



Pergamon

Journal of Insect Physiology 48 (2002) 391–400

*Journal
of
Insect
Physiology*

www.elsevier.com/locate/jinsphys

Review

Lipid melting and cuticular permeability: new insights into an old problem

Allen G. Gibbs *

Department of Ecology and Evolutionary Biology & Center for Insect Science, University of Arizona, 1041 E. Lowell St., Tucson, AZ 85721, USA

Received 14 August 2001; accepted 24 January 2002

Abstract

The idea that the physical properties of cuticular lipids affect cuticular permeability goes back over 65 years. This proposal has achieved textbook status, despite controversy and the general lack of direct supporting evidence. Recent work supports the standard model, in which lipid melting results in increased cuticular permeability. Surprisingly, although all species studied to date can synthesize lipids that remain in a solid state at environmental temperatures, partial melting often occurs due to the deposition of lipids with low melting points. This will tend to increase water loss; the benefits may include better dispersal of lipids or other compounds across the cuticle or improved communication via cuticular pheromones. In addition, insects with high melting-point lipids are not necessarily less permeable at low temperatures. One likely reason is variation in lipid properties within the cuticle. Surface lipids differ from one region to another, and biophysical studies of model mixtures suggest the occurrence of phase separation between melted and solid lipid fractions. Lipid phase separation may have important implications for insect water balance and chemical communication. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Critical temperature; Cuticular lipid; FTIR; Hydrocarbon; Phase separation; Phase transition; Pheromone; Water balance

Contents

1. Introduction	391
2. Potential problems with the lipid melting model	392
3. Fourier transform infrared spectroscopy	392
4. Is the lipid-melting model correct?	393
5. Lipid structure and melting points: what biophysicists can tell physiologists	395
6. Is lipid melting ecologically relevant?	395
7. Does T_m matter at lower temperatures?	396
8. Lipid phase separation on the insect cuticle: a hypothesis	397
9. Implications of phase separation for chemical communication	398
10. Conclusion	398

1. Introduction

Physiologists have long recognized that terrestrial arthropods face a serious problem with respect to water

balance. Because surface area scales to the 2/3 power of mass, insects and other small terrestrial animals have a relatively large surface area through which to lose a relatively small volume of water. The importance of the epicuticular lipids in reducing cuticular permeability was recognized by Ramsay (1935). A significant early finding, since repeated for numerous species, was that rates of water loss tend to be very low at moderate tempera-

* Tel.: +1-520-626-8455; fax: +1-520-621-9190.
E-mail address: agibbs@arl.arizona.edu (A.G. Gibbs).

tures, then increase rapidly above the “critical” temperature (T_C) (e.g. Ramsay, 1935; Wigglesworth, 1945; Beament, 1958; Beament, 1959; Loveridge, 1968a; Davis, 1974; Yoder and Denlinger, 1991a; Yoder and Denlinger, 1991b; Rourke and Gibbs, 1999). A cuticular model was proposed in which the increased water loss was directly attributed to melting of the surface lipids (Gibbs, 1998).

In a pair of classic papers, Wigglesworth (1945) and Beament (1945) confirmed and extended Ramsay’s work. Wigglesworth demonstrated that T_C values differed among species and were higher in insects from arid environments. Beament extracted surface lipids from many of the same species and examined their properties using capillary melting techniques. In most cases, melting points were within a few °C of the T_C values given by Wigglesworth. This work provided the first experimental evidence for the lipid melting model. Beament (1945) also used isolated cuticular patches and model membranes to further test and support this model.

Other researchers followed Beament’s lead using additional biophysical techniques, including electron diffraction (Holdgate and Seal, 1956), surface film compressibility (Lockey, 1976), electron paramagnetic resonance (Toolson et al., 1979), and differential scanning calorimetry (Machin and Lampert, 1990). In many cases, a change in cuticular properties was observed at a temperature close to the T_C . Similar results have been obtained using waterproofing layers from leaves of plants (Schreiber and Riederer, 1996a) and mammalian skin (Golden et al., 1987).

2. Potential problems with the lipid melting model

The lipid melting model has achieved textbook status (e.g. Schmidt-Nielsen, 1990; Randall et al., 1997; Chapman, 1998), although numerous researchers have pointed out its limitations. Relatively few species have been examined, particularly with respect to lipid melting properties. The model relies on correlations between two independently measured parameters, lipid phase state and cuticular permeability, both of which are open to scrutiny:

In the case of cuticular permeability, water can be lost by other routes (respiration, excretion). Recent improvements in flow-through respirometry have made it possible to distinguish between cuticular and other routes of water loss (as long as the species in question exhibits discontinuous gas-exchange cycles; Hadley (1994); Lighton (1994)). In most species studied to date, cuticular transpiration accounts for >90% of total water loss. Thus, measurements made using other methods, such as gravimetric techniques, may be reasonably accurate. It must be noted, however, that active ventilation in some species can greatly increase respiratory losses

(Loveridge, 1968b), particularly at higher temperatures. Some authors have therefore argued that the critical temperature phenomenon is simply an artefact of increased metabolism and spiracular opening, although the T_C may also be apparent when killed or when sealed specimens are used (Wigglesworth, 1945; Hadley and Quinlan, 1989; Rourke and Gibbs, 1999).

An important problem exists with many reported values for T_C , which have usually been estimated graphically from plots of water-loss rates vs. temperature. Because water loss increases so rapidly at high temperatures, the vertical scale at low temperatures is compressed. Thus, the apparent T_C can depend on the range of measurement temperatures and may simply be an artefact of using an inappropriate linear scale for data which fit a smooth exponential curve (Appel et al., 1986). Arrhenius plots are now more commonly used (e.g. Machin and Lampert, 1989; Yoder and Denlinger, 1991a; Yoder and Denlinger, 1991b; Rourke and Gibbs, 1999). These tend to be biphasic, and the presence of a breakpoint reveals that some change in cuticular permeability has occurred. The key issue is whether this breakpoint can be attributed to changes in lipid properties.

Measurements of lipid phase behavior have suffered severe technological limitations. The amount of surface lipid is small, so that *in situ* methods may actually detect physical changes in other portions of the cuticle, such as chitin or membrane phospholipids. Milligram quantities may be required, far more than is available from a single individual. Surface lipids can be extracted and combined from multiple insects, but extraction may affect lipid properties, and information about individual or regional variation is lost. The chemical complexity of surface lipids is a major problem. These mixtures can be expected to melt over a range of temperatures, rather than at a specific T_m . Calorimetry and capillary melting point techniques are widely used lipid biophysical techniques, but are most effective for samples with a narrow melting range. These difficulties can be overcome using Fourier transform infrared spectroscopy (FTIR), a technique that became available in the late 1960s (Smith, 1996).

3. Fourier transform infrared spectroscopy

Dispersive IR spectrometers use a diffraction grating to vary the wavelength of the radiation. While very useful for analytical work, these instruments are limited to a frequency precision and accuracy of only 3–5 cm^{-1} . Fourier transform IR spectrometers, on the other hand, use a system of moving mirrors and interferometry to generate infrared spectra, and can be used to locate peaks to a precision and accuracy of less than 0.1 cm^{-1} (Smith, 1996). Very small samples can be used (even individual cells when an FTIR microscope is used). These advan-

tages have made FTIR a widely used biophysical tool for the study of membranes and proteins, and FTIR spectrometers have nearly completely replaced dispersive IR instruments.

Hydrocarbons have major absorption bands at 2800–3000 cm^{-1} (Fig. 1(A)). A band at $\sim 2850 \text{ cm}^{-1}$ corresponds to symmetric stretching vibrations of $-\text{CH}_2-$ moieties along alkyl chains. In the gel phase, the chains are extended. Adjacent methylene groups are predominantly in the *trans* configuration and absorb strongly at $\sim 2849 \text{ cm}^{-1}$. As temperature rises and lipids melt, thermal motion results in formation of *cis* conformers, which absorb at $\sim 2854 \text{ cm}^{-1}$. The overall frequency of the symmetric stretching peak provides a direct indicator of the relative abundance of *trans* and *cis* conformers, and therefore of the progress of lipid melting (Fig. 1(B)). As expected, melting occurs over a broad range of temperatures, but the cumulative frequency shift allows samples with wide melting ranges to be analyzed.

My colleagues and I began using FTIR to study cuticular lipids a decade ago (Gibbs and Crowe, 1991).

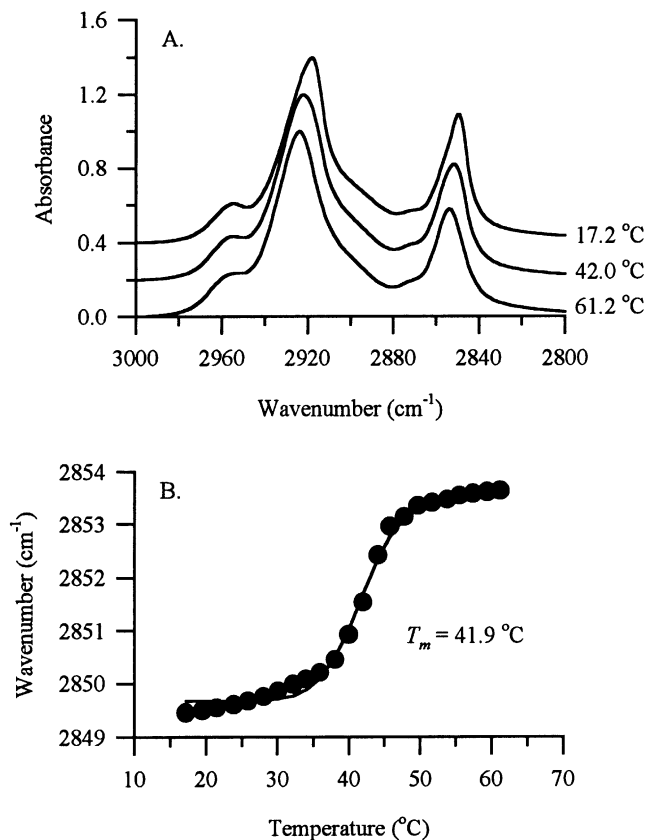


Fig. 1. Use of infrared spectroscopy to measure lipid melting temperatures. (A) Infrared spectra of surface lipids isolated from an individual *Melanoplus sanguinipes*. Note the slight shift of the two major peaks to higher frequencies as temperature increases. (B) Melting curve for the same lipid sample. The location of the $-\text{CH}_2-$ symmetric stretching vibration at each temperature was determined using the FTIR spectrometer's software. The T_m is the temperature at which the lipids are 50% melted, as calculated from the fitted logistic curve.

We felt that this improved technology would allow us to test the lipid melting model more rigorously than before, and perhaps even overturn a textbook icon. The model has fared well, but our work has also raised new questions which will probably require new techniques to answer.

4. Is the lipid-melting model correct?

Figure 2 depicts melting curves for surface lipids from several arthropod species. Melting points (T_m , defined as the midpoint of the transition) vary from $<25^\circ\text{C}$ for *Drosophila pseudoobscura* to $>80^\circ\text{C}$ for mealworms and tend to be correlated with habitat aridity. Two important points should be noted here. First, lipids in situ (on wings or exuviae) and solvent-extracted lipids have indistinguishable properties, suggesting that extraction does not affect lipid phase behavior. Second, some lipid melting may be apparent even at very low temperatures (near 0°C in the case of *D. pseudoobscura*).

Does lipid melting actually result in increased cuticular permeability? We have used three approaches to address this question: inter-specific comparisons, intra-specific studies, and model cuticle preparations. Critical temperatures have been determined for a wide variety of arthropods, and we have measured lipid T_m values for several of these. Figure 3(A) depicts the relationship between T_m and T_C for seven arthropod species. For those species with melting points below 50°C , these

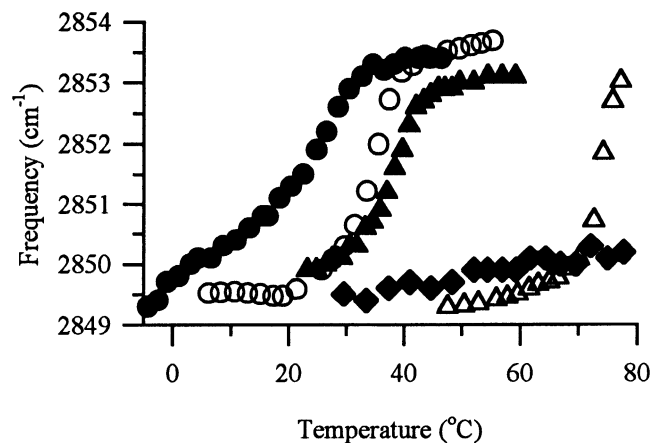


Fig. 2. Representative melting curves for five arthropod species. From left to right, the species are: *Drosophila pseudoobscura*, *Blatella germanica*, *Latrodectus hesperus* (black widow spider), *Rhodnius prolixus*, and *Tenebrio molitor* larva.

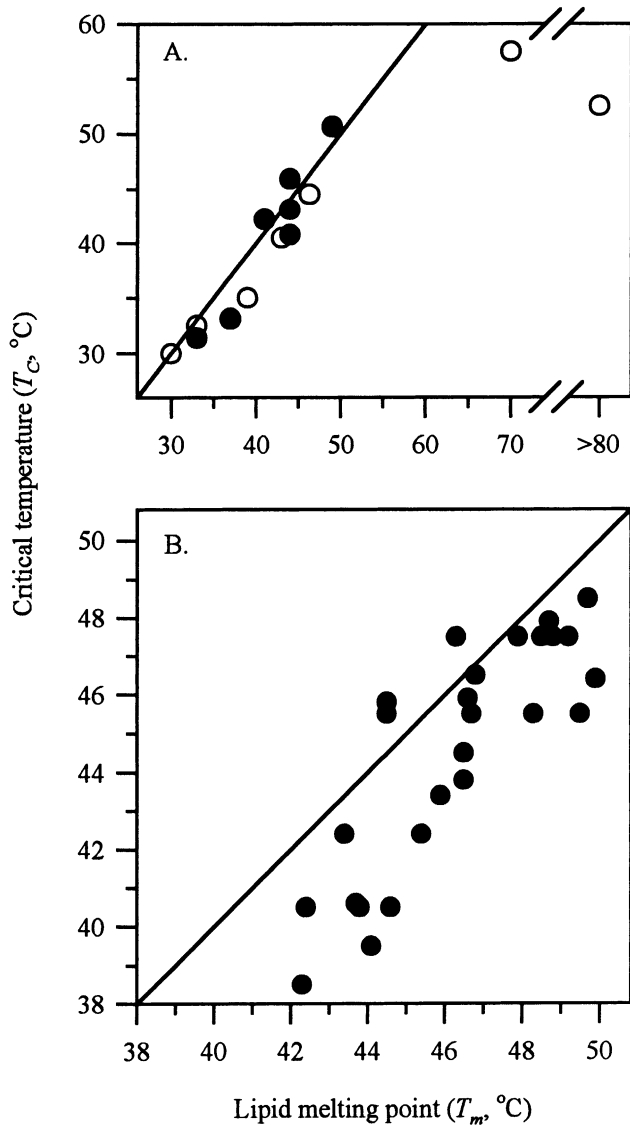


Fig. 3. Relationship between lipid melting point and critical temperature. (A) Open symbols: literature values of T_c are compared to T_m values in the same species determined using FTIR. The right-most points correspond to *Rhodnius prolixus* nymphs and *Tenebrio molitor* larvae. Filled symbols: Model membrane data from Rourke and Gibbs (1999). (B) Intra-specific variation in *Melanoplus sanguinipes*. Melting points and T_c values were measured using the same individuals. From Rourke and Gibbs (1999). Solid lines are lines of equality.

values are nearly identical. At higher temperatures, however, water-loss rates can rise without lipid melting in *Rhodnius* nymphs and *Tenebrio* larvae. Thus, data from most, but not all, species are consistent with the lipid melting model.

The lack of correspondence between T_m and T_c in some species raises the question of whether we can then ascribe changes in water loss to surface lipids in the other species. One possible explanation is intra-specific variation in lipid properties (Gibbs et al., 1991) and T_c (Beament, 1958), so that literature values for T_c may not correspond to those for individuals used in lipid

measurements. Another possibility is that thermal damage to the underlying cuticle (e.g. cuticular proteins) causes permeability to increase in these species before the lipids melt. Model membrane experiments remove these concerns and also support the lipid melting model. Gore-Tex® membranes coated with pure *n*-alkanes exhibit biphasic Arrhenius plots for water flux, with the break point occurring at the T_m (Rourke and Gibbs, 1999; Fig. 3(A)).

Although intra-specific differences can be a problem in inter-specific comparisons, they can also provide a valuable source of variation to test this model. Both genetic and environmental factors can affect lipid composition (Gibbs et al., 1991; Howard, 1993; Stennett and Eges, 1997; Liang and Silverman, 2000). In the grasshopper, *Melanoplus sanguinipes*, these cause T_m values to range between 35 and 50°C (Gibbs et al., 1991; Gibbs and Mousseau, 1994; Rourke and Gibbs, 1999; Rourke, 2000). Lipid T_m and T_c values, measured using the same individuals, are highly correlated (Rourke and Gibbs, 1999; Fig. 3(B)). This strongly supports a mechanistic connection between lipid melting and increased cuticular permeability. It is interesting to note that the T_m is consistently slightly higher than T_c . The estimated fraction of melted lipid at the T_c averages about 0.35, indicating that surface lipids need be only partially melted for cuticular permeability to increase.

In summary, the transition temperature does seem to reflect lipid melting. The only exceptions are provided by species with very high T_c values, and these can be explained in terms of damage to other cuticular components. Only a few species have been examined, however, and only one species, *M. sanguinipes*, in any detail. An important issue that needs attention is the extent of regional variation in lipid properties and its effects on organismal rates of water loss. It is clear that lipid composition varies from one region to another (Young et al., 2000), and lipid T_m values can differ by >30°C between different areas on a single individual (Gibbs and Crowe, 1991; Young et al., 2000; Fig. 4). Regional variation can make it difficult to identify critical temperatures, since

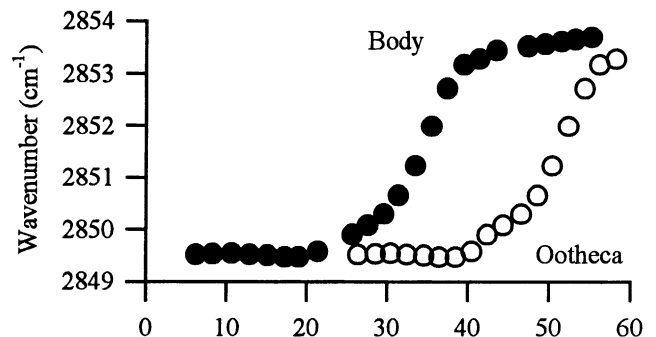


Fig. 4. Regional variation in lipid properties. Surface lipids were isolated from different portions of female cockroaches, *Blattella germanica*. Data from Young et al. (2000).

increased permeability will occur at different temperatures on different parts of the insect.

5. Lipid structure and melting points: what biophysicists can tell physiologists

Hundreds of studies have been published describing the composition of cuticular lipids (see reviews by J.S. Buckner, R.W. Howard and D.R. Nelson in Stanley-Samuelson and Nelson (1993)). It would be very useful to be able to predict T_m values from these data, and thereby gain insight into the probable effects of temperature on water balance in a given species. Most biophysical studies of pure lipids have concerned phospholipids or other constituents of cellular membranes, although a smattering of studies on compounds relevant to insect cuticles have been published (Iyengar and Schlenk, 1968; Blazyck and Rana, 1987). This basic information is also necessary in order to interpret studies that correlate lipid composition with water loss (e.g. Hadley, 1977; Toolson and Hadley, 1979; Toolson, 1982, 1984).

Hydrocarbons, phospholipids, and other lipid classes share similar structure- T_m relationships. Saturated, straight-chain molecules melt at the highest temperatures, with the T_m increasing by 1–3°C with each carbon unit. For example, *n*-tricosane, with 23 carbons, melts at 47°C, whereas *n*-tritriacontane (33 carbons) melts at 70°C. Insertion of a double bond, methyl branch, or ester link reduces T_m by 20 to 50°C, depending on where it is located (Gibbs and Pomonis, 1995; Patel et al., 2001; Fig. 5).

An important conclusion from studies of pure compounds is that chain length has relatively minor effects on T_m ; differences in lipid class (alkenes, methylalkanes,

etc.) are more important. This conclusion is supported by two studies of thermal acclimation of cuticular hydrocarbons. In *M. sanguinipes*, warm-acclimated individuals have higher T_m values than cold-acclimated ones (Gibbs et al., 1991). They also have higher proportions of *n*-alkanes relative to methylalkanes, despite the fact that the *n*-alkanes tend to be shorter (Gibbs and Mousseau, 1994). In the desert fruitfly, *Drosophila mojavensis*, warm-acclimated flies synthesize longer chain-length hydrocarbons (Markow and Toolson, 1990). However, because these are highly unsaturated, warm acclimation has no effect on T_m values (Gibbs et al., 1998).

An individual insect may contain >100 different lipid compounds on its cuticle, so it is also necessary to understand how these compounds interact with each other to affect the bulk lipid properties. Mixtures of saturated hydrocarbons exhibit fairly straight-forward physical properties. As long as the component hydrocarbons do not differ too much in chain length, mixtures of *n*-alkanes form mixed crystals (Bonsor and Bloor, 1977). As temperature rises, these crystals melt over a range of temperatures, with the midpoint (T_m) being a weighted average of the T_m values for the individual constituents (Gibbs, 1995). A similar situation appears to hold for mixtures of *n*-alkanes with branched alkanes; branched molecules co-crystallize with *n*-alkanes, and the T_m of the mixture is easily calculated from the T_m values of the components (Gibbs, 1995). Wax ester-alkane mixtures have slightly lower T_m values than predicted (Patel et al., 2001), suggesting that some packing disruption occurs in these mixtures.

Unfortunately, there appears to be very little published literature on alkene-alkane mixtures. My FTIR data suggested that these melt at much higher temperatures than predicted (Gibbs, 1995), but this interpretation now appears to be incorrect. Small (1986) outlines calorimetric, NMR, and X-ray diffraction studies indicating that unsaturated hydrocarbons do not form mixed crystals with alkanes. Instead, the alkenes and alkanes crystallize separately. The alkene crystals melt at their particular, lower T_m . As temperature increases, the alkane crystals gradually melt into the liquid alkenes, so that a condition of liquid-solid phase separation exists until the alkane crystals have melted completely (Small, 1986; Fig. 6). An important implication for insect physiologists is that lipid phase separation may occur in situ and may affect cuticular permeability (see discussion below).

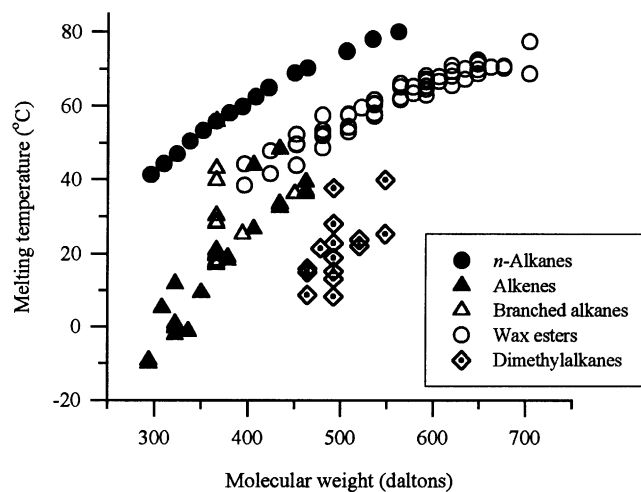


Fig. 5. Effects of structural differences on melting points of pure synthetic hydrocarbons and wax esters. Data are from Gibbs and Pomonis (1995); Patel et al. (2001) and L.L. Jackson and A.G. Gibbs (unpublished).

6. Is lipid melting ecologically relevant?

It can be argued that insects in nature avoid temperatures high enough to melt the surface lipids, so that the phenomenon is merely a laboratory curiosity. Lipid melting is clearly irrelevant for some species, like *Rhodnius* and mealworms, whose T_C values are well above their

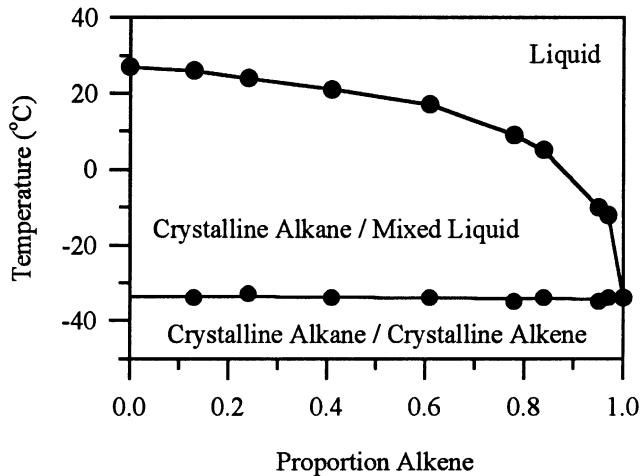


Fig. 6. Phase diagram for mixtures of *n*-octadecane and *cis*-9-octadecene. Replotted from Small (1986).

thermal tolerance limits. In other species, however, surface lipids melt within the species' thermal limits and, more importantly, at temperatures that are ecologically relevant.

Melanoplus sanguinipes uses behavioral thermoregulation to maintain body temperatures at ~40°C (Chappell, 1983; Rourke, 2000), whereas their T_m values can be as low as 35°C (Rourke, 2000), and their T_C values are even lower (Fig. 3(B)). Cactophilic *D. mojavensis* are exposed to nearly 40°C in and around their host plants (A.G. Gibbs, M.C. Perkins and T.A. Markow, unpublished), and their surface lipids melt at 35–40°C (Gibbs et al., 1998). Surface lipids of *D. pseudoobscura* begin melting below 5°C (T_m ~20°C; Fig. 2), and those of *D. melanogaster* melt at 25–30°C (Gibbs et al., 1997). Although analyses of heat-shock protein expression suggest that adult *D. melanogaster* avoid temperatures above 30°C (Feder et al., 2000), some lipid melting is certainly likely in nature.

These limited data demonstrate that lipid melting is ecologically relevant for at least some insect species. An important unanswered question is why any melting occurs at all. Nearly all species studied to date synthesize *n*-alkanes with chain lengths of at least 25 carbons (De Renobales et al. (1991). These compounds melt above 50°C (Gibbs and Pomonis, 1995), so all species have the ability to synthesize surface lipids that remain solid and water-tight at environmental temperatures. Why do insects also synthesize lipids that reduce T_m and increase the potential for water stress?

Several potential beneficial consequences of partial lipid melting come to mind: evaporative cooling at high temperatures, improved dispersal of lipids across the cuticle, or improved chemical communication mediated by contact pheromones. The first possibility can be rejected for *Drosophila*, since they are too small for effective thermoregulation by evaporative cooling

(Willmer and Unwin, 1981; Prange, 1996). Larger insects have evolved a number of more closely regulated mechanisms to control evaporative water loss (Prange, 1996), so a major role for lipid melting seems unlikely.

Ensuring lipid dispersal across the cuticle is a more likely explanation. Lipid mobility may be required to completely cover the cuticle and provide an effective water barrier. Electron micrographs reveal that high- T_m lipids, such as those found in wax blooms of whiteflies, retain their morphological structure and do not mix with the rest of the surface lipids on the cuticle (Nelson et al., 2000). They therefore may not aid much in waterproofing, but instead serve as predator deterrents or to reflect solar radiation. In addition, other hydrophobic compounds, including anti-microbial or anti-predator compounds and pheromones, can be dispersed along with the lipids.

A more direct role for lipid fluidity in pheromone reception is also possible. Surface lipids used in communication are generally branched, unsaturated, or otherwise modified in ways that will reduce T_m (Gibbs and Pomonis, 1995; Gibbs et al., 1995). Pheromones will therefore tend to reduce the overall melting point of the surface lipids, but may also be detected by conspecifics more easily if they are in a fluid state. This raises the possibility that the two major functions of cuticular lipids, water conservation and communication, may exert conflicting selective pressures on lipid composition.

7. Does T_m matter at lower temperatures?

Several studies have documented environmental effects on the composition of surface lipids, particularly greater hydrocarbon chain lengths in animals reared at higher temperatures (Hadley, 1977; Toolson and Hadley, 1979; Toolson, 1982). In each of the cited cases, acclimatory changes in rates of water loss have also been described. Similar findings have been reported in interspecific comparisons (Hadley, 1978; Hadley and Schultz, 1987). It is tempting to attribute differences in water-loss rates to lipid physical properties, but melting points were not actually measured in these cases. As noted above, chain length is perhaps the least important structural factor affecting T_m , although differences in lipid class are also usually consistent with higher T_m values in less permeable individuals.

The implicit assumption in these studies (and in numerous studies of lipid composition in which water loss was not measured) has been that higher T_m values reduce cuticular permeability at all temperatures. However, it is not clear that any relationship between T_m and water-loss rates should be found. Water-loss rates are typically measured at temperatures of 30°C or less, whereas T_m values in most species studied to date are >35°C. Evidence presented above confirms that melted

lipids are more permeable than solid ones, but if the surface lipids remain solid under measurement conditions, why should it matter what the T_m is?

Our efforts to relate variation in T_m to water-loss rates have provided conflicting results. On the positive side, populations of *M. sanguinipes* with higher T_m values lost water more slowly at temperatures from 25 to 42°C (Rourke, 2000). These populations also tended to have greater quantities of surface lipid, however, so it is impossible to distinguish rigorously between the effects of having a thicker lipid barrier and a different form of lipid barrier. In *D. mojavensis*, 14-day old flies have lower T_m values, but similar water-loss rates to younger flies (Gibbs et al., 1998). Mycophagous *Drosophila* species live in cool, moist forests and lose water more rapidly (Gibbs and Matzkin, 2001), yet they also have the highest T_m values in the genus (Gibbs, unpublished data). In a laboratory evolution system, desiccation-selected *D. melanogaster* lost water much less rapidly than unselected controls, despite having only minor differences in surface lipid properties (Gibbs et al., 1997). In houseflies, deposition of unsaturated pheromones causes T_m to decrease by ~4°C as females mature (Gibbs et al., 1995). Lipid T_m does not change in males, but both sexes exhibit similar increases in water-loss rates (K.L. Montooth and A.G. Gibbs, unpublished).

In summary, most of our studies do not support a connection between T_m and organismal water-loss rates, when water loss is measured below T_m . Changes in T_m can occur without changes in water loss, and differences in water loss can occur without any changes in T_m . Similar, negative results have been obtained in comparisons of plant species (Schreiber and Riederer, 1996b). Thus, one cannot easily attribute differences in water loss to cuticular lipids, nor is there any simple way to predict water-loss rates from lipid analyses alone.

8. Lipid phase separation on the insect cuticle: a hypothesis

Of course, the failure to find a consistent relationship between T_m and water-loss rates can also result from differences in the amount of surface lipid. Table 1 summarizes our published and unpublished data relating rates of water loss (measured at 25–30°C) to T_m and lipid quantity. Only in *M. sanguinipes* is either of the expected correlations found. Note that this is also the only taxon studied which does not contain significant quantities of cuticular alkenes. This may be coincidental, but it is intriguing that simple alkane–alkene mixtures, unlike other hydrocarbon mixtures, exhibit liquid–solid phase separation (Small, 1986). Thus, cuticular lipids of alkene-rich arthropod species may exhibit similar phase behavior. If so, phase separation could affect water balance and chemical communication, and therefore merits further investigation.

Table 1

Summary of correlations between T_m , surface lipid amounts, and water-loss rates, measured below the T_m . The predicted relationships are that higher melting points and greater lipid quantities will be associated with lower rates of water loss. “Yes” and “No” indicate whether the expected correlation was observed. The final column indicates whether the species in question have cuticular alkenes

Species or study system	Melting point?	Amount?	Alkenes?
<i>Melanoplus</i> populations	Yes	Yes	No
Thermal acclimation of <i>D. mojavensis</i>	No	No	Yes
Desiccation-selected <i>D. melanogaster</i>	No	No	Yes
<i>Drosophila</i> species	No	–	Yes
Houseflies (<i>Musca domestica</i>)	No	No	Yes
<i>Pogonomyrmex</i> species	No	No	Yes
<i>P. barbatus</i> mating status	–	No	Yes

Alkenes containing 30 or fewer carbons, like those found most often on insect cuticles, melt below 25°C, whereas cuticular *n*-alkanes generally melt above 45°C (Fig. 4). This suggests that phase separation will occur at ecologically relevant temperatures, so that the cuticles of many insects will contain regions of highly permeable, melted alkenes and poorly permeable, solid crystalline alkanes (Fig. 7). This will make it impossible to define a “bulk” lipid melting point, and therefore make it impossible to relate lipid properties to organismal water loss in any simple manner. Phase separation may therefore explain why T_m values and lipid quantities are not correlated with water loss in alkene-rich species.

This hypothesis can be tested in several ways. First, it only applies to insects which synthesize cuticular

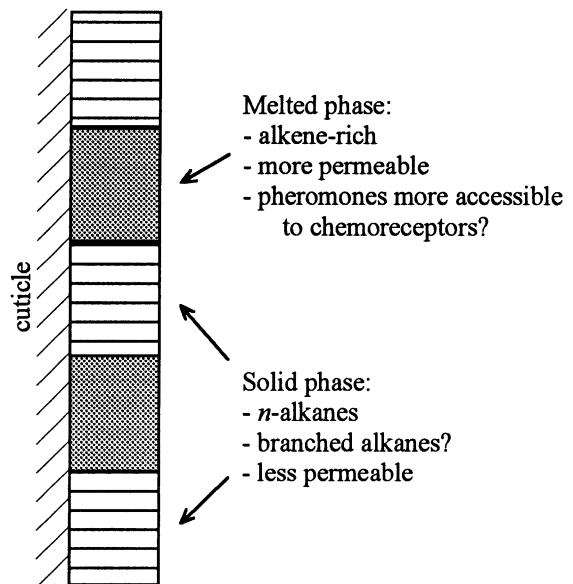


Fig. 7. Hypothetical model for phase separation of cuticular lipids. Melted (stippled) and solid (hatched) lipids are interspersed on the cuticle and differ in their chemical composition. As temperature increases, solid lipids dissolve into the melted regions.

alkenes. In species lacking alkenes, such as *M. sanguinipes*, homogeneous lipid crystals are expected, according to the limited data available from simple model mixtures. Because of this homogeneity, T_m , lipid quantity and cuticular permeability should be correlated in these species. Second, individuals or strains can differ greatly in the relative abundance of alkenes (e.g. geographic variation in *D. melanogaster*; Ferveur et al. (1996)). Those with more alkenes should lose water most rapidly, since a larger fraction of the cuticle will be coated with melted lipids.

Third, it should be possible to detect phase separation directly, using a combination of technologies. For example, FTIR microscopy may aid in examining regional variation in lipid properties. Researchers in plant surface lipids are well ahead of insect biologists in the range of techniques applied. Studies using X-ray diffraction and NMR spectroscopy reveal the presence of both highly structured and amorphous lipid regions (Reynhardt and Riederer, 1991, 1994). Outer wax layers can be selectively removed by mechanical means, with no apparent disruption to either the wax particles themselves or the underlying cuticle (Ensikat et al., 2000; Jetter et al., 2000). Cuticular properties in situ have been examined using reflectance FTIR (Merk et al., 1998), contact angle measurements (Schreiber, 1996), diffusion coefficients of hydrophobic molecules (Schreiber and Riederer, 1996a) and atomic force microscopy (Mechaber et al., 1996). Fluorescence techniques have been developed for analysis of phase separation in cell membranes (Parassasi et al., 1991; Tocanne et al., 1994; Williams, 1998), and some of these may be applicable to cuticular lipids. These methods will be most effective if more sensitive methods for measuring cuticular permeability can be developed.

9. Implications of phase separation for chemical communication

Lipid phase separation may also have significant effects on communication, since it is likely that lipid phase state will affect pheromone detection. In particular, contact pheromones may be more accessible to chemoreceptors if the pheromones are surrounded by a fluid lipid milieu, rather than a solid-phase microenvironment. Because many cuticular pheromones and kairomones are unsaturated and will melt at low temperatures, melted lipid regions may have locally high concentrations of certain pheromones. Conversely, saturated branched pheromones may tend to co-crystallize with the *n*-alkane fraction and will therefore have a lower effective concentration.

The phase separation hypothesis also predicts that temperature can have marked, complex effects on pheromone detection. As temperatures increase, saturated lip-

ids will begin to melt, and fluid regions will therefore have relatively lower levels of unsaturated pheromones and higher levels of branched ones. Thus, components of pheromone blends will exhibit changing ratios solely as a consequence of the animal's surface temperature. The thermal environment may therefore prove to be an important mediator of insect behavior. A possible example of this phenomenon may already have been observed in garter snakes (Shine et al., 2001). To test this intriguing aspect of the phase separation hypothesis, we will need to develop rigorous bioassays for contact chemoreception with well-defined lipid components, using both whole animal and neurobiological techniques.

10. Conclusion

The lipid melting model has a long history in insect physiology. This model has withstood our attempts to disprove it, but it is also clear that the phase behavior of cuticular lipids is not as simple as once thought. Lipid properties vary on regional scales, from one part of the individual to another, and on microscopic scales, due to phase separation. Variation in cuticular structure and function has been recognized for years, but the implications of this variation need to be better understood. There also needs to be better integration of behavioral and physiological studies of cuticular lipids. Chemical ecologists and physiologists have tended to ignore each other's work; this needs to change. Surface lipids provide a largely unexplored model for interacting selective pressures on reproductive success and survival.

Acknowledgements

I thank many collaborators for their insights and advice over the years. In particular, I thank John Crowe and his lab for introducing me to the FTIR technique and its potential. Research in my lab has been supported by the National Science Foundation and the Ubu Endowment.

References

- Appel, A.G., Reiersen, D.A., Rust, M.K., 1986. Cuticular water loss in the smokybrown cockroach, *Periplaneta fuliginosa*. *Journal of Insect Physiology* 32, 623–628.
- Beament, J.W.L., 1945. The cuticular lipoids of insects. *Journal of Experimental Biology* 21, 115–131.
- Beament, J.W.L., 1958. The effect of temperature on the water-proofing mechanism of an insect. *Journal of Experimental Biology* 35, 494–519.
- Beament, J.W.L., 1959. The waterproofing mechanism of arthropods. I. The effect of temperature on cuticle permeability in terrestrial insects and ticks. *Journal of Experimental Biology* 36, 391–422.
- Blazyk, J., Rana, F., 1987. An automated temperature control and data

- acquisition system of Fourier transform infrared studies: Application to phase changes in hydrocarbons and lipid bilayers. *Applied Spectroscopy* 41, 40–44.
- Bonsor, D.H., Bloor, D., 1977. Phase transitions of *n*-alkane systems, Part 2, Melting and solid state transition of binary mixtures. *Journal of Materials Science* 12, 1559–1564.
- Chapman, R.F., 1998. *The Insects*, 4th ed. Cambridge University Press, Cambridge.
- Chappell, M.A., 1983. Metabolism and thermoregulation in desert and montane grasshoppers. *Oecologia* 56, 126–131.
- Davis, M.T.B., 1974. Critical temperature and changes in cuticular lipids in the rabbit tick, *Haemaphysalis leporispalustris*. *Journal of Insect Physiology* 20, 1087–1100.
- De Renobales, M., Nelson, D.R., Blomquist, G.J., 1991. Cuticular lipids. In: Binnington, K., Retnakaran, A. (Eds.), *Physiology of the Insect Epidermis*. CSIRO, Melbourne, pp. 240–251.
- Ensikat, H.J., Neinhuis, C., Barthlott, W., 2000. Direct access to plant epicuticular wax crystals by a new mechanical isolation method. *International Journal of Plant Sciences* 161, 143–148.
- Feder, M.E., Roberts, S.P., Bordelon, A.C., 2000. Molecular thermal telemetry of free-ranging adult *Drosophila melanogaster*. *Oecologia* 123, 460–465.
- Ferveur, J.F., Cobb, M., Boukella, H., Jallon, J.M., 1996. World-wide variation in *Drosophila melanogaster* sex pheromone: Behavioural effects, genetic bases and potential evolutionary consequences. *Genetica* 97, 73–80.
- Gibbs, A.G., 1995. Physical properties of insect cuticular hydrocarbons: model mixtures and lipid interactions. *Comparative Biochemistry and Physiology B* 112, 667–672.
- Gibbs, A.G., 1998. Water-proofing properties of cuticular lipids. *American Zoologist* 38, 471–482.
- Gibbs, A., Crowe, J.H. 1991. Intra-individual variation in insect cuticular lipids studied using Fourier transform infrared spectroscopy. *Journal of Insect Physiology* 37, 743–748.
- Gibbs, A.G., Matzkin, L.M., 2001. Evolution of water balance in the genus *Drosophila*. *Journal of Experimental Biology* 204, 2331–2338.
- Gibbs, A., Mousseau, T.A., 1994. Thermal acclimation and genetic variation in cuticular lipids of the lesser migratory grasshopper (*Melanoplus sanguinipes*): effects of lipid composition on biophysical properties. *Physiological Zoology* 67, 1523–1543.
- Gibbs, A., Pomonis, J.G., 1995. Physical properties of insect cuticular hydrocarbons: the effects of chain length, methyl-branching and unsaturation. *Comparative Biochemistry and Physiology B* 112, 243–249.
- Gibbs, A., Mousseau, T.A., Crowe, J.H. 1991. Genetic and acclimatory effects on biophysical properties of insect cuticular lipids. *Proceedings of the National Academy of Sciences USA* 88, 7257–7260.
- Gibbs, A., Kuenzli, M., Blomquist, G.J., 1995. Sex- and age-related changes in the biophysical properties of cuticular lipids of the housefly, *Musca domestica*. *Archives of Insect Biochemistry and Physiology* 29, 87–97.
- Gibbs, A.G., Chippindale, A.K., Rose, M.R., 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *Journal of Experimental Biology* 200, 1821–1832.
- Gibbs, A.G., Louie, A.K., Ayala, J.A., 1998. Effects of temperature on cuticular lipids and water balance in a desert *Drosophila*: is thermal acclimation beneficial? *Journal of Experimental Biology* 201, 71–80.
- Golden, G.M., Guzek, D.B., Kennedy, A.H., McKie, J.E., Potts, R.O., 1987. Stratum corneum lipid phase transitions and water barrier properties. *Biochemistry* 26, 2382–2388.
- Hadley, N.F., 1977. Epicuticular lipids of the desert tenebrionid beetle, *Eleodes armatus*: seasonal and acclimatory effects on chemical composition. *Insect Biochemistry* 7, 277–283.
- Hadley, N.F., 1978. Cuticular permeability of desert tenebrionid beetles: correlations with epicuticular lipid composition. *Insect Biochemistry* 8, 17–22.
- Hadley, N.F., 1994. Ventilatory patterns and respiratory transpiration in adult terrestrial insects. *Physiological Zoology* 67, 175–189.
- Hadley, N.F., Quinlan, M.C., 1989. Cuticular permeability of the black widow spider *Latrodectus hesperus*. *Journal of Comparative Physiology B* 159, 243–248.
- Hadley, N.F., Schultz, T.D., 1987. Water loss in three species of tiger beetles (*Cicindela*): correlations with epicuticular hydrocarbons. *Journal of Insect Physiology* 33, 677–682.
- Holdgate, M.W., Seal, M., 1956. The epicuticular wax layers of the pupa *Tenebrio molitor* L. *Journal of Experimental Biology* 33, 82–108.
- Howard, R.W., 1993. Cuticular hydrocarbons and chemical communication. In: Stanley-Samuels, D.W., Nelson, D.R. (Eds.), *Insect Lipids: Chemistry, Biochemistry and Biology*. University of Nebraska Press, Lincoln, NE, pp. 179–226.
- Iyengar, B.T.R., Schlenk, H., 1968. Melting points of synthetic wax esters. *Lipids* 4, 28–30.
- Jetter, R., Scahher, S., Riederer, M., 2000. Leaf epicuticular waxes are arranged in chemically and mechanically distinct layers: evidence from *Prunus laurocerasus* L. *Plant. Cell and Environment* 23, 619–628.
- Liang, D., Silverman, J., 2000. “You are what you eat”: Diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften* 87, 412–416.
- Lighton, J.R.B., 1994. Discontinuous ventilation in terrestrial insects. *Physiological Zoology* 67, 142–162.
- Lockey, K.H., 1976. Cuticular hydrocarbons of *Locusta*, *Schistocerca* and *Periplaneta*, and their role in waterproofing. *Insect Biochemistry* 6, 457–472.
- Loveridge, J.P., 1968a. The control of water loss in *Locusta migratoria* R. & F. I. Cuticular water loss. *Journal of Experimental Biology* 49, 1–13.
- Loveridge, J.P., 1968b. The control of water loss in *Locusta migratoria* R. & F. II. Water loss through the spiracles. *Journal of Experimental Biology* 49, 15–29.
- Machin, J., Lampert, G.J., 1989. Energetics of water diffusion through the cuticular water barrier of *Periplaneta*: The effect of temperature, revisited. *Journal of Insect Physiology* 35, 437–445.
- Machin, J., Lampert, G.J., 1990. Evidence for water-organized structure in the cuticular water barrier of the American cockroach, *Periplaneta americana*. *Journal of Comparative Physiology B* 159, 739–744.
- Markow, T.A., Toolson, E.C., 1990. Temperature effects on epicuticular hydrocarbons and sexual isolation in *Drosophila mojavensis*. In: Barker, J.S.F., Starmer, W.T., MacIntyre, R.J. (Eds.), *Ecological and Evolutionary Genetics of Drosophila*. Plenum Press, New York, pp. 315–331.
- Mechaber, W.L., Marshall, D.B., Mechaber, R.A., Jobe, R.T., Chew, F.S., 1996. Mapping leaf surface landscapes. *Proceedings of the National Academy of Science USA* 93, 4600–4603.
- Merk, S., Blume, A., Riederer, M., 1998. Phase behaviour and crystallinity of plant cuticular waxes studied by Fourier transform infrared spectroscopy. *Planta* 204, 44–53.
- Nelson, D.R., Freeman, T.P., Buckner, J.S., 2000. Waxes and lipids associated with the external waxy structures of nymphs and pupae of the giant whitefly, *Aleurodicus dugesii*. *Comparative Biochemistry and Physiology Part B* 125, 265–278.
- Parasassi, T., De Stasio, G., Ravagnan, G., Gratton, G., 1991. Quantitation of lipid phases in phospholipid vesicles by the generalized polarization of laurdan fluorescence. *Journal of Biophysics* 60, 179–189.
- Patel, S., Nelson, D.R., Gibbs, A.G. 2001. Chemical and physical analyses of wax ester properties. *Journal of Insect Science*. 1.4, 8 pp. Available online: insectscience.org/1.4.

- Prange, H.D., 1996. Evaporative cooling in insects. *Journal of Insect Physiology* 42, 493–499.
- Ramsay, J.A., 1935. The evaporation of water from the cockroach. *Journal of Experimental Biology* 12, 373–383.
- Randall, D.J., Burggren, W., French, K., 1991. *Eckert Animal Physiology: Mechanisms and Adaptations*, 4th ed. W.H. Freeman & Co, New York.
- Reynhardt, E.C., Riederer, M., 1991. Structure and molecular dynamics of the cuticular wax from leaves of *Citrus aurantium* L. *Journal of Physics D. Applied Physics* 24, 478–486.
- Reynhardt, E.C., Riederer, M., 1994. Structures and molecular dynamics of plant waxes. II. Cuticular waxes from leaves of *Fagus sylvatica* L. and *Hordeum vulgare* L. *European Biophysics Journal* 23, 59–70.
- Rourke, B.C., 2000. Geographic and altitudinal variation in water balance and metabolic rate in a California grasshopper, *Melanoplus sanguinipes*. *Journal of Experimental Biology* 203, 2699–2712.
- Rourke, B.C., Gibbs, A.G., 1999. Effects of lipid phase transitions on cuticular permeability: model membrane and in situ studies. *Journal of Experimental Biology* 202, 3255–3262.
- Schmidt-Nielsen, K., 1990. *Animal Physiology: Adaptation and Environment*, 4th ed. Cambridge University Press, Cambridge.
- Schreiber, L., 1996. Wetting the upper needle surface of *Abies grandis*: influence of pH, wax chemistry and epiphyllic microflora on contact angles. *Plant, Cell and Environment* 19, 455–463.
- Schreiber, L., Riederer, M., 1996a. Determination of diffusion coefficients of octadecanoic acid in isolated cuticular waxes and their relationship to cuticular water permeabilities. *Plant, Cell and Environment* 19, 1075–1082.
- Schreiber, L., Riederer, M., 1996b. Ecophysiology of cuticular transpiration: comparative investigation of cuticular water permeability of plant species from different habitats. *Oecologia* 107, 426–432.
- Shine, R., Phillips, B., Waye, H., LeMaster, M., Mason, R.T., 2001. Benefits of female mimicry in snakes. *Nature* 414, 267.
- Small, D.M. (Ed.), 1986. *Physical Chemistry of Lipids. Handbook of Lipid Research*, vol. 4. Plenum Press, New York.
- Smith, B.C., 1996. *Fundamentals of Fourier Transform Infrared Spectroscopy*. CRC Press, Boca Raton, FL.
- Stanley-Samuels, D.W., Nelson, D.R. (Eds.), 1993. *Insect Lipids: Chemistry, Biochemistry and Biology*. University of Nebraska Press, Lincoln, NE.
- Stennett, M.D., Etges, W.J., 1997. Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. III. Epicuticular hydrocarbon variation is determined by use of different host plants in *Drosophila mojavensis* and *Drosophila arizonae*. *Journal of Chemical Ecology* 23, 2803–2824.
- Tocanne, J.F., Cézanne, L., Lopez, A., Pikhova, B., Schram, V., Tournier, J.F., Welby, M., 1994. Lipid domains and lipid/protein interactions in biological membranes. *Chemistry and Physics of Lipids* 73, 139–158.
- Toolson, E.C., 1982. Effects of rearing temperature on cuticle permeability and epicuticular lipid composition in *Drosophila pseudoobscura*. *Journal of Experimental Zoology* 222, 249–253.
- Toolson, E.C., 1984. Interindividual variation in epicuticular hydrocarbon composition and water loss rates of the cicada *Tibicen dealbatus* (Homoptera: Cicadidae). *Physiological Zoology* 57, 550–556.
- Toolson, E.C., Hadley, N.F., 1979. Seasonal effects on cuticular permeability and epicuticular lipid composition in *Centruroides sculpturatus* Ewing 1928 (Scorpiones: Buthidae). *Journal of Comparative Physiology B* 129, 319–325.
- Toolson, E.C., White, T.R., Glaunsinger, W.S., 1979. Electron paramagnetic resonance spectroscopy of spin-labelled cuticle of *Centruroides sculpturatus* (Scorpiones: Buthidae): correlation with thermal effects on cuticular permeability. *Journal of Insect Physiology* 25, 271–275.
- Wigglesworth, V.B., 1945. Transpiration through the cuticle of insects. *Journal of Experimental Biology* 21, 97–114.
- Williams, E.E., 1998. Membrane lipids: What membrane physical properties are conserved during physiochemically-induced membrane restructuring? *American Zoology* 38, 280–290.
- Willmer, P.G., Unwin, D.M., 1981. Field analyses of insect heat budgets: reflectance, size and heating rates. *Oecologia* 50, 250–255.
- Yoder, J.A., Denlinger, D.L., 1991a. Water balance in flesh fly pupae and water vapor absorption associated with diapause. *Journal of Experimental Biology* 157, 273–286.
- Yoder, J.A., Denlinger, D.L., 1991b. A comparison of the water balance characteristics of temperate and tropical fly pupae. *Physiological Entomology* 16, 375–380.
- Young, H.P., Larabee, J.K., Gibbs, A.G., Schal, C., 2000. Relationship between tissue-specific hydrocarbon profiles and lipid melting temperatures in the cockroach *Blattella germanica*. *Journal of Chemical Ecology* 26, 1245–1263.