Review

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

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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.

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

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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-

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ures, 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 ~2850 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 \textit{trans} configuration and absorb strongly at ~2849 cm\(^{-1}\). As temperature rises and lipids melt, thermal motion results in formation of \textit{cis} conformers, which absorb at ~2854 cm\(^{-1}\). The overall frequency of the symmetric stretching peak provides a direct indicator of the relative abundance of \textit{trans} and \textit{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). 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°C for \textit{Drosophila pseudoobscura} to >80°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 \textit{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
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 Etges, 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 $\geq 30^\circ C$ 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. 3](https://example.com/fig3.png)

**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 rightmost 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.

![Fig. 4](https://example.com/fig4.png)

**Fig. 4.** Regional variation in lipid properties. Surface lipids were isolated from different portions of female cockroaches, *Blatella 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 straightforward 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).

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 \( T_m \) values are well above their
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 $T_m$ cannot 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>
<thead>
<tr>
<th>Species or study system</th>
<th>Melting point?</th>
<th>Amount?</th>
<th>Alkenes?</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Melanoplus</em> populations</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Thermal acclimation of <em>D. mojavensis</em></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Desiccation-selected <em>D. melanogaster</em></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Drosophila</em> species</td>
<td>No</td>
<td>–</td>
<td>Yes</td>
</tr>
<tr>
<td>Houseflies (<em>Musca domestica</em>)</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Pogonomymex</em> species</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><em>P. barbatus</em> mating status</td>
<td>–</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

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 lipids 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.

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