomass of males). Female *C. tarsalis* show striking changes in both absolute abundance and composition of their cuticular hydrocarbons between emergence from the cocoon and 3 wk of age, the greatest change occurring at 1 wk (Fig. 2). The major factor effecting this dramatic increase in total hydrocarbons at day 7 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.

Host feeding is a common strategy among parasitoids (Heimpel and Collier 1996) and is thought to be important for both somatic maintenance and reproductive development. The hemolymph of larval insects varies widely across taxon and develop-

mental stage, and has generally been thought to be poor in carbohydrates and lipids and rich in free amino acids and proteins (Woodring 1985). Later studies have shown, however, that hemolymph-borne lipophorins transport substantial quantities of lipids, especially hydrocarbons (Katase and Chino 1982, Chino 1985, Van der Horst et al. 1993). Although no explicit studies on the hemolymph of the sawtoothed grain beetle have been conducted, it is reasonable to assume that its hemolymph is similar in composition to that of the vast majority of other insects. Whatever the exact biochemical nature of this hemolymph, its constituents are undoubtedly broken down by the *C. tarsalis* females and absorbed, translocated, and reassembled into fat body, muscle, and developing oocytes (Heimpel and Collier 1996).

Cephalonomia tarsalis females are several days posteclosion when they emerge from their cocoon (Powell 1938) and are ready to mate and soon thereafter to commence oviposition. But because all known host-feeding parasitoids are synovigenic (possess ovaries in which eggs are matured throughout all or most of adult life) (Heimpel and Collier 1996), any eggs present are not likely to be well developed and will require the female to host-feed to mature them (Bartlett 1964, Jarvis and Kidd 1986, van Lenteren et al. 1987, Collier 1995). If prevented from host feeding, then there is ample precedent for the female reabsorbing the developing egg and using the nutrients for either her own maintenance or the development of future eggs (Heimpel and Collier 1996). Could this reabsorption be related to the observed large increase in the cuticular hydrocarbons of *C. tarsalis* females not allowed to host feed?

Although I know of no studies directly addressing this question with parasitic Hymenoptera, related studies have been conducted by Schal and his colleagues using the German cockroach, *Blattella germanica* (L.) (Schal et al. 1994). They clearly showed that hydrocarbon biosynthesis in this insect cycles with the reproductive cycle of the female, and that at any given time, most of the hydrocarbon is found internally, stored at the site of biosynthesis, deposited at target sites other than the epicuticle, or bound to carrier proteins such as lipophorin in the hemolymph. They further showed that developing oocytes were a primary target for much of the biosynthesized hydrocarbon at any given time, and that these hydrocarbons were identical to those found on the cuticle of this insect. Most significantly, Schal et al. (1994) showed that in the absence of an ovarian sink for internal hydrocarbons, deposition of hydrocarbons on the cuticle increased substantially. I propose that when *C. tarsalis* females are prevented from host feeding, they reabsorb their developing eggs, thus causing the internal hydrocarbons which would normally be deposited in the ovaries to be shunted to the cuticle of the females. When the *C. tarsalis* females are held for extended periods of time without access to a host, then the amount of cuticular hydrocarbon on their cuticle

decreases, and with time approaches that of females who had earlier been allowed to host-feed (Fig. 2A). This reduction in cuticular hydrocarbons could come either from reabsorption or from simple loss from abrasion. In the absence of host feeding, it is unlikely that hydrocarbon biosynthesis would continue (Schal et al. 1994), and I would thus not expect the high levels of hydrocarbon to persist on these females' cuticle. The above argument of necessity entails a considerable amount of speculation, and the extremely small size of these parasitoids will make it difficult to conduct the requisite biochemical and physiological studies needed to clarify the issue.

Male *C. tarsalis* emerge from their cocoons several days before the females and actively search out female cocoons. Males might distinguish female from male cocoons by gender- and species-specific hydrocarbon profiles on the silk. I have earlier shown that cocoons of *C. waterstoni* (Howard 1992) possess species-specific hydrocarbons, but in that study I did not examine male and female cocoons separately. Cocoons of female and male *C. tarsalis* also contain substantial amounts of the same hydrocarbons as occur on adult *C. tarsalis*, but in somewhat different relative abundances, n-alkanes now being the predominant components (Fig. 5). Although there are some apparent hydrocarbon differences between female and male cocoons, they are certainly not as striking as the differences between sexes of the adult wasps. I therefore conducted a simple bioassay to test the hypothesis that the males could distinguish between female and male cocoons. Preliminary studies using natural cocoons from which live *C. tarsalis* had just emerged showed that males spent significantly more time examining and rapidly antennating cocoons from which females had emerged than they did from cocoons from which males had just emerged. Hexane extracts of these female and male cocoons applied to artificial cotton cocoons yielded the same general results. When these hexane extracts were fractionated into a hydrocarbon fraction and a polar fraction, and then rebioassayed, all activity was in the polar fraction. Males are frequently attracted to female cocoons just before the female emerges, suggesting that the females may be releasing a volatile sex pheromone (unpublished data).

The presence of such a pheromone complicates the interpretation of short-range species or gender recognition using cuticular hydrocarbons as cues. I hypothesized, however, that the male might use the female cuticular hydrocarbons as a secondary gender recognition cue after he had been exposed to female pheromone. Such seems to be the case as is clear from the amount of time males spent investigating artificial cocoons treated with female pheromone and cuticular hydrocarbon (Fig. 6). I suspect that in the case of live females, the ability of the male to use the female cuticular hydrocarbons as a final gender recognition cue is even more dramatic. Experiments are in progress to test this hypothesis.

Clearly much remains to be learned about the physiological and semiochemical functions of the cuticular hydrocarbons of parasitoids. In many parasitoids, males and females possess what appear to be sex-specific hydrocarbon profiles, but until now behavioral evidence that males can make the gender-based distinction using these cues has been lacking. Because cuticular hydrocarbons are usually thought to be rather nonvolatile, these chemicals may be serving not as long-range volatile pheromones but as short-range secondary cues to reaffirm that the male (or female) has made the correct choice in the mating sequence. Likewise, physiological studies on the roles of cuticular hydrocarbons in parasitoids are sparse. The small size of these organisms makes them difficult to work with, but that very smallness probably increases the importance of cuticular hydrocarbons as barriers to environmental stresses. Female *C. tarsalis* cuticular hydrocarbons make up almost 3% of their wet biomass, a substantial amount indeed compared with many larger insects. Will they show abundance changes to other environmental or ecological factors, as occurs in their host, the sawtoothed grain beetle (Howard et al. 1995)? Only a very small proportion of the parasitoids of the world have been examined, and undoubtedly many unusual and interesting phenomena related to the myriad of ecological niches that these organisms fill remain to be discovered. Knowledge of these phenomena will greatly increase our understanding of the biological roles of hydrocarbons among arthropods.

Acknowledgments

I thank M. Charlton for skilled technical assistance. I also thank J. Baker, G. Blomquist, C. D. Howard, and L. Jackson for critically reviewing an early draft of the manuscript.

References Cited

- Abraham, Y. J., D. Moore, and G. Godwin. 1990. Rearing and aspects of biology of *Cephalonomia stephanoderis* and *Prorops nasuta* (Hymenoptera: Bethyilidae) parasitoids of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Bull. Entomol. Res.* 80: 121-128.
- Baker, P. S. 1984. Some aspects of the behaviour of the coffee berry borer in relation to its control in Southern Mexico. *Folia Entomol. Mex.* 61: 9-24.
- Baker, P. S., and J. F. Barrera. 1993. A field study of a population of coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae), in Chiapas, Mexico. *Trop. Agric.* 70: 351-355.
- Barrera, J. F., J. Gomez, F. Infante, A. Castillo, and W. de la Rosa. 1989. Biologie de *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethyilidae) en laboratoire. I. Cycle biologique, capacité d'oviposition et emergence du fruit du caféier. *Cafe Cacao* The 33: 101-108.
- Bartlett, B. R. 1964. Patterns in the host-feeding habit of adult parasitic Hymenoptera. *Ann. Entomol. Soc. Am.* 57: 344-350.
- Chino, H. 1985. Lipid transport: biochemistry of hemolymph lipophorin, pp. 115-135. In G. A. Kerkut and L. I. Gilbert [eds.], *Comprehensive insect physiology, biochemistry and pharmacology*, vol. 10. Pergamon, Oxford.
- Collier, T. R. 1995. Host-feeding, egg maturation, resorption, and longevity in the parasitoid *Aphytis melinus*. *Ann. Entomol. Soc. Am.* 88: 206-214.
- Evans, H. E. 1964. A synopsis of the American Bethyilidae (Hymenoptera, Aculeata). *Mus. Comp. Zool. (Harv. Univ.) Bull.* 132.
- Finlayson, L. H. 1950. The biology of *Cephalonomia waterstoni* Gahan (Hym., Bethyilidae), a parasite of *Laemophloeus* (Col., Cucujidae). *Bull. Entomol. Res.* 41: 79-97.
- Flinn, P. W., and D. W. Hagstrum. 1995. Simulation model of *Cephalonomia waterstoni* (Hymenoptera: Bethyilidae) parasitizing the rusty grain beetle (Coleoptera: Cucujidae). *Environ. Entomol.* 24: 1608-1615.
- Flinn, P. W., D. W. Hagstrum, and W. H. McGaughey. 1996. Suppression of beetles in stored wheat by augmentative releases of parasitic wasps. *Environ. Entomol.* 25: 505-511.
- Francis, G. W., and K. Veland. 1981. Alkylthiolation for the determination of double-bond positions in linear alkenes. *J. Chromatogr.* 219: 379-384.
- Heimpel, G. E., and T. R. Collier. 1996. The evolution of host-feeding behaviour in insect parasitoids. *Biol. Rev.* 71: 373-400.
- Howard, R. W. 1992. Comparative analysis of cuticular hydrocarbons from the ectoparasitoids *Cephalonomia waterstoni* and *Laelius utilis* (Hymenoptera: Bethyilidae) and their respective hosts, *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) and *Trogoderma variable* (Coleoptera: Dermestidae). *Ann. Entomol. Soc. Am.* 85: 317-325.
1993. Cuticular hydrocarbons and chemical communication, pp. 179-226. In D. W. Stanley-Samuels and D. R. Nelson [eds.], *Insect lipids: chemistry, biochemistry and biology*. University of Nebraska Press, Lincoln.
- Howard, R. W., and R. D. Akre. 1995. Propaganda, crypsis and slavemaking, pp. 364-424. In W. J. Bell and R. T. Cardé [eds.], *Chemical ecology of insects II*. Chapman & Hall, New York.
- Howard, R. W., and P. W. Flinn. 1990. Larval trails of *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) as kairomonal host-finding cues for the parasitoid *Cephalonomia waterstoni* (Hymenoptera: Bethyilidae). *Ann. Entomol. Soc. Am.* 83: 239-245.
- Howard, R. W., and F. Infante. 1996. Cuticular hydrocarbons of the host-specific ectoparasitoid *Cephalonomia stephanoderis* (Hymenoptera: Bethyilidae) and its host the coffee berry borer (Coleoptera: Scolytidae). *Ann. Entomol. Soc. Am.* 89: 700-709.
- Howard, R. W., C. A. McDaniel, and G. J. Blomquist. 1978. Cuticular hydrocarbons of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). *J. Chem. Ecol.* 4: 233-245.
- Howard, R. W., C. D. Howard, and S. Colquhoun. 1995. Ontogenetic and environmentally induced changes in cuticular hydrocarbons of *Oryzaephilus surinamensis* (Coleoptera: Cucujidae). *Ann. Entomol. Soc. Am.* 88: 485-495.
- Jackson, L. L., and G. J. Blomquist. 1976. Insect waxes, pp. 201-233. In P. E. Kolattukudy [ed.], *Chemistry and biochemistry of natural waxes*. Elsevier, Amsterdam.

- Jervis, M. A., and N. A. C. Kidd. 1986. Host-feeding strategies in hymenopteran parasitoids. *Biol. Rev.* 61: 395-434.
- Katase, H., and H. Chino. 1982. Transport of hydrocarbons by the lipophorin of insect hemolymph. *Biochem. Biophys. Acta* 710: 341-348.
- Lockey, K. H. 1988. Lipids of the insect cuticle: origin, composition and function. *Comp. Biochem. Physiol. B* 89: 595-645.
- Mertins, J. W. 1985. *Laelius utilis* [Hym.: Bethyridae], a parasitoid of *Anthrenus fuscus* [Col.: Dermestidae]. *Entomophaga* 30: 65-68.
- Nakanishi, K. 1962. Infrared absorption spectroscopy. Holden-Day, San Francisco, CA.
- Nelson, D. R. 1978. Long-chain methyl-branched hydrocarbons: occurrence, biosynthesis and function. *Adv. Insect Physiol.* 13: 1-33.
- Powell, D. 1938. The biology of *Cephalonomia tarsalis* (Ash.), a vespid wasp (Bethyridae: Hymenoptera) parasitic on the sawtoothed grain beetle. *Ann. Entomol. Soc. Am.* 31: 44-48.
- Rilett, R. O. 1949. The biology of *Cephalonomia waterstoni* Gahan. *Can. J. Res. Sect. D Zool. Sci.* 27: 93-111.
- Schal, C., X. Gu, E. L. Burns, and G. J. Blomquist. 1994. Patterns of biosynthesis and accumulation of hydrocarbons and contact sex pheromone in the female German cockroach, *Blattella germanica*. *Arch. Insect Biochem. Physiol.* 25: 375-391.
- Van der Horst, D. J., P. M. M. Weers, and W. J. A. Van Marrewijk. 1993. Lipoproteins and lipid transport, pp. 1-24. In D. W. Stanley-Samuelson and D. R. Nelson [eds.], *Insect lipids: chemistry, biochemistry and biology*. University of Nebraska Press, Lincoln.
- van Lenteren, J. C., A. van Vianen, H. F. Gast, and A. Kortenhoff. 1987. The parasite-host relationship between *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). XVI. Food effects on oögenesis, lifespan and fecundity of *Encarsia formosa* and other Hymenopterous parasites. *J. Appl. Entomol.* 103: 69-84.
- Woodring, J. P. 1985. Circulatory systems, pp. 5-58. In M. S. Blum [ed.], *Fundamentals of insect physiology*. Wiley, New York.

Received for publication 3 June 1997; accepted 4 August 1997.
