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INTRA-INDIVIDUAL VARIATION IN CUTICULAR LIPIDS STUDIED USING FOURIER TRANSFORM INFRARED SPECTROSCOPY

ALLEN GIBBS* and JOHN H. CROWE
Department of Zoology, Storer Hall, University of California, Davis, CA 95616, U.S.A.

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Abstract—We report here a method for studying phase transitions in cuticular lipids of insects. This technique, Fourier transform infrared spectroscopy (FTIR), is more sensitive than previous biophysical methods and is applicable to either lipid extracts or lipids *in situ* (in cast skins or intact wings). We used FTIR to compare biophysical properties of cuticular lipids in different regions of individual insects. Lipid melting points varied by approx. 10°C in different body regions of the tropical cockroach, *Blaberus craniifer*, and by almost 30°C in the grasshopper, *Melanoplus sanguinipes*. Using cast skins, we followed the time course of lipid acclimation to temperature in single individuals of *M. sanguinipes*. We conclude that FTIR is useful for studies of spatial and temporal differences in cuticular lipids of arthropods.

Key Word Index: Cuticle; Fourier transform IR; hydrocarbon; phase transition; surface lipid

INTRODUCTION

Epicuticular lipids provide the primary passive barrier to water loss in terrestrial arthropods (Edney, 1977; Hadley, 1981). The physical properties of surface lipids help determine rates of water loss from intact organisms, and several investigators have postulated that lipid phase transitions are responsible for "critical" temperatures for water loss (Beament, 1945, 1964; Wigglesworth, 1945, 1986; Locke, 1965; Davis, 1974). The chemical composition of surface lipids has been studied extensively (Blomquist and Jackson, 1979), but only on rare occasions have physical properties been examined (Beament, 1945; Lockey, 1976; Toolson *et al.*, 1979; Gilby, 1980; Machin and Lampert, 1990). A major difficulty has been that methods used to study phase transitions in other lipid systems (differential scanning calorimetry, fluorescence polarization, electron paramagnetic resonance, capillary melting point) suffer from one or more of the following problems when applied to cuticles:

(1) Lipids must be extracted from the cuticle, raising the question of whether the results are applicable to the *in vivo* situation.
(2) The methods are imprecise when applied to complex mixtures with wide phase transitions, as is commonly the case for cuticular lipids.
(3) Lipid probe molecules may partition into a subfraction of the lipid layer, or may themselves affect lipid properties.

Finally, arthropods are small, whereas the amount of lipid required for most techniques is relatively large. It may be necessary to pool lipids from several individuals in order to obtain sufficient material, thus hiding inter-individual lipid variation (Toolson, 1984). Even if a technique is sensitive enough to analyse lipids from individual insects, water flux (and possibly lipid composition and physical properties) may not be the same over the entire cuticle (Hadley and Quinlan, 1987; Toolson and Hadley, 1987; Hadley *et al.*, 1989; Machin and Lampert, 1989). Regional lipid variation is difficult or impossible to study with available techniques.

We describe here a new technique for the study of cuticular lipids, Fourier transform infrared spectroscopy (FTIR). The advantages of FTIR spectrometers over older dispersive infrared machines have been detailed elsewhere (Braiman and Rothschild, 1988). Briefly, extremely precise and accurate frequency determination allows resolution of peaks to within 0.1 cm^{-1}, and higher light intensities allow examination of small, optically dense samples. Previous uses of FTIR to study lipid phase transitions in living cells (Cameron *et al.*, 1983; Crowe *et al.*, 1989a, b) and isolated stratum corneum from porcine skin (Golden *et al.*, 1987; Potts and Francoeur, 1990) suggested to us that the technique might be applicable to arthropod cuticles. In this paper, we demonstrate the applicability of FTIR for the study of cuticular

*To whom all correspondence should be addressed.

lipids, and we use FTIR to examine lipid variability in individual insects.

MATERIALS AND METHODS

Species studied

We used four insect species in this study. Mealworms (*Tenebrio molitor*) were purchased locally and maintained at 25°C. Grasshoppers (*Melanoplus sanguinipes*), a cricket (*Allonemobius fasciatus*) and a tropical cockroach (*Blaberus craniifer*) were from laboratory populations.

Sample preparation

Two types of sample were examined by FTIR. Lipid extracts were prepared by treatment of cast skins, wings or intact insects with chloroform or hexane. Lipids were deposited on infrared-transparent BaF_2 windows, the solvent was evaporated away, and the windows were mounted in a temperature-controlled cell holder (Crowe *et al.*, 1989b). For *in situ* lipid studies, cast skins, wings, or intact pieces of cuticle were simply sandwiched between two BaF_2 windows.

Fourier transform infrared spectroscopy

Methylene (—CH_2—) and methyl (—CH_3) moieties of lipids absorb infrared radiation at frequencies of 3000–2800 cm^{-1}. The absorbance maximum of the —CH_2— symmetric stretch occurs at ~2850 cm^{-1}. As lipids proceed through the gel-to-liquid crystalline melting transition, this peak shifts to higher wavenumbers (higher frequencies) (Crowe *et al.*, 1989b). The change in wavenumber reflects the isomerization of hydrocarbon chains from an all *trans*, straight-chain conformation in the gel phase to a predominantly *gauche* conformation in the liquid crystalline phase. Less than 5 cm^{-1} separates the —CH_2— symmetric stretch absorbance maxima above and below the phase transition. This difference is too small to be measured with dispersive infrared spectrometers, which typically have peak resolutions of several wavenumbers. With Fourier transform infrared spectrometers, however, peak locations can be determined with an accuracy and precision of better than 0.1 cm^{-1}, allowing the shift in the —CH_2— absorbance maximum to be used as an indication of lipid melting.

The FTIR spectrometer used in these experiments was a Perkin–Elmer model 1750 instrument, in the transmittance configuration, assisted by a Perkin–Elmer 7500 workstation. Data were analysed essentially as described previously (Crowe *et al.*, 1989b). Transmittance spectra (10–15 scans averaged) were converted to absorbance spectra, and baseline drift was removed using the instrument's software. The absorbance scale was arbitrarily expanded to one full-scale absorbance unit, for easy visualization and manipulation of the spectra. Spectra were of such high quality that no smoothing procedures were required. The location of the absorbance peak at about 2850 cm^{-1} was determined by eye to the nearest 0.1 cm^{-1}. Peak maxima were plotted against temperature, and midpoints of lipid phase transitions (T_m) were determined by probit analysis.

RESULTS

A typical infrared transmittance spectrum for a cast skin is shown in Fig. 1(A). Drift in the baseline indicates the presence of some light scattering, as might be expected in a sample composed largely of a repetitive polymer such as chitin. The portion of the spectrum enclosed by the box is the region of interest for this study. Figure 1(B) depicts absorbance spectra

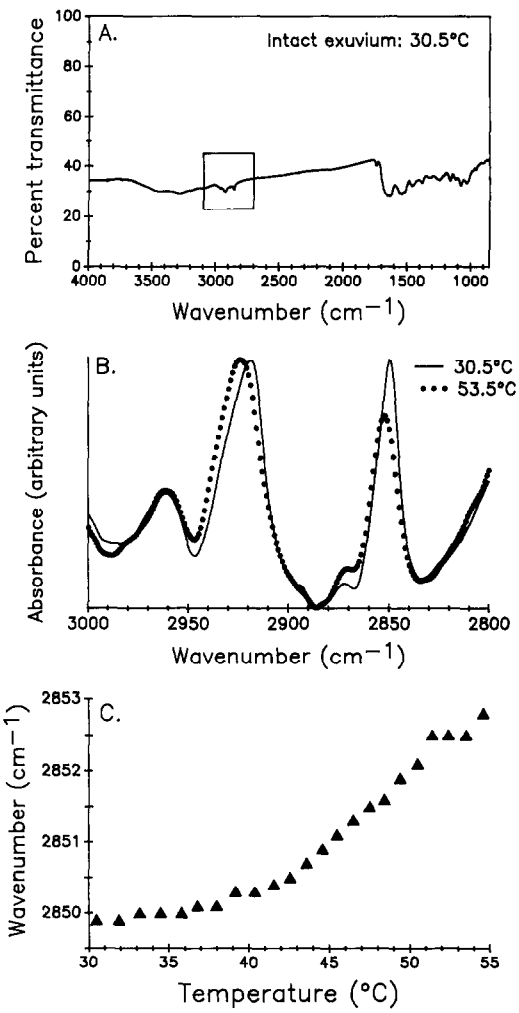

Fig. 1. Analysis of FTIR data. (A) Infrared transmittance spectrum for a cast skin from a grasshopper (*M. sanguinipes*). Box indicates the hydrocarbon infrared absorbance region. (B) Absorbance spectra for the same cast skin after data transformation (see Materials and Methods). Note the shift in the peak at about 2850 cm^{-1}. (C) Effect of temperature on the —CH_2— absorbance peak. The midpoint of the phase transition (T_m, calculated by probit analysis) was 45.6°C.

obtained from the same exuvium, following data manipulation (see Materials and Methods). These results demonstrate that adequate infrared spectra can be obtained even from a complex, optically dense sample such as a cast skin. Figure 1(C) depicts the effect of temperature on the frequency at which the absorbance maximum of the $-CH_2-$ symmetric stretch occurs. These data are qualitatively similar to lipid melting curves obtained in other systems, in which a shift in wavenumber corresponds to a gel-to-liquid crystalline melting transition of component lipids (Mantsch *et al.*, 1982; Cameron *et al.*, 1983; Crowe *et al.*, 1989a, b).

For three species of insect, we compared lipid extracts (from cast skins) with exuviae from the same species. In each species studied, the effects of temperature on wavenumber were similar in cast skins and lipid extracts (Fig. 2). The close correspondence between the data for intact skins and isolated lipids indicates that the shift to higher wavenumbers seen in the skins represented a lipid phase transition.

Fourier transform infrared spectra of de-lipidated (chloroform-extracted) exuviae were similar to the spectrum shown in Fig. 1(A) for an untreated sample (data not shown). The major difference was that absorbance in the hydrocarbon region [3000–2800 cm^{-1}, box in Fig. 1(A)] was barely detectable above background absorbance. A small peak at about 2850 cm^{-1} was observed, as a minor peak on the shoulder of a peak at about 2875 cm^{-1}. These peaks presumably represented absorbance by non-lipid components of the cuticle and trace amounts of unextracted lipid.

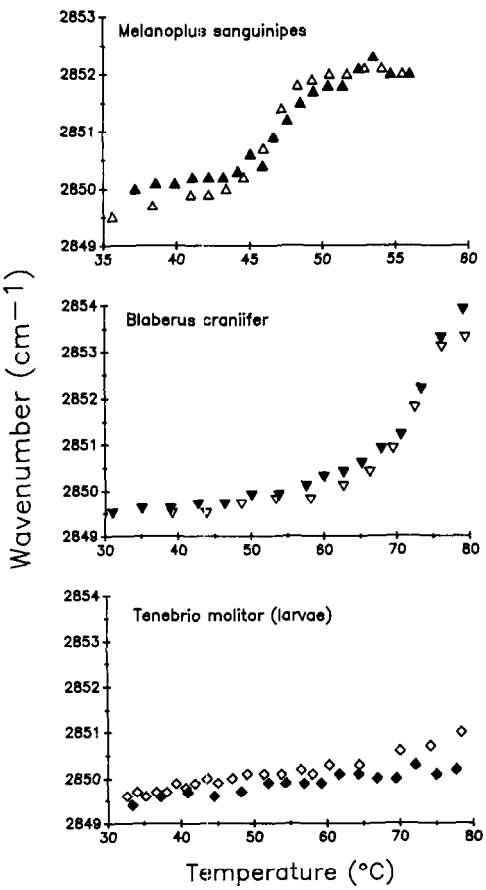

Fig. 2. Comparison of cast skins and lipid extracts from cast skins, in three insect species. Solid symbols: exuviae. Open symbols: lipid extracts from exuviae.

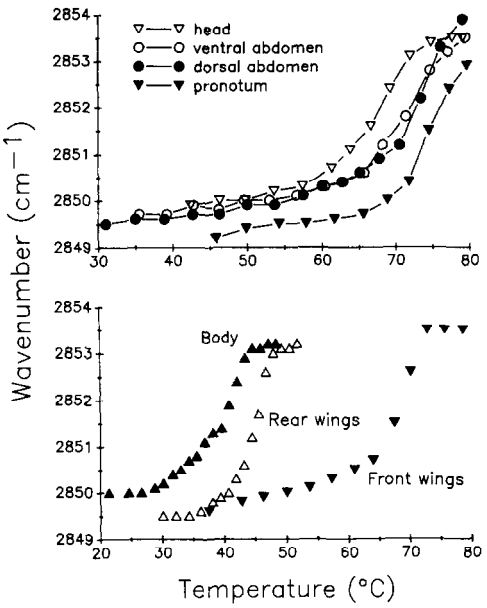

Fig. 3. Regional variation in lipid biophysical properties. Upper panel: lipid melting curves for four different areas of a single exuvium from a tropical cockroach, *B. craniifer*. Lower panel: lipid melting curves for lipid extracts from different parts of a single adult grasshopper, *M. sanguinipes*. Melting points were: body lipids, 37.9°C, rear wings, 43.4°C, front wings, 66.2°C.

We used FTIR to examine regional variation in cuticular lipids in two species (Fig. 3). In Fig. 3(A), lipid melting curves are shown for four areas of a single exuvium from a cockroach, *B. craniifer*. Melting points (T_m) varied by approx. 10°C, but were too high to determine accurately in our system. Regional lipid variation in the grasshopper, *M. sanguinipes*, was even greater. Melting points varied by almost 30°C between whole body surface lipids ($T_m = 37.9$°C) and lipids associated with the front pair of wings [$T_m = 66.2$°C; Fig. 3(B)].

Figure 4 depicts the effects of rearing regime on melting points in *M. sanguinipes*. In this example, two individuals were reared at 27°C through the second instar, at which point one each was transferred to "summer" (34°C, 15 h light–9 h dark) or "fall" (29°C, 11 h light–13 h dark) conditions [details in Dingle *et al.* (1990)]. Exuviae were collected after each moult, and melting points were determined in cast skins using FTIR. Lipid melting in each of these samples occurred over a 15–20°C range. The actual melting curve for the fourth-instar moult from the

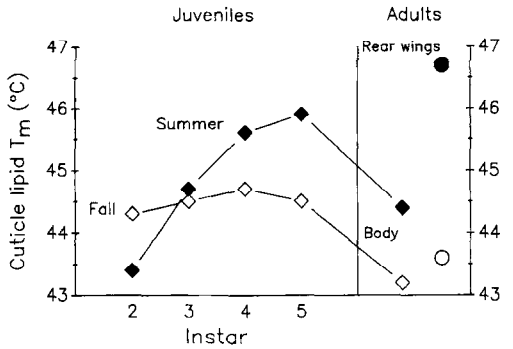

Fig. 4. Environmental effects on cuticular lipids in individual grasshoppers, *M. sanguinipes*, reared under "summer" or "fall" conditions (see text). For juveniles, melting points were determined in the cast skins deposited following each instar. For the adults, the wings were dissected out, and melting points were determined separately in intact front and rear wings. Melting points for front wings were 68.4 (summer) and 65.6°C (fall). Body lipids were extracted with hexane from de-winged adults and analysed separately.

summer-reared individual ($T_m = 45.6$°C) is shown in Fig. 1(C). For the adults, the front and rear pairs of wings were removed and examined separately. Body lipids of the de-winged adults were extracted with two 10 min hexane washes for analyses.

Lipid melting temperatures varied little in the fall-reared grasshopper, ranging from 44.3 to 44.7°C. In the summer-reared individual, melting points increased with each instar, from 43.4°C for the second-instar moult to 45.9°C for the fifth and final instar. In the adults, melting points for body surface lipids were lower than for the final-instar exuviae. Regional lipid variation was observed in both adults, similar to the results shown for a different individual in Fig. 3(B). Lipids on the rear pair of wings melted at somewhat higher temperatures than the body lipids, and melting points for lipids on the front wings were much higher (65.6 and 68.4°C, respectively, for fall- and summer-reared adults).

DISCUSSION

Previous infrared spectroscopic studies of cuticular lipids used older dispersive infrared instruments to describe general changes in classes of cuticular lipids. Fourier transform infrared spectroscopy provides orders of magnitudes improvements in accuracy, precision and sensitivity over dispersive infrared (Braiman and Rothschild, 1988). These improvements make FTIR an appropriate tool for biophysical measurements, not just a qualitative indicator of lipid composition.

Fourier transform infrared scans of exuviae provided adequate spectra in the hydrocarbon absorbance range (3000–2800 cm^{-1}, Fig. 1). The effects of temperature were similar in cast skins and lipid extracts (Fig. 2), suggesting that lipid phase transitions can be examined *in situ*, in exuviae. The order of increasing melting point for adult insects was the same as for increasing habitat temperature [*A. fasciatus* (cricket, not shown) < *M. sanguinipes* < *B. crenifer*]. These differences were consistent with previous interspecific comparisons of cuticular lipid composition (Toolson and Hadley, 1977; Hadley, 1978).

The case for the mealworm, *T. molitor* [Fig. 2(C)], is especially interesting. Our FTIR data indicate that cuticular lipids in this species had just begun to melt at the highest temperatures we could attain in our apparatus (80°C). Bursell and Clements (1967) found that epicuticular lipids of *T. molitor* larvae contain over 50% pentacosane-8,9-diol, which melts at 115°C. Cuticular lipid extracts from adult *T. molitor* melted at 44.5°C (data not shown), consistent with the lipid composition described for adults of this species (Lockey, 1978). The correspondence between our results and previous studies provides support for the idea that FTIR is a valid biophysical technique for lipid studies, in exuviae or in lipid extracts.

An alternative explanation is that some other cuticular component (e.g. —CH_2— moieties of cuticular proteins) absorbed at similar frequencies, and that the putative lipid phase transitions observed in exuviae actually represented a non-lipid phenomenon. To test this idea, we examined cast skins from which lipids had been removed by chloroform extraction. Transmittance spectra were similar to that shown in Fig. 1(A), except that absorbance by methylene moieties in the 3000–2800 cm^{-1} range was almost indetectable (data not shown). In particular, the peak at about 2850 cm^{-1} was greatly reduced; it appeared as only a minor peak on the shoulder of a peak at about 2875 cm^{-1} (data not shown). A minor 2875 cm^{-1} peak appeared in exuviae and lipid extracts [Fig. 1(B)] and probably represented the —CH_3 symmetric stretch. The small size of the 2850 cm^{-1} peak in de-lipidated exuviae made it difficult to reliably determine its location, but there was no clear transition in wavenumber, particularly not in the range of temperatures over which putative lipid phase transitions were observed in cast skins. We conclude that, in exuviae, non-lipid absorbance at about 2850 cm^{-1} made little contribution to the total. Thus, the change in wavenumber of this peak indicated a gel-to-liquid crystalline thermotropic phase transition of the cuticular lipids, not the effects of temperature on some other cuticular component. Similar studies extended these conclusions to lipid phase transitions in intact wings of insects.

The results presented above lead to an obvious question: can FTIR be applied to intact cuticles? Our attempts to do so using cuticle pieces from intact insects were unsuccessful. The presence of subsurface lipids, presumably epidermal and other cell membranes, made it impossible to distinguish phase transitions of epicuticular lipids from those of other components. Even vigorous chloroform rinsing of the internal side did not remove enough lipid from this side of the cuticle. Since FTIR provides a cumulative description of the effects of temperature on phase properties of all lipid classes, the signal from the

major lipid fraction present tends to swamp the responses of other components. We conclude that transmittance FTIR methods are not applicable to intact integuments. Reflectance FTIR techniques, which examine only surface properties, may be more useful for *in vivo* studies.

A major advantage of FTIR over other biophysical methods is its much greater sensitivity, both quantitatively (amount of sample required) and qualitatively (detail of information obtained). With regard to quantitative considerations, FTIR can be applied to samples containing less than 50 μg of lipid. We were able to compare different regions on a given individual (Fig. 3), and we obtained good-quality spectra and lipid melting curves from second-instar moults from grasshoppers which were less than 5 mm long (Fig. 4). Thus, FTIR is more sensitive than other biophysical techniques.

The data presented in Fig. 3 have important implications for physiological studies of water balance. Differences in lipid physical properties necessarily reflect compositional differences. Water loss rates can vary greatly between areas on the same animal (Hadley and Quinlan, 1987; Toolson and Hadley, 1987; Hadley *et al.*, 1989; Machin and Lampert, 1989), but the role of lipid variation in these differences has not been examined. Regional lipid variation implies that water fluxes, or the effects of temperature on water flux, measured on one portion of the cuticle may not accurately reflect the whole animal situation. Also, our findings have obvious implications for attempts to correlate surface lipid composition with organismal water loss (Toolson and Hadley, 1979; Toolson, 1982, 1984; Toolson and Kuper-Simbrón, 1989).

Fourier transform infrared spectroscopy also has qualitative advantages over other biophysical techniques. Complex mixtures of lipids, such as are found on insect cuticles, would be expected to melt over a wide temperature range, with low cooperativity. Differential scanning calorimetry, for example, would detect only broad isotherms, and melting points would be difficult to estimate reliably. By contrast, FTIR is an additive method in that melting of a portion of the lipid results in an increase in the average frequency for the $-CH_2-$ vibrations. Further warming leads to melting of additional lipid species and another increase in wavenumber. Thus, the method conceptually can be used to quantify the proportion of surface lipid that has melted.

Figure 1(C) shows a lipid phase transition in *M. sanguinipes* occurring over a range of more than 15°C. The midpoint of the phase transition was calculated to be 45.6°C, and is plotted in Fig. 4 as the melting point for the fourth-instar moult from the summer-reared individual. Phase transitions for other exuviae from the fall- and summer-reared individuals, and for wings and lipid extracts from adults, were equally broad. The consistency in the melting point for four separate exuviae from the fall-reared grasshopper (Fig. 4, range = 0.4°C) indicates that FTIR can be used to determine lipid melting points with excellent precision, even in cast skins.

Precise estimation of the melting point allows a fine-scale study of lipid acclimation to environmental conditions. Habitat conditions, particularly temperature, affect surface lipid composition within a species (Hadley, 1977; Toolson and Hadley, 1979; Toolson, 1982). The differences are similar to those observed in interspecific comparisons: longer average hydrocarbon chain lengths in individuals from warmer conditions. These differences are analogous to those described for cell membranes, in accordance with the theory of homeoviscous adaptation described for these systems (Cossins, 1983). Unlike the case for membranes, intraspecific changes in biophysical properties of cuticular lipids are not well-documented.

Figure 4 shows data suggesting changes in cuticular lipids in response to a difference in temperature of only 5°C. A more detailed description and analysis of surface lipid acclimation in *M. sanguinipes* will be presented elsewhere (Gibbs *et al.*, in press). However, we point out that this temperature difference is the smallest for which lipid acclimation has been described, for any lipid system.

In spite of its advantages, Fourier transform infrared does have limitations. It is a physical method; detailed information regarding surface lipid composition cannot be obtained, nor does FTIR provide quantitative information regarding the amount of lipid present. Other methods are available to study these questions. At present, the price of an FTIR spectrometer is relatively high.

We have had two principle aims in this paper: to demonstrate the general applicability of FTIR for biophysical studies of cuticular lipids, and to show the utility of FTIR for studies of a particular problem, namely, intra-individual lipid variation. Our data suggest that: (1) FTIR is applicable to cuticular lipid studies in cast skins or lipid extracts (Figs 1 and 2); (2) lipid physical properties may vary within a single insect, both regionally (Fig. 3) and temporally (Fig. 4); (3) small differences in lipid properties can be examined, even in exuviae containing complex mixtures of lipids with broad phase transitions (Fig. 4).

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REFERENCES

Beament J. W. L. (1945) The cuticular lipoids of insects. *J. exp. Biol.* **21**, 115–131.

Beament J. W. L. (1964) Active transport and passive movement of water in insects. *Adv. Insect Physiol.* **2**, 67–129.

Blomquist G. J. and Jackson L. L. (1979) Chemistry and biochemistry of insect waxes. *Prog. Lipid Res.* **17**, 319–345.

Braiman M. S. and Rothschild K. J. (1988) Fourier transform infrared techniques for probing membrane protein structure. *A. Rev. Biophys. biophys. Chem.* **17,** 541–570.

Bursell E. and Clements A. N. (1967) The cuticular lipids of larvae of *Tenebrio molitor* L. (Coleoptera). *J. Insect Physiol.* **13,** 1671–1678.

Cameron D. G., Martin A. and Mantsch H. H. (1983) Membrane isolation alters the gel to liquid crystalline transition of *Acholeplasma laidlawii* B. *Science* **219,** 180–182.

Cossins A. R. (1983) *Cellular Acclimitisation to Environmental Change* (Eds Cossins A. R. and Sheterline P.), pp. 3–32. Cambridge University Press, Cambridge.

Crowe J. H., Hoekstra F. A. and Crowe L. M. (1989a) Membrane phase transitions are responsible for imbibitional damage in dry pollen. *Proc. natn. Acad. Sci., U.S.A.* **86,** 520–523.

Crowe J. H., Hoekstra F. A., Crowe L. M., Anchordoguy T. A. and Drobnis E. (1989b) Lipid phase transitions measured in intact cells with Fourier transform infrared spectroscopy. *Cryobiology* **26,** 76–84.

Davis M.-T. B. (1974) Critical temperature and changes in cuticular lipids in the rabbit tick, *Haemaphysalis leporispalustris*. *J. Insect Physiol.* **20,** 1087–1100.

Dingle H., Mousseau T. A. and Scott S. M. (1990) Altitudinal variation in life cycle syndromes of California populations of the grasshopper, *Melanoplus sanguinipes* (F.). *Oecologia* **84,** 199–206.

Edney E. B. (1977) *Water Balance in Land Arthropods*, Springer, Berlin.

Gilby A. R. (1980) Transpiration, temperature and lipids in insect cuticle. In *Advances in Insect Physiology* (Eds Berridge M. J., Treherne J. E. and Wigglesworth V. B.), Vol. 15, pp. 1–33. Academic Press, New York.

Golden G. M., Guzek D. B., Kennedy A. H., McKie J. E. and 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 armata*: seasonal and acclimatory effects on composition. *Insect Biochem.* **7,** 277–283.

Hadley N. F. (1978) Cuticular permeability of desert tenebrionid beetles: correlations with epicuticular hydrocarbon composition. *Insect Biochem.* **8,** 17–22.

Hadley N. F. (1981) Cuticular lipids of terrestrial plants and arthropods: a comparison of their structure, composition, and waterproofing function. *Biol. Rev.* **56,** 23–47.

Hadley N. F. and Quinlan M. C. (1987) Permeability of arthrodial membrane to water: a first measurement using *in vivo* techniques. *Experientia* **43,** 164–166.

Hadley N. F., Toolson E. C. and Quinlan M. C. (1989) Regional differences in cuticular permeability in the desert cicada *Diceroprocta apache*: implications for evaporative cooling. *J. exp. Biol.* **141,** 219–230.

Locke M. (1965) Permeability of insect cuticle to water and lipids. *Science* **147,** 295–298.

Lockey K. H. (1976) Cuticular hydrocarbons of *Locusta*, *Schistocerca*, and *Periplaneta*, and their role in waterproofing. *Insect Biochem.* **6,** 457–472.

Lockey K. H. (1978) The adult cuticular hydrocarbons of *Tenebrio molitor* L. and *Tenebrio obscurus* F. (Coleoptera: Tenebrionidae). *Insect Biochem.* **8,** 237–250.

Machin J. and Lampert G. J. (1989) Energetics of water diffusion through the cuticular water barrier of *Periplaneta*: the effect of temperature, revisited. *J. Insect Physiol.* **35,** 437–445.

Machin J. and Lampert G. J. (1990) Evidence for water-organized structure in the cuticular water barrier of the American cockroach, *Periplaneta americana*. *J. comp. Physiol.* **159B,** 739–744.

Mantsch H. H., Cameron D. G., Tremblay P. A. and Kates M. (1982) Phosphatidylsulfocholine bilayers: an infrared spectroscopic characterization of the polymorphic phase behavior. *Biochim. biophys. Acta* **689,** 63–72.

Potts R. O. and Francoeur M. L. (1990) Lipid biophysics of water loss through the skin. *Proc. natn. Acad. Sci., U.S.A.* **87,** 3871–3873.

Toolson E. C. (1982) Effects of rearing temperature on cuticle permeability and epicuticular lipid composition in *Drosophila pseudoobscura*. *J. exp. Zool.* **222,** 249–253.

Toolson E. C. (1984) Interindividual variation in epicuticular hydrocarbon composition and water loss rates of the cicada *Tibicen dealbatus* (Homoptera: Cicadidae). *Physiol. Zool.* **57,** 550–556.

Toolson E. C. and Hadley N. F. (1977) Cuticular permeability and epicuticular lipid composition in two Arizona vejovid scorpions. *Physiol. Zool.* **50,** 323–330.

Toolson E. C. and Hadley N. F. (1979) Seasonal effects on cuticular permeability and epicuticular lipid composition *Centruroides sculturatus* Ewing 1928 (Scorpiones: Buthidae). *J. comp. Physiol.* **129B,** 319–325.

Toolson E. C. and Hadley N. F. (1987) Energy-dependent facilitation of transcuticular water flux contributes to evaporative cooling in the Sonoran Desert cicada, *Diceroprocta apache* (Homoptera: Cicadidae). *J. exp. Biol.* **131,** 439–444.

Toolson E. C. and Kuper-Simbrón R. (1989) Laboratory evolution of epicuticular hydrocarbon composition and cuticular permeability in *Drosophila pseudoobscura*: effects of sexual dimorphism and thermal-acclimation ability. *Evolution* **43,** 468–473.

Toolson E. C., White T. R. and Glaunsinger W. S. (1979) Electron paramagnetic resonance spectroscopy of spin-labelled cuticle of *Centruroides sculpturatus* (Scorpiones: Buthidae): correlation with thermal effects on cuticular permeability. *J. Insect Physiol.* **25,** 271–275.

Wigglesworth V. B. (1945) Transpiration through the cuticle of insects. *J. exp. Biol.* **21,** 97–114.

Wigglesworth V. B. (1986) Temperature and the transpiration of water through the cuticle. *Tissue Cell* **18,** 99–115.