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RELATIONSHIP BETWEEN TISSUE-SPECIFIC HYDROCARBON PROFILES AND LIPID MELTING TEMPERATURES IN THE COCKROACH *Blattella germanica*

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Abstract—Hydrocarbons (HC) are the most important waterproofing barrier on the cuticle of most terrestrial insects. Yet, the relationships among the type, amount, biophysical properties, and water retardation capacity of constituent HC are poorly understood. Melting temperatures and gas chromatographic profiles of HC of German cockroach tissues of various ages and stages were compared. The melting temperature (T_m) of oothecal HC was highest, T_m of epicuticular HC was substantially lower, and that of hemolymph HC was lowest. The epicuticular HC of older nymphs and adults had higher T_m than HC of the same sex and stage soon after the molt. The HC of females had higher T_m than did male HC. Principal components analysis suggested that normal and 3- and 5-methylalkanes, which were more prevalent on the epicuticle, were associated with higher T_m, implicating these components of the HC blend in waterproofing roles. The cockroach ootheca is particularly well protected by an abundance of *n*-alkanes and its external HC exhibit the highest T_m of any HC blend. The methyl ketone sex pheromone components, which are derived from HC, appear to only slightly reduce the T_m of the epicuticular HC, probably because the methyl ketones represent only 1.12% of the mass of epicuticular HC. We suggest that the evolution of polar epicuticular chemical signals may be constrained by their tendency to increase water transpiration.

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Key Words—*Blattella*, German cockroach, hydrocarbon, cuticle, melting temperature, pheromone, water relations.

INTRODUCTION

Epicuticular lipids have a central role in waterproofing the cuticle of terrestrial arthropods (Nelson and Blomquist, 1995). Hydrocarbons (HC) are usually the most abundant cuticular lipid, and their role has been intensely studied, especially in insects and arachnids adapted to xeric habitats; both the gross quantity of HC as well as the relative abundance of various HC components varies with habitat and season (Hadley, 1977, 1978; Hadley and Schultz, 1987; Toolson and Hadley, 1977, 1979). These effects have been noted both within species (Toolson, 1982; Markow and Toolson, 1990; Gibbs and Mousseau, 1994) and across species within genera or families (Hadley, 1977, 1978; Hadley and Schultz, 1987; Toolson and Hadley, 1979, 1977).

The probable roles of HC of various chain lengths, methyl-branching patterns, and degrees of saturation have been deduced from their relative abundance in arthropods from various habitats. Cuticular permeability increases rapidly above a species-dependent transition or critical, temperature (Ramsay, 1935; Wigglesworth, 1945), which is caused by the melting of the epicuticular lipid barrier (Gibbs, 1998; Rourke and Gibbs, 1999). Changes in HC structure that should increase melting tempertaures, such as longer chain lengths and reduced branching and unsaturation, are associated with more xeric environments. Only recently, however, has work been done to more directly determine the effect that individual components of the HC blend have on the melting temperature (T_m) of the native HC blend and presumably, upon the waterproofing ability of the epicuticular HC. The effects on T_m of HC chain length, the presence and location of methyl branches, and the presence or absence of double bonds have been revealed by Fourier transform infrared spectrometry (FTIR) on artificial blends of varied proportions of known HC (Gibbs, 1995; Gibbs and Pomonis, 1995). Increasing chain length results in higher melting temperatures, and introducing methyl branches lowers T_m, as does shifting the location of the methyl branch from the end of the chain towards the interior of the molecule. The T_m of a simple blend of two alkane components with different T_m is the proportionally weighted average of their respective T_m but alkane–alkene blends have higher T_m values (Gibbs and Pomonis, 1995).

Cuticular HC also serve as important chemical mediators of interactions among insects and between insects and plants (Howard, 1993; Eigenbrode and Espelie, 1995), in many cases as part of a blend that includes oxygenated derivatives of HC (Blomquist et al., 1993; Nelson and Blomquist, 1995). Polar lipids tend to disrupt the orderly arrangement of HC, and their sex- and stage-spe-

cific production and accumulation on the epicuticle might compromise the waterproofing capacity of HC. The interaction between epicuticular HC and HC sex pheromones has been studied in only two insect species, *Drosophila mojavensis* and *Musca domestica* (Markow and Toolson, 1990; Gibbs et al., 1995, 1998).

The German cockroach is a good model for evaluating the effects of varied HC profiles found on or in ecologically relevant tissues. Epicuticular HC differ at various ages, between the adult sexes, and HC coating the ootheca vary even more; furthermore, hemolymph lipophorin carries a rather large load of HC, yet is not in intimate contact with the environment (Schal et al., 1998b). As an experimental system, *B. germanica* is amenable to T_m and gas–liquid chromatographic (GLC) analyses and dissection of the HC profile for three reasons: The HC are well characterized (Augustynowicz et al., 1987; Carlson and Brenner, 1988; Jurenka et al., 1989) and are somewhat varied in both chain length and branching pattern, yet not so varied as to be too complex. Secondly, the German cockroach has only alkanes, which have more predictable physical properties than unsaturated alkenes (Gibbs, 1995; Gibbs and Pomonis, 1995), which are absent. Thirdly, the female German cockroach produces oxygenated sex pheromone components derived from 3,11-dimethylnonacosane and 3,11-dimethylheptacosane (Nishida and Fukami, 1983; Schal et al., 1990).

In this research we have examined the roles played by the major HC components relative to the melting temperature (and presumably the waterproofing capacity), the implications of their presence in various external and internal tissues, and the effect of epicuticular methyl ketone sex pheromone components on the biophysical properties of the HC.

METHODS AND MATERIALS

Insects. Insects were from an American Cyanamid insecticide-susceptible strain raised in glass battery jars at 27°C and 50% relative humidity on a 12L : 12D cycle. Insects were fed Purina Rat Chow #5012 (Purina Mills, St. Louis, Missouri) and water ab libitum. All insects were staged to within 2 hr after-ecdysis in mid-photophase and they did not eat their exuviae. Ten insects were placed in clean 150- × 25-mm plastic Petri dishes with water and chow and reared to the appropriate ages.

Chemicals. Chemicals were from Sigma (St. Louis, Missouri), Bio-Rad (Richmond, California), or Fisher Scientific (Pittsburgh, Pennsylvania).

Extraction of Lipids for Analysis. All insects were killed by freezing at −20°C overnight. External lipids were removed from the epicuticle by immersing thawed insects in 2 ml of *n*-hexane, mixing gently 5 min, decanting the solvent, repeating, then rinsing the vial and insects with 1 ml hexane. Hemolymph was taken from clipped cerci of CO_2-anesthetized insects with a 5-μl glass cap-

illary and extracted by a modified Bligh and Dyer (1959) method (Young and Schal, 1997). Epicuticular and hemolymph extractions effectively removed 98% and 99%, respectively, of the available HC (Young and Schal, 1997).

Extracts were evaporated under N_2, taken up in hexane, loaded onto silica gel (Biosil-A, Bio-Rad) minicolumns, and the HC fraction eluted with 7 ml hexane. The methyl ketone fraction of the epicuticular extract of 7-day-old virgin females was then eluted with 7 ml of 5% diethyl ether in hexane. Fractions were dried under N_2 and injected in hexane into a splitless injector leading to a 25-m × 0.32-mm × 1-μm Hewlett-Packard (Avondale, Pennsylvania) HP-1 capillary column in a HP5890II GLC interfaced with a HP3365II Chemstation. The column was held at 150°C for 2 min, then heated at 10°C/min to 245°C, then at 1°C/min to 260°C, and finally, at 10°C/min to 280°C and held for 5 min. Injector and flame-ionization detector were at 280°C and 300°C, respectively.

Fourier-Transform Infrared Spectroscopy. The melting temperatures of samples extracted and purified as above were determined with a Perkin-Elmer Systems 2000 FTIR spectrometer by the method of Gibbs and Crowe (1991). This technique uses the frequency of —CH_2— symmetric stretching vibrations as an indicator of lipid melting. Samples (5–100 μg) were placed in a CaF_2 cell, and the temperature was raised in increments of 2°C from well below to well above the melting range, with five infrared spectra being collected and averaged at each temperature. Vibrational frequencies increased from ~2850 cm^{-1} to ~2854 cm^{-1} and were determined with instrument software to a precision of 0.01 cm^{-1}.

Plots of frequency vs. tempertaure were fitted by logistic regression, and four parameters were calculated. The melting point (T_m) is defined as the midpoint of the melting transition, where half of the lipid is in a fluid state. The temperatures at which the lipids were 5% and 95% melted ($T_{0.05}$ and $T_{0.95}$, respectively) were also determined from these curves. The temperature range over which melting occurred (ΔT) was calculated as the difference between $T_{0.05}$ and $T_{0.95}$.

Statistical Analyses. The peak areas as determined by the GLC data-logging program were converted to relative percentages, and the replicates of each treatment were averaged and standard errors determined. Statistical analyses were performed by either Microsoft Excel 98 or SAS StatView version 5.0. Hydrocarbons occurring at 1% or more of the total HC fraction were used for principal component analysis and compared with a two-tailed, unpaired Student's t test. Each treatment was replicated three times with 10 insects or oothecae in each replicate. All data are presented as means ± SEM.

RESULTS

Chromatographic HC Profiles. Table 1 and Figure 1 show the 20 HC analyzed in this study and identified in subsequent figures and text by the peak number assigned them by Jurenka et al. (1989). The locations of the methyl branches

TABLE 1. HYDROCARBONS OF *Blattella germanica*

Peak number[a]	Hydrocarbons	Branch sites[b]
1	*n*-heptacosane	none
2	11- and 13-methylheptacosane	middle
3	5-methylheptacosane	end
4	11,15-dimethylheptacosane	middle, middle
5	3-methylheptacosane	end
6	5,9- and 5,11-dimethylheptacosane	end, middle
7	*n*-octacosane	none
8	3,9- and 3,11-dimethylheptacosane	end, middle
9	12- and 14-methyloctacosane	middle, middle
10	2-methyloctacosane	end
11	4-methyloctacosane	end
13	*n*-nonacosane	none
15	9-, 11-, 13-, and 15-methylnonacosane	middle
16	7-methylnonacosane	middle
17	5-methylnonacosane	end
18	11,15- and 13,17-dimethylnonacosane	middle, middle
20	3-methylnonacosane	end
21	5,9- and 5,11-dimethylnonacosane	end, middle
22	3,7-, 3,9-, and 3,11-dimethylnonacosane	end, middle
24	11-, 13-, and 15-methyltriacontane	middle

[a] The 20 peaks are as identified and numbered by Jurenka et al. (1989) and comprise >95% of the HC mass.

[b] In our results HC with methyl branches at carbon 5 behaved as though they were end branches, although they are usually classified as being sufficiently far from the chain terminus as to be internal.

are also given; one should note that carbon 5 is herein described as being at the end of the carbon backbone, although it is more usual to classify only carbons 2, 3, and 4 as being end-chain. Other results suggest that the biophysical properties of a methyl branch at the 5 position are more similar to an end branch than a mid-chain branch. The nymphal cuticle profile shown (Figure 1) is from nymphs 10 days after ecdysis (2 days before adult eclosion). The male cuticular chromatogram was from 1-day-old adult males, and the female epicuticle and hemolymph were from 25-day-old adults. The egg case chromatograms are from the oothecae of those females at 25 days after eclosion.

Principal Components Analysis. The relative peak areas of the 20 most abundant HC, which constituted over 95% of all HC, for all tissues, physiological ages, and sexes, were subjected to principal components analysis (Figure 2). The first component separated the HC into two distinctly separate groups: (1) The positively-loaded *n*-alkanes and (2) the monomethyl-branched alkanes having the methyl group near the end of the carbon chain on carbons 2–4. Associating with these relatively straight-chain HC were the melting temperature param-

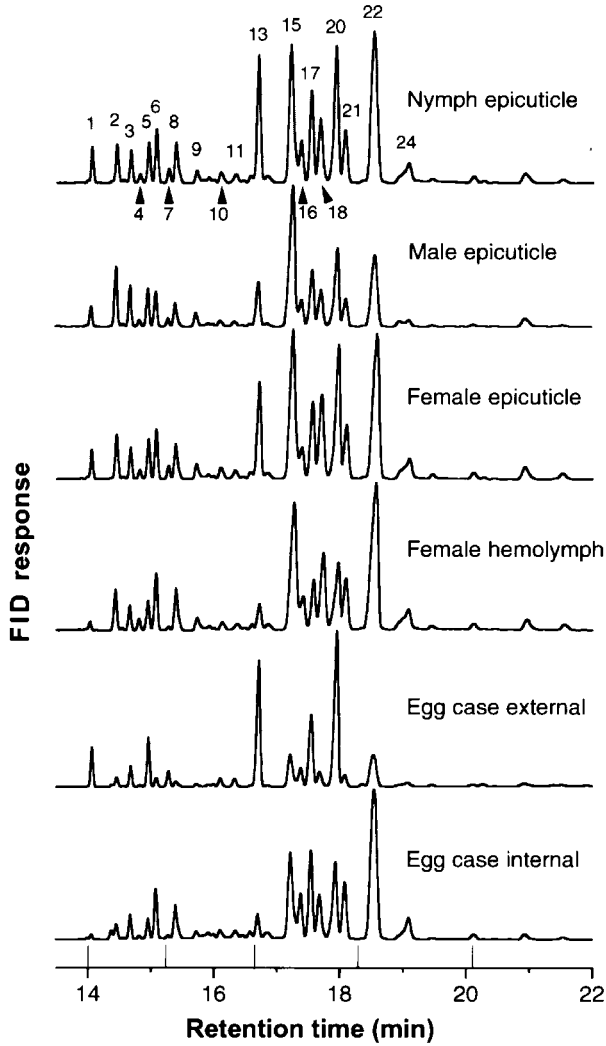

FIG. 1. Representative gas–liquid chromatograms of hydrocarbons extracted from *Blattella germanica*. Hydrocarbons shown are from the epicuticle of a 10-day-old female nymph, a 25-day-old adult male and female, the hemolymph of a 7-day-old adult female, and from the exterior and interior of the egg case of a 25-day-old adult female. The vertical lines above the *x* axis represent retention times for *n*-C27, *n*-C28, *n*-C29, *n*-C30, and *n*-C31, respectively, from left to right. Peak numbers refer to the HC identified in Table 1.

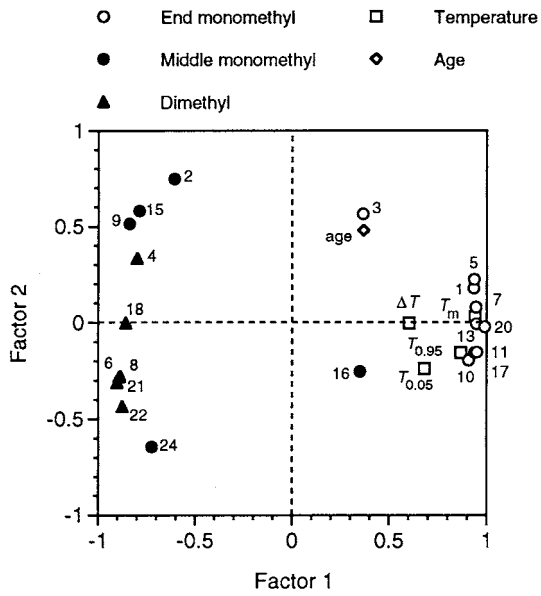

FIG. 2. Principal components analysis of the relative peak areas of the 20 most abundant HC, which constituted over 95% of all HC, for all tissues, physiological ages, and sexes, and the biophysical parameters T_m, $T_{0.05}$, $T_{0.95}$, and ΔT.

eters T_m, $T_{0.05}$, $T_{0.95}$, and ΔT. Slightly separated from this group were HC 3 and 16, with 5- and 7-monomethyl branches. The other major grouping of HC, with negative loadings, was of HC having a methyl group near the middle of the carbon backbone. Factor 2 separated this latter group into monomethyl- and dimethyl-branched alkane groups. Factor 2 had little effect on the normal and end-branched monomethyl alkanes.

Effect of Age in Nymphs and Adults. The relative amounts of HC on the epicuticle of female nymphs at days 4 and 10 after-ecdysis reflect the effects of aging in nonreproductive insects (Figure 3). *n*-Nonacosane (peak 13), and 3-methylnonacosane (peak 20) were more prevalent in older females, and the total amount of *n*-alkanes almost doubled in the same period. Concomitantly, a significant decline was evident in peak 22 (dimethylnonacosanes). Epicuticular T_m thus increased significantly by 4°C over this six-day interval (Table 2), and the gain was spread evenly over the entire melting range (ΔT), which remained constant.

A comparison of the epicuticular HC of 1-, 10-, and 25-day-old adult females showed the effects of age on HC profile and FTIR parameters. The largest effect was an increase in the abundance of 3-methylnonacosane (peak 20)

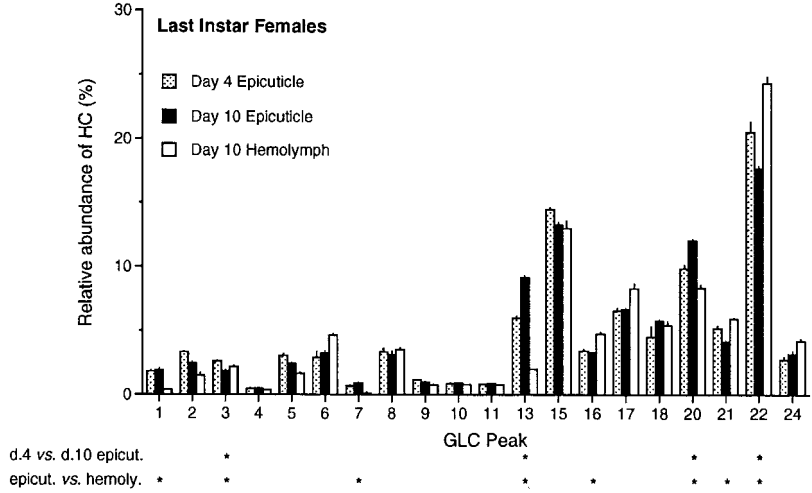

FIG. 3. The relative abundance of HC on the epicuticle of 4-day-old and 10-day-old last-instar females and in the hemolymph (lipophorin-bound) of the same 10-day-old females. $N = 3$ groups of 10 insects each. $^{*}P < 0.001$ in a comparison of day 4 to day 10 epicuticular HC (unpaired t test), and in a comparison of day 10 epicuticular HC and hemolymph HC from the same females (paired t test).

and a decrease in peak 22 (dimethylnonacosanes), as in nymphs (Figure 4). The relative abundance of peak 15, the internally branched monomethylnonacosanes, also increased significantly with age. The effect of aging, sexual maturation, and environmental exposure on the melting characteristics of HC were more modest

TABLE 2. MELTING TEMPERATURE PARAMETERS OF HC FROM EPICUTICLE AND HEMOLYMPH OF DAY 4 AND 10 LAST-INSTAR FEMALES[a]

FTIR parameter (°C)	Day 4 epicuticle	Day 10 epicuticle[b]	Day 4 Hemolymph[c]	Day 10 Hemolymph[d]	Day 10 epicuticle vs hemolymph[e]
T_m	33.81 ± 0.59	37.78 ± 0.26**	27.18 ± 0.47**	27.22 ± 0.62 NS	***
$T_{0.05}$	25.86 ± 0.36	29.73 ± 0.18**	19.38 ± 0.46***	18.43 ± 0.66 NS	***
$T_{0.95}$	44.22 ± 1.36	48.02 ± 0.81 NS	38.13 ± 0.47*	40.22 ± 0.40*	**
ΔT	18.36 ± 1.36	18.29 ± 0.92 NS	18.75 ± 0.25 NS	21.79 ± 0.28**	*

[a] NS: $P > 0.05$; $^{*}0.01 < P < 0.05$; $^{**}0.001 < P < 0.01$; $^{***}P < 0.001$.
[b] Comparison (unpaired t test) of epicuticular HC of day 4 and day 10.
[c] Comparison (paired t test) of epicuticular and hemolymph HC of day 4 nymphs.
[d] Comparison (unpaired t test) of hemolymph HC of day 4 and day 10.
[e] Comparison (paired t test) of epicuticular and hemolymph HC of day 10 nymphs.

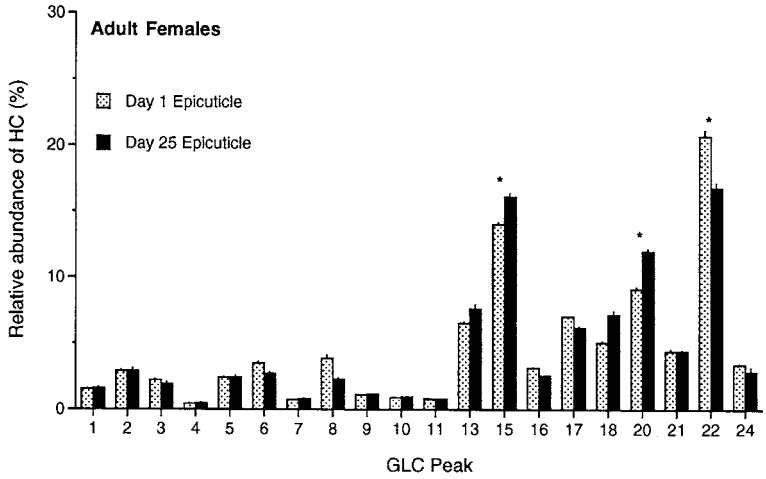

FIG. 4. The relative abundance of HC on the epicuticle of 1-day-old and 25-day-old adult females. $N = 3$ groups of 10 insects each. *denotes $P < 0.001$ (unpaired t test).

than seen in nymphs, T_m increasing by only 1.6°C ($P = 0.014$) over 24 days (Table 3). This modest increase was attributed to an increase in $T_{0.95}$, resulting is a greater ΔT.

In adult males the epicuticular HC changed little between days 1 and 25 (not shown). A slight decline in T_m and $T_{0.05}$ between days 1 and 10 was followed by a correction by day 25 (Table 4).

Effect of Sex. Male and female epicuticular HC were compared 1, 10, and 25 days after-eclosion, at which age both sexes were sexually mature and females carried an ootheca (which was not included in the extraction). On day 25, females had more *n*-alkane (peak 13) and dimethylnonacosanes (peak 22) than did males; the latter peak includes the sex pheromone precursor 3,11-dimethylnonacosane (Figure 5). Males, in turn, had much more HC peak 15, a mid-chain monomethylnonacosane. Female HC also exhibited a higher T_m than did male HC (by 3.2°C, $P = 0.0016$), as a result of increases in both $T_{0.05}$ and $T_{0.95}$, but no difference in ΔT (Tables 3 and 4).

Effect of Epicuticular Sex Pheromone. Coincident with vitellogenesis, adult females produce a courtship-inducing contact sex pheromone, composed of methyl ketone derivatives of 3,11-dimethylheptacosane and 3,11-dimethylnonacosane (Nishida and Fukami, 1983, Schal et al., 1990). 3,11-Dimethylnonacosan-2-one, the most abundant component attains a mass of 1.5 μg on the epicuticular surface, while total HC reach ca. 170 μg (Schal et al., 1994). We sought to determine whether the methyl ketones compromised the waterproofing properties of the HC in which they are presumably dissolved. The melting temperature

TABLE 3. MELTING TEMPERATURE PARAMETERS OF HC FROM EPICUTICLE AND OOTHECAE OF DAY 1, 10, AND 25 ADULT FEMALES[a]

FTIR parameters (°C)	Day 1 epicuticle	Day 10 epicuticle[b]	Day 25 epicuticle[c]	Day 10 ootheca[d]	Day 25 ootheca[e]	Day 10 vs. day 25 oothecae
T_m	33.92 ± 0.27	33.32 ± 1.19 NS	35.49 ± 0.37*	48.74 ± 0.28***	45.11 ± 0.60***	**
$T_{0.05}$	26.61 ± 0.32	25.45 ± 0.99 NS	27.92 ± 0.45 NS	40.20 ± 0.75***	30.04 ± 0.62*	***
$T_{0.95}$	43.25 ± 0.23	43.63 ± 1.43 NS	45.11 ± 0.44*	59.14 ± 1.32***	67.88 ± 3.07**	NS
ΔT	16.64 ± 0.23	18.17 ± 0.49*	17.19 ± 0.54 NS	18.95 ± 2.01 NS	37.83 ± 3.66**	*

[a] NS: $P > 0.05$; $^{*}0.01 < P < 0.05$; $^{**}0.001 < P < 0.01$; $^{***}P < 0.001$.
[b] Comparison (unpaired t test) of epicuticles of day 1 and day 10.
[c] Comparison (unpaired t test) of epicuticles of day 1 and day 25.
[d] Comparison (paired t test) of epicuticle and oothecal surface of day 10 females.
[e] Comparison (paired t test) of epicuticle and oothecal surface of day 25 females.

TABLE 4. MELTING TEMPERATURE PARAMETERS OF HC FROM EPICUTICLE OF DAY 1, 10, AND 25 ADULT MALES[a]

FTIR parameters (°C)	Day 1 epicuticle	Day 10 epicuticle[b]	Day 25 epicuticle[c]	Day 1 male vs. female	Day 10 male vs. female	Day 25 male vs. female
T_m	33.96 ± 0.82	31.16 ± 0.28*	32.32 ± 0.34 NS	NS	NS	**
$T_{0.05}$	26.42 ± 0.86	23.08 ± 0.55*	24.73 ± 0.01 NS	NS	NS	**
$T_{0.95}$	43.65 ± 0.69	42.11 ± 1.02 NS	42.25 ± 0.89 NS	NS	NS	*
ΔT	17.23 ± 0.18	19.03 ± 1.48 NS	17.52 ± 0.90 NS	NS	NS	NS

[a] NS: $P > 0.05$; *$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.001$.
[b] Comparison (unpaired t test) of epicuticles of day 1 and day 10.
[c] Comparison (unpaired t test) of epicuticles of day 1 and day 25.

of the methyl ketone fraction was not significantly ($P = 0.099$) lower than that of HC (Table 5). Interestingly, however, $T_{0.05}$ of the methyl ketones was 10°C lower ($P = 0.011$), and $T_{0.95}$ was 23°C higher ($P = 0.026$) than the respective values for HC, resulting in a ΔT of methyl ketones that was 33°C greater than the melting range of HC ($P = 0.020$). Because the methyl ketone fraction rep-

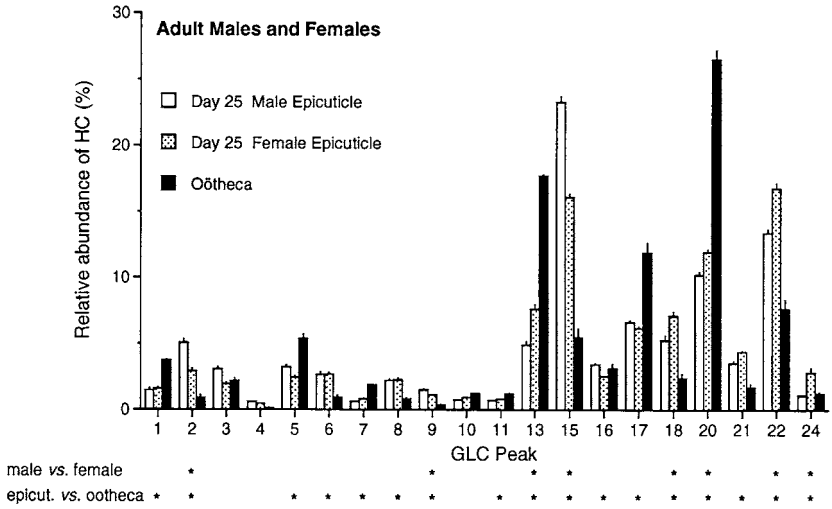

FIG. 5. The relative abundance of HC on the epicuticle of 25-day-old adult males and females and on the surface of the oothecae of the same 25 day-old females. $N = 3$ groups of 10 insects each. *$P < 0.001$ in a comparison of day 25 male and female epicuticular HC (unpaired t test), and in a comparison of day 25 female epicuticular HC and oothecal HC from the same females (paired t test).

TABLE 5. MELTING TEMPERATURE PARAMETERS OF HC AND METHYL KETONES FROM EPICUTICLE OF DAY 7 ADULT FEMALES[a]

FTIR parameters (°C)	Hydrocarbons (HC)	Methyl ketones (MK)[b]	Hydrocarbons and methylketones (HC + MK)[c]
T_m	33.88 ± 0.17	32.98 ± 0.43 NS	34.35 ± 0.04 NS
$T_{0.05}$	26.66 ± 0.29	16.75 ± 2.32*	27.21 ± 0.05 NS
$T_{0.95}$	43.06 ± 0.04	65.97 ± 7.45*	43.35 ± 0.17 NS
ΔT	16.39 ± 0.33	49.22 ± 9.76*	16.14 ± 0.22 NS

[a] NS: $P > 0.05$; *$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.001$.
[b] Comparison (unpaired t test) of HC vs. MK.
[c] Comparison (unpaired t test) of HC vs. HC + MK.

resented approximately 1.12% of the epicuticular lipids (Schal et al., 1994), all four melting temperature parameters of HC did not change ($P > 0.05$) when the HC and methyl ketones were recombined in the ratio at which they normally occurred on the epicuticle. Thus, it appears that, at physiological amounts, the sex pheromone does not interfere with the physical characteristics of HC and, hence, water relations.

Transport of Hydrocarbons in Nymphs and Adults. We compared the relative abundance of HC on the epicuticle and in the hemolymph of 10-day-old last-instar females (Figure 3). All hemolymph HC in nymphs was lipophorin-bound (Young et al., 1999). The abundance of six HC peaks differed between the two sites: Normal (peaks 1, 13) and 3-monomethyl (peak 20) HC were more prominent on the epicuticle, whereas peaks 16, 21, and 22 (7-methyl and 3,x- and 5,x-dimethyl-branched HC) were more represented in the hemolymph. FTIR data (Table 2) showed that as the T_m of epicuticular HC increased between days 4 and 10 ($P = 0.004$), the T_m of hemolymph HC remained unchanged ($P = 0.958$). Thus, a 6.6°C higher T_m for epicuticular HC than for hemolymph HC on day 4 ($P = 0.001$) increased to a difference of 10.5°C six days later ($P < 0.001$). The higher melting temperature of epicuticular HC was achieved by raising both the lower and upper ends of the melting range, while keeping the melting range unchanged. The alkanes indicated in Figure 3 as more abundant on the epicuticle (and less so in the blood) are associated with higher melting temperatures (Gibbs, 1995; Gibbs and Pomonis, 1995).

The Ootheca. The ootheca, which shelters the developing embryos outside of the mother's body for 20 days, had the most unusual HC profile and biophysical characteristics of all HC blends we examined. A comparison of the relative abundance of external oothecal HC and the epicuticular HC of the 25-day-old mother showed that 13 of the 20 peaks differed between mothers and oothecae (Figure 5). n-Alkanes (peaks 1, 7, and 13) and 3-methyl- (peaks 5 and 20), and

5-methyl-branched (peak 17) alkanes were much more common on the surface of oothecae. Conversely, epicuticular HC exhibited greater relative amounts of mid-chain monomethyl alkanes (peaks 2, 9, and 15), mid-chain dimethyl alkanes (peak 18), and dimethylnonacosanes (peaks 21 and 22). The HC in the interior of the egg case, within the developing embryos, were similar to the epicuticular HC of the mother, and not to the external surface of the ootheca (Figure 1).

The melting temperature of oothecal HC was 15.4°C higher than that of epicuticular HC on day 10 ($P < 0.001$) and 10°C higher on day 25 ($P < 0.001$) (Table 3). Most of these gains were made at the upper end of the melting temperature range, $T_{0.95}$, resulting in a ΔT that was more than double that of epicuticular HC on day 25 ($P = 0.003$).

DISCUSSION

Features of the microhabitat, including wind velocity, air temperature, and humidity affect the rate of evaporative water loss from the cuticle. A transition, or critical, temperature defines a point at which there is an abrupt increase in permeability of the cuticle, and this temperature correlates with the microhabitat of the insect (Ramsay, 1935; Wigglesworth, 1945). Recent results have provided compelling evidence that the transition temperature is related to a phase change in the epicuticular lipids, which form the main permeability barrier (Noble-Nesbitt, 1991; Gibbs, 1998; Rourke and Gibbs, 1999). The physicochemical phase change is defined by T_m, the melting temperature. The steepness or smoothness of this change is represented by ΔT, the range over which HC melt. This range is determined by the degree of homogeneity (low ΔT) or heterogeneity (high ΔT) of the epicuticular lipids. Because waterproofing depends on the molecular packing of epicuticular lipids, greater water retardation is accomplished by greater amounts of long-chain, saturated compounds, with less branching and bending.

The epicuticle of *B. germanica* is covered by normal, mono-, and dimethyl-branched long-chain alkanes, which form an excellent water barrier. Indeed, the same types of HC predicted to have T_m-increasing effects by Gibbs and Pomonis (1995) appear to be elevated in tissues that require greater waterproofing. Normal and end-branched monomethyl HC are more abundant on the epicuticle than in internal tissues, on aged epicuticle, and on the oothecal exterior. The hemolymph is not only a pool of significant amounts of HC, but serves to transport HC to sites remote from the abdominal integument where HC are synthesized (Gu et al., 1995; Schal et al., 1998b; Young et al., 1999). The hemolymph is notably depleted of normal and end-branched monomethyl HC, and concomitantly has the lowest T_m.

Tissue-Specific HC Profiles. We examined two major changes in the external HC profiles of nymphs and adults. First, age affects the HC profile and melt-

ing characteristics in both life stages. Although it spends only 12 days in the last stadium, and will soon abandon the nymphal cuticle and the HC associated with it as a result of the imaginal molt (Young et al., 1997, 1999), the nymph appears to selectively deposit normal and end-methyl branched alkanes on its epicuticle. The age effect is still present, although less obvious, in adult females as well. Conversely, in older adult males 9-, 11-, 13-, and 15-methylnonacosanes (peak 15) increase substantially (not shown), and the melting temperature of their HC declines slightly with age.

Specific types of HC may be found on particular locations on the insect body (Howard, 1993; Nelson and Blomquist, 1995). The HC on the exterior of the ootheca of *B. germanica* have a dramatically different profile and different melting temperature values compared with the epicuticle of the same insect. The oothecal HC have the most marked increase in relative content of the normal and 3-methyl alkanes, known to have elevating effects on T_m (Gibbs, 1995; Gibbs and Pomonis, 1995). Interestingly, the oothecal HC are unique in having a broadened ΔT, the high-end ($T_{0.95}$) melting temperature being greatly increased without a corresponding increase in the melting-onset temperature, $T_{0.05}$, as is seen on the epicuticle.

The adaptive significance of efficient waterproofing of the ootheca is quite apparent. *Blattella germanica* females incubate the egg case for the total duration of embryogenesis, approximately 20 days (Roth and Stay, 1962; Schal et al., 1997). Whereas the embryos lose water during embryogenesis in oviparous cockroaches that abandon the egg case, in *B. germanica* the mother provisions the embryos with water through her genital vestibulum (Roth, 1967). Presumably, supplementary waterproofing, especially with *n*-alkanes, would reduce water loss for both embryos and mother. In this context, it is interesting to note that the T_m of the oothecal HC declines 3.5°C ($P = 0.005$) between day 10 (freshly oviposited) and day 25 (three to four days before hatch), while during the same period the T_m of the epicuticular HC of the mother increases 2°C. It is possible that the mother, who drinks sparingly during gestation (Cochran, 1983), maximizes the waterproofing of her cuticle. Conversely, embryos have added their own HC to those derived from the mother (Schal et al., 1998b; Schal, unpublished), developed a cuticle within the ootheca, and are about to abandon the ootheca, all of which are consistent with lack of adaptive changes in oothecal HC. Moreover, T_m is still >10°C above ambient, so the lipids remain in the solid phase.

How Are Tissue-Specific HC Profiles Generated? Based on the adaptive value of being able to direct waterproofing HC constituents to vulnerable regions, pheromones to tissues that are presented to conspecifics, and allomonal HC to other body parts, one would predict that physiological mechanisms would evolve to accomplish this. While postdeposition mechanisms, including grooming, selective abrasion, and selective reinternalization of HC, probably play significant roles, the appearance of differences even in young insects suggests that

this disparity may be due to a selective deposition of HC. HC are formed in internal tissues, either in association with the integument or in oenocytes in the hemocoel (Romer, 1991) and are shuttled by lipophorin to their final destination, which may include the epicuticle, specialized glands, the gonads, and the fat body (Schal et al., 1998b). It is thus possible that a common HC blend in the hemolymph is selectively unloaded, resulting in unique HC profiles on various tissues.

The hemolymph HC chromatographic profile is constant across the entire last stadium (not shown), while at precisely the same time the epicuticle is being supplied with a changing mixture of HC components. In the adult, too, while the epicuticle changes over time and the ootheca is coated with a unique HC blend, little change occurs in hemolymph HC (not shown). Differences in the HC profiles and their melting parameters between the hemolymph and epicuticle, as a function of age, and on the incubating ootheca, lend support to the idea that both nymphs and adults have precise regulation of the various HC blends on different body parts. We suggest that the HC transport system is able to differentiate not only between targets for deposition of lipids as diverse as sterols, diacylglycerols, phospholipids, and HC (Chino, 1985; Blacklock and Ryan, 1994), but also between components as similar as various HC constituents.

Recently, we found support for this idea in the termite *Zootermopsis nevadensis* (Sevala et al., 2000). Despite differing patterns of epicuticular HC, the hemolymph lipophorin-bound HC profile was nearly identical in soldiers, nymphs, and male and female reproductives. However, the clearest example of such tissue specialization of HC profiles is in the tiger moth, *Holomelina aurantiaca*. The adult female synthesizes short-chain HC that serve as volatile sex pheromone constituents and long-chain HC that are deposited on the epicuticle (Schal et al., 1998a). All HC are loaded onto a hemolymph high-density lipophorin, but only 2-methylheptadecane and related pheromone homologs of similar chain length are specifically deposited by lipophorin into tubular pheromone glands. The long-chain HC, on the other hand, appear on the epicuticular surface. In this insect, the epicuticular HC profile is enriched with long-chain compounds, whereas the pheromone gland is essentially devoid of these HC. The hemolymph HC represent both groups.

Do Sex Pheromone Constituents Interfere with Waterproofing Function of HC? The cuticular HC of adult female *B. germanica* serve as precursors for polar courtship-inducing contact sex pheromone components (Jurenka et al., 1989; Chase et al., 1992). Because interaction between the polar groups of the HC derivatives and water molecules could alter the intermolecular spacing and therefore the movement of water, we examined their melting temperature parameters. The methyl ketone pheromone components have significantly lower T_m than the combined cuticular HC and methyl ketones, and their phase transition is progressive over a range of 49°C compared with 16°C for HC. However,

because the pheromone blend constitutes only 1.12% of the epicuticular HC, it appears not to interefere with the water-retarding properties of the HC.

The effects of cuticular pheromones on water loss may be important in other species. (*Z*)-9-Tricosene, the sex pheromone of the house fly, *Musca domestica*, is relatively abundant on the female's epicuticle (Ahmad et al., 1989). The T_m of the fly's alkene fraction is significantly lower than that of the alkanes, and a significant decline in T_m occurs as females attain sexual maturity and produce more (*Z*)-9-tricosene and the methylalkanes that enhance its pheromonal activity (Gibbs et al., 1995). Similarly in *Drosophila*, Markow and Toolson (1990) showed that acclimation to higher temperatures reduced mating success, apparently through a trade-off in cuticular lipids.

On the other hand, Gibbs et al. (1998) found little or no correlation among rearing temperature, T_m, and water-loss rates of *D. mojavensis* raised under several different temperature regimes. Indeed, the entire issue of what, if any, lipid-melting criteria are the most appropriate proxies for actual water transpiration rates needs to be more thoroughly explored. In grasshoppers, *Melanoplus sanguinipes*, the transition point for water loss occurs when the surface lipids are ~1/3 melted (Rourke and Gibbs, 1999). As described above, the oothecal HC T_m is 10°C above the ambient tempertures usually chosen by *B. germanica*, and furthermore, the epicuticular HC $T_{0.05} \approx T_{\text{ambient}}$. In this case, 95% of the HC are still in the solid, crystalline phase at physiological temperatures. The fact that only partial melting results in increased water loss suggests that $T_{0.05}$ may be a more appropriate measure of lipid properties than T_m, defined as the point at which half of the HC have left the solid phase. This is one physical aspect of cuticular HC that needs to be further addressed. In addition, the fact that the T_m of the lipophorin-associated HC was at ambient temperature suggests that thermodynamic considerations of remaining in or moving freely in and out of the solid, crystalline phase are important in determining the role a given HC mixture can perform. The HC at the interface with the environment must form a barrier, while at the same temperature, the HC associated with lipophorin must be able to bind reversibly to the lipid transporter. The potential importance of lipid physical properties for lipophorin function remains unexplored.

It is possible that the evolution of semiochemicals may be constrained by their tendency to alter water relations. If so, insects could minimize the quantity of these compounds by maximizing their sensory signal-to-noise ratio, limit their deposition to only small regions of the body, or sequester them in specialized, often internal, glands with small openings to the exterior. Examples of these strategies are common among insects.

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