Review

Thermodynamics of cuticular transpiration☆

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ABSTRACT

Water conservation is a significant physiological problem for many insects, particularly as temperature increases. Early experimental work supported the concept of a transition temperature, above which water-loss rates increase rapidly as temperature increases. The transition phenomenon was hypothesized to result from melting of epicuticular lipids, the main barrier to cuticular transpiration. This explanation has been challenged on theoretical grounds, leading to thermodynamic analyses of cuticular transpiration based on reaction rate theory. These studies have not directly addressed the mechanistic basis of the transition temperature. Models developed in the context of cell membrane transport provide potential explanations that can be tested experimentally. These models include changes in the activation entropy for diffusion through the cuticular lipids, increased solubility of water in melted lipids, and lateral heterogeneity of the cuticle.

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1. Introduction

One of the most important environmental challenges facing terrestrial animals is scarcity of water. The chemical activity of water outside the animal is generally lower than inside, so that water will tend to leave via transpiration through the integument. These problems are exacerbated in insects and other small animals that have a relatively large surface area:volume ratio; i.e. a relatively large surface through which the small internal water volume may escape. In spite of this fundamental physical constraint, insects are abundant and diverse, even in the driest deserts.

The problem of water balance in insects has been recognized for decades (Ramsay, 1935; Beament, 1945; Wigglesworth, 1945;

Edney, 1977; Hadley, 1994; Gibbs and Rajpurohit, 2010). Insects can lose water by evaporation through the spiracles, secretion from the mouth or anus, or transpiration through the cuticle. The relative importance of each route varies depending on insect activity and other factors, but cuticular transpiration typically accounts for >80% of water loss from quiescent animals (Quinlan and Gibbs, 2006). One of the major structures allowing insects to survive and thrive is a thin layer of lipids on the surface of the cuticle. The composition of these lipids has been investigated in numerous taxa (Blomquist and Bagneres, 2010). Long-chain hydrocarbons are usually the most abundant, but a variety of other compounds (esters, ketones, fatty acids, etc.) have also been described.

The insect cuticle is multilayered, consisting of the procuticle and the epicuticular lipids (Neville, 1975). The procuticle contains the inner endocuticle and outer exocuticle, which differ in their chemical and physical properties. Transpiration of water through the cuticle minimally requires three steps: movement of water from epidermal cells into the procuticle, diffusion across the procuticle into the epicuticular lipids, and evaporation from the

epicuticle. Diffusion into and through the epicuticular lipids is likely to be the slowest step, due to the hydrophobic nature of the lipids. The barrier function of the procuticle cannot be ignored, however. Deposits of hydrophobic melanin granules in the exocuticle may have a significant effect on overall water balance. Recent studies of *Drosophila* species have found that reduced water-loss rates are not correlated with greater amounts of epicuticular lipids, but are associated with increased melanization of the procuticle (Rajpurohit et al., 2008; Parkash et al., 2011).

2. Effects of temperature on cuticular transpiration

Temperature has dramatic effects on water loss from insects, and in many cases a relatively sharp transition is apparent. Ramsay (1935) was the first to note this phenomenon, and proposed that it was the result of melting of the epicuticular lipids. Below the transition temperature, lipids would be in a solid, impermeable state. Melting at the transition temperature would cause cuticular permeability to increase, because melted lipids would provide less of an energetic barrier to water flux than solid lipids. This hypothesis became widely accepted, but a number of criticisms have been raised.

The first critique is that the transition phenomenon itself is a visual artefact created by the manner in which data are presented. The saturation vapor pressure of water increases exponentially with temperature. Insect tissues typically have a chemical activity of water >0.99; i.e. insects will tend to lose water whenever ambient humidity is below 99% RH. Thus, transition temperatures might simply be caused by an exponentially increasing gradient for water loss. To correct for this, water-loss rates are usually divided by the vapor pressure deficit, the difference between the saturating and actual absolute humidity at the experimental temperature. Fig. 1 depicts water-loss from a grasshopper, *Melanoplus sanguinipes*, with and without correction for the vapor pressure deficit. When the correction is done, a transition appears to occur at ~47 °C. Perhaps coincidentally, cuticular lipids extracted from this individual melted at ~47 °C.

Toolson (1978, 1980) argued that correction for vapor pressure deficit was inappropriate, and that a linear relationship exists between temperature and the gradient for water loss across the cuticle, at least under typical experimental conditions. Thus, apparent transitions in water loss might be graphical artefacts. This conclusion was challenged by Monteith and Campbell (1980) and led to further theoretical work by Gelman et al. (1988) and Gelman and Machin (1994). They concluded that correction for vapor pressure deficit was valid. Their work did not, however, address the issue of the existence and cause of transitions in water

loss. Temperature was incorporated directly into thermodynamic equations, but the effects of temperature itself on cuticular permeability were not examined, and their models assumed a simple, homogeneous cuticle.

3. Arrhenius plots and water-loss rates

Thermodynamic analyses of water loss ultimately derive from the work of Svante Arrhenius on chemical reactions in the late 19th century. The general formula for Arrhenius relationships is:

$$k = A \times \exp\left(\frac{-\Delta G^{\ddagger}}{RT}\right) = A \times \exp\left(\frac{-(\Delta H^{\ddagger} - T\Delta S^{\ddagger})}{RT}\right) \quad (1)$$

where k is the rate of reaction, R is the gas constant; $\Delta G^{\ddagger}$ is the *apparent* free energy of activation (i.e. the apparent difference in Gibbs free energy between the transition and ground states of the reaction, sometimes written as E_a), T is the absolute temperature, and A is a reaction-specific factor that takes into account steric factors (i.e. the likelihood that the reactants will be in the correct orientation or striking each other at an appropriate angle to react successfully) and other variables, including properties of the medium in which the reaction occurs. $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are, respectively, the apparent enthalpy and entropy of activation. Each of the parameters A, $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ may depend upon temperature, even in simple chemical reactions (Berry et al., 1980). The Arrhenius equation can be rearranged and transformed as a linear equation:

$$\ln(k) = \ln(A) \frac{-\Delta G^{\ddagger}}{RT} = \ln(A) \frac{-\Delta H^{\ddagger}}{RT} + \frac{\Delta S^{\ddagger}}{R} \quad (2)$$

For *simple* chemical reactions, Arrhenius plots of the logarithm of the reaction rate *vs.* the inverse of the absolute temperature are linear, and the slope equals $-\Delta G^{\ddagger}/R$ (= $\Delta H^{\ddagger}/R + \Delta S^{\ddagger}/RT$). Biological reactions are not simple, however, and non-linear Arrhenius plots are common. This is particularly true for membrane processes, such as the activity of integral membrane enzymes or transporters. Typically, Arrhenius plots are steeper at low temperatures (i.e. they are concave downward). Approximately 30 years ago, numerous publications explained biphasic Arrhenius plots as the result of changes in the physical state of membrane lipids (Klein, 1982). The general idea was that $\Delta H^{\ddagger}$ was high when the membrane was in a viscous gel state, then decreased as lipids melted and allowed proteins to undergo conformational changes more easily. Thus, non-linear Arrhenius plots would be caused by changes in the enthalpy of activation. In many cases, no supporting biophysical measurements were performed, and theoretical analysis suggested
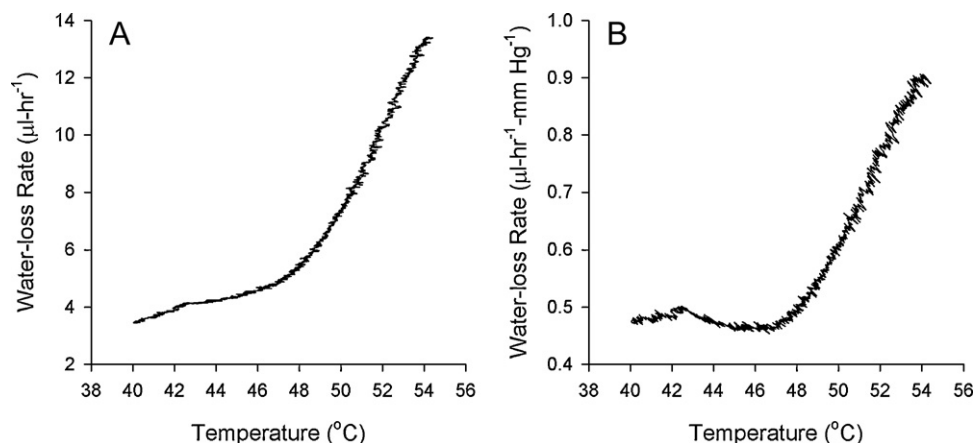

Fig. 1. Effects of temperature on water loss from a cyanide-killed grasshopper, *Melanoplus sanguinipes*. (A) Uncorrected water-rates. (B) Water-loss rates corrected for saturation deficit. Original data from Rourke and Gibbs (1999).
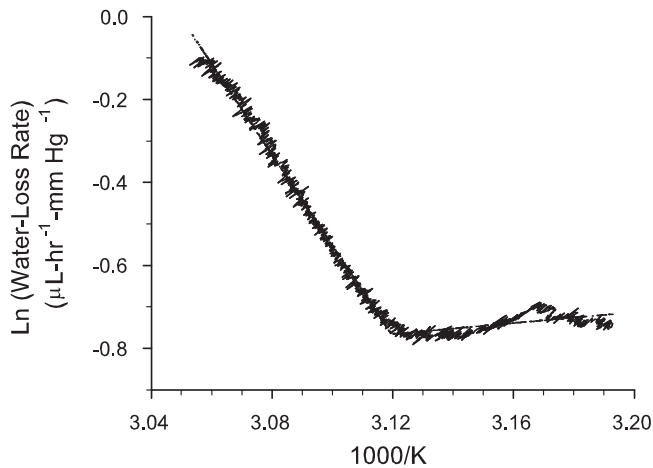
Fig. 2. Data from Fig. 1B, re-plotted as an Arrhenius plot.

that sharp breakpoints in membrane protein activity were unlikely (Silvius and McElhaney, 1981).

When water-loss rates from insects are plotted using the Arrhenius equation, transition temperatures can disappear, suggesting that transpiration through the cuticle is a relatively simple process. In many cases, however, Arrhenius plots are non-linear. Fig. 2 depicts the same data as Fig. 1B, re-plotted as an Arrhenius plot. The plot is biphasic, with a transition ('breakpoint') at ~47 °C. Note that the slope ($-\Delta G^{\ddagger}/R$) increases at high temperature, a common observation for cuticular transpiration. Many authors have assumed that rapidly increasing water-loss rates are observed because it is "easier" for water molecules to move through fluid lipids. Yoder et al. (2005b) argued that this pattern does not make sense. They point out that if molecules with less kinetic energy are able to penetrate the surface lipids, then the slope of an Arrhenius plot should decrease, not increase, at high temperatures. This argument is intuitively appealing, but it takes into consideration only the enthalpic component of the overall activation energy. As discussed below, thermodynamic models indicate that non-linear Arrhenius plots can arise when the entropic component of the Gibbs free energy is temperature dependent.

4. Thermodynamics of transport processes

In a review, Klein (1982) summarized a large number of experimental and theoretical studies on the effects of temperature
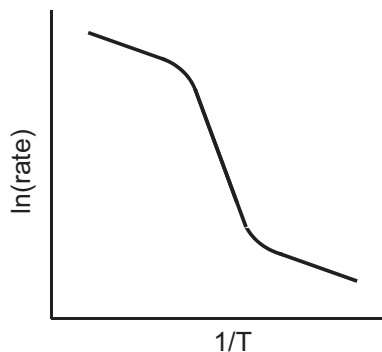

Fig. 3. Effects of temperature on membrane transportation rates, using the partition model described by Klein (1982). The model assumes that membrane transporters preferentially partition into fluid membranes, activation enthalpy ($\Delta H^{\ddagger}$) is the same in solid and fluid membranes, and activation entropy ($\Delta S^{\ddagger}$) is higher in fluid membranes.

on membrane proteins. Included is an extensive discussion of possible causes of non-linear Arrhenius plots for membrane enzymes, some of which can be extended to cuticular transpiration. In particular, a partition model, in which membrane transport proteins preferentially occur in fluid or gel phase regions of the membrane, can result in triphasic Arrhenius plots (Fig. 3). Depending on the experimental range of temperatures, one may find downwardly concave, upwardly concave, or linear Arrhenius plots. This model assumes that $\Delta H^{\ddagger}$ (the activation enthalpy) is the same in fluid and gel phase regions, but $\Delta S^{\ddagger}$ (the activation entropy) is higher in the melted phase. In other words, the entropic contribution to the activation energy changes, and this is what causes non-linearity. Transporters preferentially begin to enter fluid regions of the membrane (where activity is higher) as it starts to melt, causing the upward inflection. Once the membrane is completely melted, partitioning between phases is no longer an issue, and the apparent $\Delta G^{\ddagger}$ returns to its original value (downward inflection). The general shape of the relationship is consistent for a variety of model parameters (including the partition coefficient, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and the range of temperatures over which lipid melting occurs), but the overall curve will shift left or right depending upon the partition coefficient. Neither of the inflection points need correspond to the melting temperature.

Klein's partition model was developed in the context of membrane transport proteins. To extend the model to cuticular transpiration, we need to think in terms of transport sites for water rather than protein transporters. Water diffuses through phospholipid bilayers via defects in packing between adjacent fatty acyl chains (Carruthers and Melchior, 1983; Deamer and Bramhall, 1986). Cuticular hydrocarbons such as n-alkanes do not form bilayers, but the chains do pack tightly in the solid state (Bonsor and Bloor, 1977; Maroncelli et al., 1982). As hydrocarbons melt, their carbon backbone undergoes trans-cis isomerizations, creating spaces into which water molecules can enter and diffuse through the lipid layer. In terms of the model, water will tend to partition into melted lipids relative to non-melted lipids.

It is important to emphasize that Klein's model does not require any differences between solid and fluid lipids in activation enthalpy ($\Delta H^{\ddagger}$). Instead, it assumes a higher activation entropy ($\Delta S^{\ddagger}$) in fluid membranes. In insect cuticle, water diffusing through melted lipids will be moving through a high-entropy, disordered environment relative to densely packed lipids below the melting point. High-entropy states are favored under the Second Law of Thermodynamics, so entry of water into fluid lipids (the transition state of the process) will be more favorable thermodynamically. Note that most studies of insect water loss find only the upward inflection point, while Klein's model predicts a second, downward inflection at higher temperatures. This is likely a result of the limited range of experimental temperatures used in most studies.

Klein's (1982) model does not consider the effects of a change in transporter number, but an effective increase in transport capacity may occur as cuticular lipids melt. This is because the total number of lipid defects will increase, so that the total capacity to hold water increases. In effect, water is more soluble in melted than solid lipids. Increased water content of the lipid layer will result in greater evaporation from the cuticle, independently of changes in thermodynamic parameters. This is important in relation to the theory developed by Gelman and Machin (1994), which assumes a low, constant water concentration in the lipid layer.

An alternative explanation for non-linear, concave-upward Arrhenius plots is spatial non-homogeneity of the cuticle. For example, arthrodial membrane is more permeable than other cuticular regions (Hadley and Quinlan, 1987), but accounts for only a small percentage of the total surface area. If the activation energy for transpiration through the arthrodial membranes is greater than for bulk cuticle, arthrodial transpiration will increase more rapidly

with temperature than bulk flux, and eventually will exceed bulk water loss. This will cause an upward inflection on an Arrhenius plot. It must be noted that this example does not rely on any changes in the physical properties of cuticular lipids. Non-linear plots have been observed in solvent-extracted ticks (Yoder et al., 2005a), consistent with this idea.

Cuticular lipids can also exhibit spatial variation across the cuticle. Infrared spectroscopy detected differences in lipid physical properties between the body and wings of *M. sanguinipes* (Gibbs and Crowe, 1991), and recent work has detected differences in composition within individual fruitflies (Yew et al., 2008) and beetles (Hughes et al., 2011). Because cuticular lipids are the main barrier to transpiration, variation in physical properties, composition, or amounts could easily result in varying cuticular permeability.

At a smaller spatial scale, the cuticular lipids themselves may form a non-homogeneous barrier to water loss. Small (1986) described biophysical measurements of mixtures of alkanes and alkenes differing in melting point by >50 °C, and reported that these compounds exhibit phase separation over a wide range of temperatures. The higher melting alkanes form uniform crystals alongside liquid regions of alkenes. These hydrocarbons are the most common lipids found on insect cuticle, suggesting that phase separation may be a widespread phenomenon. Unfortunately, Small (1986) does not provide any information on the spatial scale of phase separation, which is necessary information to judge whether phase separation may be biologically relevant.

5. Summary

One must use care in extending membrane phenomena to cuticular lipids, as the eventual discrediting of Beament (1958) monolayer hypothesis illustrates. I argue here that a modified version of Klein's (1982) model is consistent with observations of transition temperatures and can be interpreted in light of how we think water moves through lipid layers. It provides a potential thermodynamic explanation for Ramsay's (1935) hypothesis that melting of cuticular lipids causes transition temperatures. However, my arguments are not rigorous thermodynamic demonstrations, and more theoretical work is certainly needed. Other thermodynamic models may explain non-linear Arrhenius plots, and Klein (1982) discusses several of these. Inhomogeneity of the cuticle, at multiple scales, might also account for transitions in organismal transpiration rates. Our understanding of the structure and properties of insect cuticle is limited, and new experimental techniques, perhaps borrowed from nanotechnology and materials science (e.g. Sun et al., 2008), should be explored.

Acknowledgements

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