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# Genetic and acclimatory variation in biophysical properties of insect cuticle lipids

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**ABSTRACT** **Epicuticular lipids provide the primary barrier to water loss in insects and other terrestrial arthropods. Using Fourier transform infrared spectroscopy, we found that the melting temperatures for these lipids in the grasshopper *Melanoplus sanguinipes* varied by over 10°C between individuals. The most significant determinant of lipid melting temperature was geographic population, followed by family effects and rearing regime. The width of the phase transition also showed population and family effects. Differences in lipid phase properties were correlated with habitat temperature. Our results provide evidence for genetically based intraspecific variation in epicuticular lipids and have important implications for physiological studies of water balance in arthropods.**

A rapid increase in water loss above a certain "critical" temperature has led several investigators to conclude that phase transitions of epicuticular lipids are important in determining rates of evaporative water loss in terrestrial arthropods (1–5). According to this hypothesis, as the lipids melt they lose their waterproofing abilities. The biophysical properties of surface lipids necessarily reflect their chemical composition, which is mainly long-chain saturated hydrocarbons, as well as other hydrophobic compounds (6, 7). There are tremendous differences in lipid composition between species, and this variation presumably is due in part to genetic differences, an idea exploited in efforts to use surface lipid composition as a taxonomic character (8). Environmental conditions, particularly temperature, also affect lipid composition, and these changes are correlated with interspecific differences in organismal water balance (9, 10).

Surface lipid composition also varies within species, as a result of both genetic (11) and environmental (12, 13) factors. These changes have been used to infer differences in lipid properties, but the actual biophysical consequences of intraspecific variation in lipid composition have not been described. In this study, we used Fourier transform infrared (IR) spectroscopy to study genetic and acclimatory variation in cuticular lipid phase transitions, in cast skins of the lesser migratory grasshopper, *Melanoplus sanguinipes*.

## MATERIALS AND METHODS

**Specimens.** The grasshoppers used in these studies were first-generation descendants of individuals collected from the field in the summer of 1989. Grasshoppers from 18 populations all over California were studied (Table 1, Fig. 1). Field-caught animals were maintained and bred in the laboratory, and the eggs were collected and hatched. Nymphs were reared on a diet of lettuce and wheat bran under three environmental regimes: summer = 34°C, 15:9 LD (15 hr of light and 9 hr of dark); fall = 29°C, 11:13 LD; average = 32°C, 13:11 LD. Summer and fall conditions were chosen to simulate actual field conditions during these seasons, as indicated by weather station data (14). Cast skins (exuvae) were collected daily and were stored at −20°C under $N_2$ until analyzed. Storage had no apparent effects on lipid properties. Almost all exuvae used in these studies were from the final, fifth-instar, molt.

**Fourier Transform IR Spectroscopy.** Fourier transform IR spectroscopy was performed on intact cast skins, as described elsewhere (15, 34). Thoracic and abdominal portions of exuvae were sandwiched between two infrared-transparent $BaF_2$ windows and placed in a temperature-controlled cell holder. The holder was placed in a Perkin–Elmer model 1750 Fourier transform IR spectrometer, and the temperature was increased in 1°–2°C increments from 25°–30°C to 55°–60°C. Typically, 15 scans were averaged at each temperature. Transmittance spectra were converted to absorbance spectra, baseline drift was removed by using the instrument's software, and the frequency of the $-CH_2-$ symmetric stretch absorbance maximum at $\approx$2850 $cm^{-1}$ was determined by eye to within 0.1 $cm^{-1}$. This peak shifts to higher frequencies (higher wavenumbers) as lipids go through the gel-to-liquid-crystalline phase transition (15). Lipid melting curves for intact exuvae and for lipid extracts from exuvae are very similar (34).

**Analysis of Lipid Melting Curves.** Typical lipid melting curves for cast skins are shown in Fig. 2. Midpoints ($T_m$) of lipid phase transitions were estimated by probit analysis. To estimate the widths ($\Delta T$) of phase transitions, data were fitted to logistic equations (Fig. 2). Widths of the phase transitions were estimated as the difference in temperature between the 95% and 5% points on the logistic curves. $T_m$ values calculated from logistic curves were within 0.1°C of those calculated by probit analysis.

## RESULTS

Lipid melting temperatures (the midpoint of the lipid phase transition, $T_m$) ranged from 39.1°C to 49.6°C (Fig. 3). Using analyses of variance (16), we found significant effects of population, family (nested within population), and rearing regime on variation in $T_m$ (Table 2). Variance components were estimated by using the restricted maximum likelihood methods (16). Population and family (nested within population) accounted for 49% and 20%, respectively, of the total variance in $T_m$. Rearing regime also significantly affected $T_m$ (explained variance = 16%); in all but one family, $T_m$ values for summer-reared individuals were on average higher than those of siblings reared under fall-like conditions (Fig. 4). There were no significant interactions between rearing regime and either family or population, suggesting that individuals from all populations and families responded similarly to the two rearing environments. Variation among populations in $T_m$ was significantly associated with the environment from which populations were collected (Table 2); populations

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Table 1. Description of collection sites

| Collection site* | Latitude | Altitude, m | Description |
|---|---|---|---|
| 1. Bear Valley | 39°17′ | 1400 | Montane |
| 2. Big Sur | 36°20′ | 15 | Coastal |
| 3. Carmel Valley Rd. | 32°55′ | 10 | Coastal |
| 4. Damfine Spring | 39°28′ | 2000 | Montane |
| 5. Davis | 38°32′ | 15 | Inland valley |
| 6. Julian | 33°05′ | 1250 | Montane |
| 7. Laing Rd. | 39°17′ | 1500 | Montane |
| 8. Manila | 40°50′ | 10 | Coastal |
| 9. Mineral | 40°19′ | 1300 | Montane |
| 10. Morro Bay | 35°19′ | 10 | Coastal |
| 11. Mt. Laguna | 32°51′ | 1300 | Montane |
| 12. Mt. Palomar† | 33°20′ | 1950 | Montane |
| 13. Mt. Shasta | 41°20′ | 1150 | Montane |
| 14. Point Reyes | 38°00′ | 90 | Coastal |
| 15. Santa Barbara | 34°24′ | 90 | Coastal |
| 16. Tom's Place | 37°32′ | 2260 | Montane |
| 17. Whispering Palms | 32°57′ | 100 | Inland valley |

*Numbers are used to identify sites in Figs. 1 and 3. All sites are in California.

†Two sites, a and b, separated by 1000 m, were sampled from Mt. Palomar.

collected from low latitudes had significantly higher $T_m$ values than those from high latitudes. Also, there was an interaction between latitude and altitude, reflecting the observation that grasshoppers collected from northern montane locations had the lowest $T_m$ values.

The widths of the lipid phase transitions ($\Delta T$) ranged from 4.7°C to 24.8°C. Family and population significantly affected $\Delta T$ (explained variance: family = 53%, population = 14%), but rearing regime did not (Table 3). There were, however, significant regime × population interaction effects, which were not seen within families [regime × family (population)], suggesting that $\Delta T$ was affected by rearing environment in different ways in different populations. This effect may be related to the pattern of variation observed in $\Delta T$ with respect to latitude and altitude (Table 3). As with $T_m$, $\Delta T$ varied significantly with latitude (broader phase transitions in northern populations), and there was a significant interaction between latitude and altitude, with the largest $\Delta T$ values observed in populations from northern montane sites.

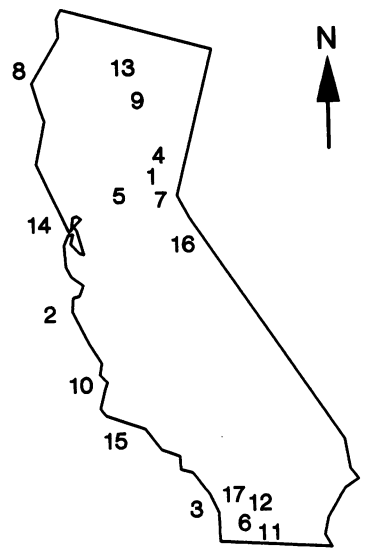


FIG. 1. Locations in California of collection sites for populations of grasshoppers used in this study. Sites are described in Table 1.

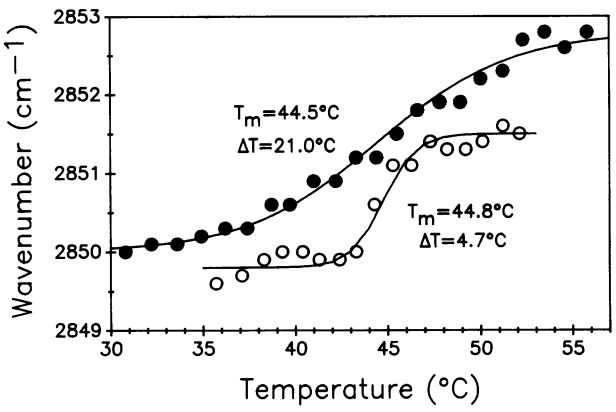


FIG. 2. Typical cuticular lipid melting curves for cast skins from two grasshoppers, with fitted logistic curves. The —$CH_2$— symmetric stretch absorbance maximum at ≈2850 $cm^{-1}$ shifts to higher frequencies as component lipids go through the gel-to-liquid-crystalline phase transition (15). Lipid phase properties in exuvae are similar to those of extracted lipids (34).

There was a significant correlation between $T_m$ and $\Delta T$ in our grasshoppers ($r = 0.40$, $n = 99$, $P < 0.0001$), although $\Delta T$ could differ by over 15°C between grasshoppers with similar $T_m$ values (Fig. 2). Individuals with lower $T_m$ values tended to have broader phase transitions. To investigate the possible significance of this association, we performed (*i*) a multivari-

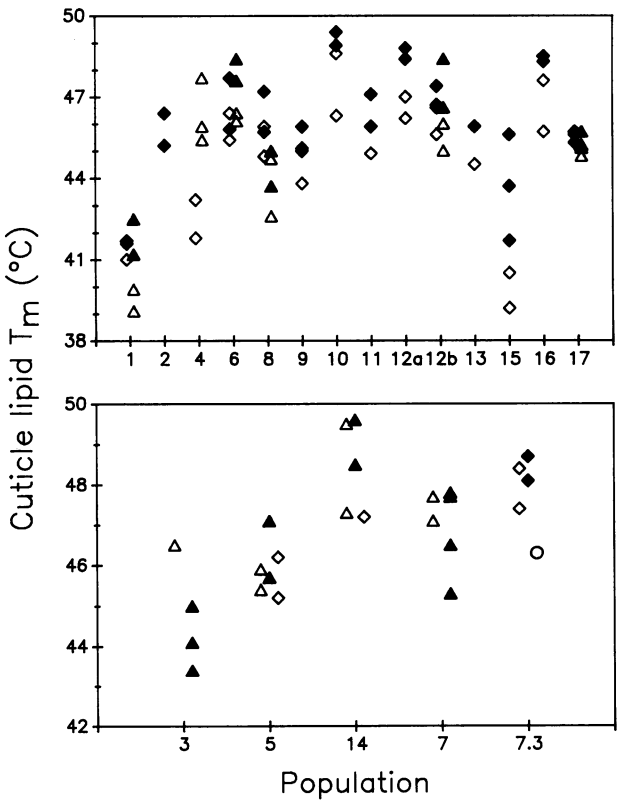


FIG. 3. Melting temperatures of cuticular lipids (midpoint of lipid phase transition, $T_m$) in individual grasshoppers. First-generation offspring of field-collected adults were reared under three habitat regimes (details in *Materials and Methods* and ref. 14). (*Upper*) Grasshoppers reared under "summer" (filled symbols, 34°C, 15:9 LD) and "fall" (open symbols, 29°C, 11:13 LD) conditions. Diamonds and triangles indicate different families (i.e., siblings) within a population. (*Lower*) All grasshoppers reared under "average" (32°C, 13:11 LD) conditions. Different symbols indicate families from a given population. Population 7.3 included three families of third-generation offspring from the Laing Rd. site.

Table 2. Analysis of variance results for $T_m$

| Source of variance | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Family and population effects | | | | | |
| Family (population) | 6 | 27.9 | 4.7 | 5.3 | 0.0005* |
| Population | 13 | 310.8 | 23.9 | 27.4 | 0.0001* |
| Rearing regime | 1 | 31.8 | 31.8 | 36.5 | 0.0001* |
| Regime × population | 11 | 11.3 | 1.0 | 1.2 | 0.3335 |
| Regime × family (population) | 5 | 3.2 | 0.6 | 0.7 | 0.6080 |
| Error | 37 | 32.3 | 0.9 | | |
| Corrected total | 73 | 409.4 | | | |
| Environmental effects | | | | | |
| Latitude | 1 | 29.4 | 29.4 | 7.6 | 0.0074* |
| Altitude | 1 | 2.2 | 2.2 | 0.6 | 0.4528 |
| Latitude × altitude | 1 | 66.6 | 66.6 | 17.2 | 0.0001* |
| Rearing regime | 1 | 18.1 | 18.1 | 4.7 | 0.0337* |
| Error | 69 | 266.8 | 3.9 | | |
| Corrected total | 73 | 409.4 | | | |

Only individuals reared under "fall" or "summer" conditions were included in order that some measure of balance might be contained in data set (i.e., families reared under "average" conditions were not included because members of these families were not reared under fall or summer conditions). df, Degrees of freedom; SS, sum of squares; MS, mean squares; *, $P < 0.05$.

ate analysis of variance of $T_m$ and $\Delta T$, (*ii*) a univariate analysis of variance of the eigenvectors associated with the first principal component from a principal components analysis of $T_m$ and $\Delta T$, and (*iii*) a univariate analysis of variance of the temperatures at which lipid melting started (i.e., the estimated onset of the phase transition, equal to $T_m - 0.5\Delta T$). All three analyses gave results qualitatively similar to those obtained for $T_m$ alone (results not shown).

## DISCUSSION

The differences in cuticular lipid phase behavior ($T_m$, $\Delta T$) between populations, and between families within populations, suggest that intraspecific variation in cuticular lipids has a genetic basis. Genetic differences in epicuticular lipid composition have been associated with differences in cuticular permeability in a laboratory population of *Drosophila pseudoobscura* (11). Our results provide evidence for genetic differences in the biophysical properties of surface lipids in natural populations.

Maternal effects have been shown to be an important contributor to life history variation in insects (17). Since the grasshoppers in these experiments were first-generation offspring of field-caught animals, we cannot rigorously exclude parental influences on properties of cuticular lipids. We do not think they were a major factor, for the following reasons. First, $T_m$ values in third-generation members of the Laing Rd. population were similar to those of first-generation offspring (from different families; Fig. 3 *Lower*). Also, population- and family-related differences in $T_m$ were much greater than environmental effects (explained variance = 49%, 20%, and 16% respectively, for population, family, and rearing environment), suggesting that parental influences would have had to predominate over the direct effects of the environment on an individual. Since direct environmental effects are generally stronger than indirect maternal environmental effects in insects (18), we conclude that most of the variation in $T_m$ was likely to be genetically based.

Given this genetic influence, one might expect a relationship between $T_m$ and the climatic conditions of the habitats from which our study populations were collected, similar to the variation in lipid composition documented by interspecific studies (9, 10). In accordance with this hypothesis, melting points tended to be higher in populations from lower

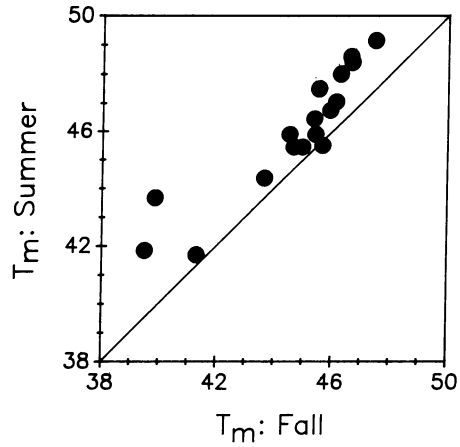


FIG. 4. Effect of rearing regime on $T_m$. Data points represent mean $T_m$ values for 17 families from 12 populations, for which both summer- and fall-reared siblings were analyzed. Solid line represents identical summer and fall $T_m$ values.

latitudes (Table 2), locations in which ground temperatures in summer months tend to be warmer than at other sites.

Several authors have described temperature-related differences in cuticular lipid composition (12, 13, 19), in a manner consistent with the theory of homeoviscous adaptation described for acclimation of cell membranes (20, 21). We found similar changes in lipid melting points; $T_m$ values in grasshoppers raised under summer conditions were higher than in fall-reared siblings (Fig. 4). An analysis of variance found no significant interactions between family and rearing regime, suggesting little genotype by environment interaction. Using full-sibling family means, we obtained a rough estimate of the genetic correlation between $T_m$ under fall and summer rearing conditions [$r_g = 0.92$, 95% confidence interval = 0.84–0.98, using 1000 bootstraps (22)]. This finding suggests little genetic variance for the reaction norms underlying phenotypic plasticity in $T_m$. In other words, rearing regimes affected $T_m$ in all individuals in a similar manner.

The grasshoppers were raised under regimes designed to simulate actual field conditions in summer and autumn, as indicated by weather station data (14). Temperature and photoperiod were varied simultaneously, so we cannot distinguish which factor, or both, was responsible for the

Table 3. Analysis of variance results for width of lipid phase transitions, $\Delta T$

| Source of variance | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Family and population effects | | | | | |
| Family (population) | 6 | 212.2 | 35.4 | 9.6 | 0.0001* |
| Population | 13 | 508.8 | 39.1 | 10.6 | 0.0001* |
| Rearing regime | 1 | 3.4 | 3.4 | 0.9 | 0.3431 |
| Regime × population | 11 | 96.5 | 8.8 | 2.4 | 0.0243* |
| Regime × family (population) | 5 | 20.9 | 4.2 | 1.1 | 0.3617 |
| Error | 37 | 136.6 | 3.7 | | |
| Corrected total | 73 | 978.4 | | | |
| Environmental effects | | | | | |
| Latitude | 1 | 49.7 | 49.7 | 4.1 | 0.0467* |
| Altitude | 1 | 10.9 | 10.9 | 0.9 | 0.3460 |
| Latitude × altitude | 1 | 65.9 | 65.9 | 5.4 | 0.0226* |
| Rearing regime | 1 | 1.4 | 1.4 | 0.1 | 0.7374 |
| Error | 69 | 836.4 | 12.1 | | |
| Corrected total | 73 | 978.4 | | | |

Only individuals reared under "fall" or "summer" conditions were included in order that some measure of balance might be contained in data set (i.e., families reared under "average" conditions were not included because members of these families were not reared under fall or summer conditions). *, $P < 0.05$.

environmental effects on $T_m$. Acclimation of lipid systems to temperature has been described often (20, 21), but we are not aware of any similar research on photoperiod acclimation. Preliminary results (not shown) suggest that temperature effects greatly outweighed any effects of photoperiod on $T_m$. For all families for which data were available, the average efficacy of homeoviscous adaptation (20) was 26%, similar to values obtained for cell membranes.

Higher $T_m$ values result from greater average hydrocarbon chain lengths of the component lipids. Fourier transform IR spectroscopy can also provide estimates of the widths of lipid phase transitions ($\Delta T$). These ranged from 4.7°C to 24.8°C and varied as a function of population and family, but not rearing regime. Population- and family-related variation in $\Delta T$ provides additional evidence for genetic effects on lipid composition. It is important to note that, although $T_m$ and $\Delta T$ were strongly correlated, these measures describe very different aspects of lipid phase behavior. The biochemical basis for differences in $\Delta T$ is unknown, but we hypothesize that wider phase transitions are associated with greater heterogeneity of hydrocarbon chain lengths.

The discovery of so much variation in surface lipid properties raises the question of the physiological relevance of this variability. The argument could be made that changes in phase behavior are of no consequence to the organism, as long as the lipids remain in a gel state. In accordance with this idea, for all individuals studied, the beginning of the phase transition was above the growth temperature. Surface lipids would not even have begun to melt *in vivo*, under laboratory conditions. Field conditions may be much different, however. Temperatures are extremely variable, and soil surface temperatures may reach 50°C (14). At these temperatures, cuticular lipids from most individuals would have been almost completely melted. Microhabitat selection will mitigate some of the effects of climate, but grasshoppers in the field may experience much higher temperatures than those in our laboratory experiments. The fact that higher $T_m$ values were correlated with lower latitude, and thus with greater presumed environmental temperature, is consistent with the idea that higher lipid melting points have adaptive value under these conditions (i.e., there is local adaptation) and that selective pressure is great enough to offset gene flow in this highly mobile insect.

Our findings have important implications for physiological studies of water balance in arthropods. Although the driving force for water loss has been disputed (23–26), the biophysical properties of epicuticular lipids are clearly important in reducing water loss, and there is general agreement that lipid phase transitions markedly affect permeability. Intraspecific variation in $T_m$ may explain discrepancies between different researchers' estimates of critical points for water loss, and differences in $\Delta T$ may be partially responsible for disagreements regarding the existence of sharp transitions in water loss (5, 27).

We did not measure water loss from *M. sanguinipes*, so it remains to be seen whether individual variation in surface lipid properties is reflected in differences in water loss. We do note that water loss from a congener, *Melanoplus bivittatus*, shows a broad transition over 43°–50°C (1), in the range of $T_m$ values we measured for *M. sanguinipes*. Transition temperatures for transpiration from other acridid insects are similar [*Locusta migratoria*, 46°–48°C (28); *Schistocerca gregaria*, 48°C (29)].

Previous studies have documented considerable intraspecific variation in water loss rates, which can be correlated with differences in surface lipid composition (13, 30). Genetic variation in water loss rates has been described in *Drosophila* species (31–33), but rarely has this been correlated with differences in lipid composition (11). Our results indicate that, although there are significant environmental effects, much of the variation in cuticular lipids is genetically based. Family level variation suggests significant heritability to $T_m$ and $\Delta T$ within wild populations, and population level variation correlated to differences in habitat temperature suggests that natural selection has molded epicuticular lipids in a manner appropriate to local environmental conditions. Thus, surface lipids provide an example of a trait with a strong genetic basis, which demonstrates considerable phenotypic plasticity. Given their well-documented physiological significance, epicuticular lipids are an excellent system in which to address fundamental questions regarding adaptation and acclimation.

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