

SHORT COMMUNICATION

ENERGY-DEPENDENT FACILITATION OF TRANSCUTICULAR WATER FLUX CONTRIBUTES TO EVAPORATIVE COOLING IN THE SONORAN DESERT CICADA, *DICEROPROCTA APACHE* (HOMOPTERA: CICADIDAE)

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Terrestrial arthropods exhibit an array of impressive adaptations for conserving water, including highly waterproof cuticles, the ability to reduce excretory losses by resorption of water from the faeces and, in some species, the ability to absorb water vapour from subsaturated atmospheres (Edney, 1977). The possibility of an additional adaptation has been inferred from the observation that gravimetric water loss rates of many terrestrial arthropods increase significantly following death, even when body openings, e.g. spiracles, are sealed. This increase, which may be as large as five-fold (Toolson & Hadley, 1979), has been interpreted as evidence for an energy-dependent process that actively retards loss of water through the cuticle (Winston & Beament, 1969). The mechanistic basis for the phenomenon, however, is controversial (Berridge, 1970; Riddle, 1981; Machin, Lampert & O'Donnell, 1985).

In contrast with the typical situation, whole-animal gravimetric water loss rates of cicadas decrease following death. In *Tibicen dealbatus*, the decrease averages more than 40% at 25°C, and the amount of decrease in each cicada is proportional to its surface area. Based on these findings, Toolson proposed that the higher water loss rates of live cicadas result from activity of an energy-dependent process that increases rates of transcuticular water flux (TWF), and that the facilitation of TWF plays an important role in evaporative cooling (Toolson, 1984). The present study was undertaken as part of a project investigating evaporative cooling in *Diceroprocta apache*, and was designed to test the hypothesis of active facilitation of TWF by cicadas.

Individual *D. apache* were collected during July and August, 1986, on the campus of Arizona State University, Tempe, Arizona. Cicadas were taken to the laboratory and used immediately. TWF was measured *in vivo* by monitoring the moisture content of air passed through a ventilated capsule placed in direct contact with the

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cuticle. Dry air entering the capsule inlet tube acquires water that has diffused through the cuticle and exits *via* an outlet tube leading to an aluminium oxide sensor. Data are obtained in the form of continuous recordings of sensor output (V) as a function of time, and are converted to TWF values ($\text{mg H}_2\text{O cm}^{-2} \text{h}^{-1}$) using an empirically derived calibration curve. Details of the system's design, operation and calibration are given in Hadley, Machin & Quinlan (1986).

Test cicadas were anaesthetized by a 1-min exposure to CO_2 and the capsule was placed in firm contact with the dorsal mesonotum (Fig. 2A). The base of the capsule was sealed to the cuticle using quick-setting epoxy resin applied around the outer edge of the capsule. The temperature and humidity to which each cicada was exposed were controlled externally by placing the cicada and attached capsule inside a sealed Plexiglas[®] cylinder which was submerged in a temperature-controlled ($\pm 0.1^\circ\text{C}$) water bath set initially at 25.0°C . The temperature of the capsule (and, therefore, the air immediately surrounding the mesonotal cuticle) was measured with a copper-constantan thermocouple connected to a BAT-12 thermometer. Flow rates through the ventilated capsule were monitored with a soap bubble flowmeter. TWF was recorded only after the system had stabilized. The chamber and capsule temperature was then rapidly raised to 30.0°C ($\pm 0.2^\circ\text{C}$) and TWF recorded for a minimum of 10 min following stabilization. TWF determination was repeated at 35°C , and subsequently at intervals of 2°C from 37 to 43°C . Attempts to measure TWF at 45°C and above usually resulted in breaching of the epoxy seal between the capsule and the cuticular surface. In all, 10 live cicadas (six males, four females) and six dead cicadas (three males, three females, killed by exposure to cyanide vapour) were used.

An illustrative tracing from a live male *D. apache* is shown in Fig. 1. At temperatures of 39°C and below, the typical *D. apache* pattern of (i) small increases in TWF with each increase in temperature and (ii) relatively constant TWF values at each temperature is evident. Increasing the temperature to 39°C , however, resulted in a transient surge in TWF to $9.4 \text{ mg H}_2\text{O cm}^{-2} \text{h}^{-1}$. Such a surge was not always observed, but was not an uncommon feature of the tracings at 39°C . The results of increasing temperature to 41°C are typical of all live cicadas used in our experiments. TWF increased rapidly to a plateau at about $36 \text{ mg H}_2\text{O cm}^{-2} \text{h}^{-1}$. When temperature was increased to 43°C , TWF rapidly increased to values as high as $70.4 \text{ mg H}_2\text{O cm}^{-2} \text{h}^{-1}$. The cyclic changes in TWF apparent at 41 and 43°C were observed only in living cicadas. The process responsible for the increase in TWF is energy-dependent; intrathoracic injection of aqueous NaCN was followed by an immediate disappearance of the cyclic fluctuations in TWF and a rapid decline in TWF to values typical to those of the dead controls.

Summary statistics of the data from the 10 live and six dead (control) cicadas are presented in Table 1. Because all live cicadas exhibited cyclic patterns of TWF at 41 and 43°C (and at 45°C when data could be obtained), average minima and maxima of the TWF cycles rather than overall average TWF values are presented. Even at 25°C , TWF values of *D. apache* are considerably greater than any hitherto reported for xeric-adapted arthropods, in which values less than $0.5 \text{ mg cm}^{-2} \text{h}^{-1}$ are the rule (Edney, 1977). No intersexual difference in TWF was detected.

Increasing temperature affects TWF in *D. apache* somewhat differently than it does in other terrestrial arthropods. Between 25 and 39°C, TWF of both live and dead cicadas increased less rapidly ($Q_{10} = 1.5$) than is usually observed in terrestrial arthropods. Between 39 and 43°C, TWF of dead cicadas continues to increase relatively slowly ($Q_{10} = 1.7$), but in live individuals, TWF increases by nearly 600% over the same temperature range with a four- to five-fold increase occurring between 39 and 41°C.

The TWF-facilitating process is not the result of thermal disruption of the epicuticular lipids or other normal cuticular function, as is demonstrated by the low Q_{10} values in dead individuals and the effect of cyanide on the process. Furthermore, during isothermal runs at 41°C, cessation of cycling and significant decreases in TWF were observed in some individuals after 1 or 2 h (E. C. Toolson & N. F. Hadley, unpublished data). Whether this phenomenon represents a response to

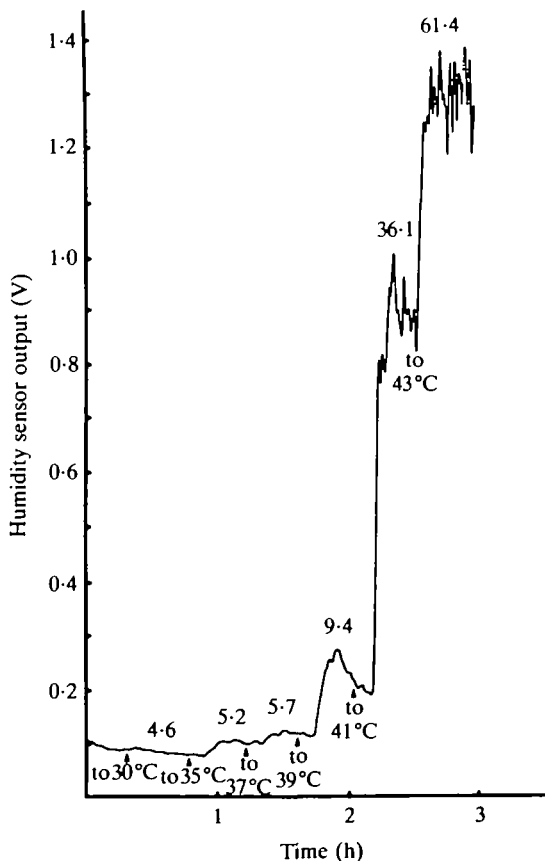


Fig. 1. Effects of temperature on transcuticular water flux (TWF) in a live male *Diceroprocta apache*. The ordinate presents humidity sensor output (in volts). Arrows indicate ramping of temperature to the next higher value. Horizontal bars above the tracing indicate the interval that the cicada's temperature was stable at the desired value. Numbers above horizontal bars represent average TWF ($\text{mg H}_2\text{O cm}^{-2} \text{h}^{-1}$) during each interval.

Table 1. *Effects of temperature on transcuticular water flux in Diceroprocta apache*

Temperature (°C)	Transcuticular water flux (mg H ₂ O cm ⁻² h ⁻¹)	
	Live (N = 10)	Dead (N = 6)
25.0	5.3 ± 1.23	6.4 ± 1.49
30.0	5.8 ± 1.11	7.6 ± 1.84
35.0	6.8 ± 1.32	9.2 ± 2.12
37.0	7.3 ± 1.42	9.8 ± 2.16
39.0	8.8 ± 1.64	11.0 ± 2.13
41.0	Minima: 33.4 ± 5.49	12.1 ± 2.19
	Maxima: 47.6 ± 6.44	
43.0	Minima: 46.4 ± 7.35	13.7 ± 2.35
	Maxima: 65.7 ± 12.88	

Values are presented as $\bar{x} \pm \text{S.E.M.}$

dehydration is unknown, but it is further evidence that the TWF-facilitating process can be regulated, even at high ambient temperatures.

Although physiological regulation of cuticular permeability and water flux through the cuticle of terrestrial arthropods was proposed years ago (Winston & Beament, 1969), previous studies have yielded negative or equivocal results (Riddle, 1981; Machin *et al.* 1985), and our direct demonstration of short-term physiological regulation of TWF is unique. When the TWF-facilitating process is activated, whole-animal gravimetric water loss rates (WLR) in excess of 10 mg cm⁻² h⁻¹ are observed (Toolson, 1987). Since average masses of adult male and female *D. apache* are only 0.6 ± 0.02 g and 0.7 ± 0.02 g, respectively, of which 66.2 ± 0.4 % is water, such high WLRs can result in loss of more than 30 % of a cicada's total body water in a single hour.

The adaptive function of TWF facilitation in *D. apache* (and other cicadas) appears to be related to evaporative cooling at high ambient temperatures. Activation of the TWF-facilitating process occurs when body temperature reaches 39–41 °C, which corresponds precisely to the temperature range at which evaporative cooling commences in this species (Toolson, 1987). Because *D. apache* can increase TWF so markedly, it is surprisingly effective at evaporatively cooling and thermoregulating at high ambient temperatures. In laboratory experiments at an ambient temperature of 45.5 °C, individual *D. apache* can maintain body temperatures from 2–5 °C below ambient for over an hour, even while denied access to water. This ability is crucial to the survival and reproduction of *D. apache* in its natural habitat, where high ambient temperatures (often in excess of 42–43 °C) and radiant heat loadings would tend to result in a rapid increase of its body temperature to a level in excess of its upper critical temperature of 45.6 °C (Heath & Wilkin, 1970).

Although the mechanism underlying the TWF facilitation is unclear, we have tentatively identified the route by which the water moves through the cuticle. In the dorsal mesonotum of *D. apache*, there are three tracts of pores, one along the mid-dorsal line and two lateral tracts approximately 1–2 mm to either side of the central tract (Fig. 2A). These large pores (average diameter approx. 7 µm; Fig. 2B,C) are

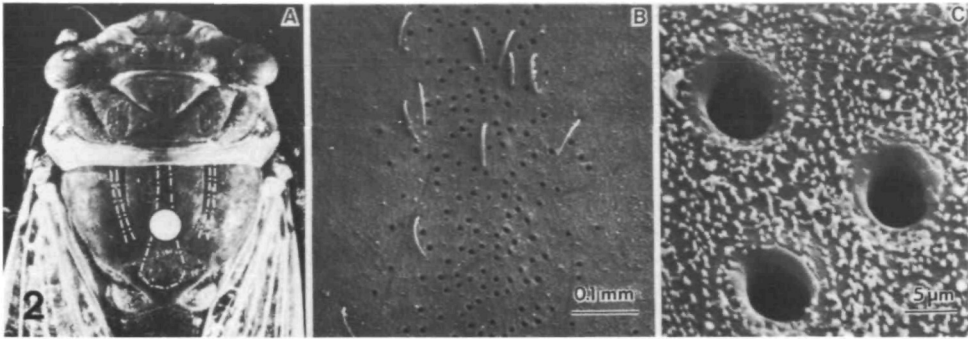


Fig. 2. (A) Scanning light microscopic view ($\times 5$) of dorsal aspect of an adult *Diceroprocta apache*. Dashed lines indicate the three tracts of mesonotal pores discussed in the text. The circle indicates placement and diameter (1 mm) of the *in vivo* capsule opening. (B) Low-magnification scanning electron microscope view of part of the central tract. (C) High-magnification scanning electron microscope view of three pores from the central tract.

also widely distributed over the abdominal surface and are probably the openings to dermal gland ducts which originate in the epidermis and typically penetrate the cuticle surface. Dermal gland ducts are thought to be the route by which components of the epicuticular cement layer reach the surface and they may also be a pathway for ion transport through the cuticle (Neville, 1975). In cicadas, these dermal gland ducts may serve as low-resistance pathways through which water is extruded onto the cuticle surface.

The data raise some interesting questions. For example, between 25 and 39°C, TWF rates of live cicadas were consistently less than that of dead individuals. Although not statistically significant at any one temperature (Mann–Whitney U-test; $P > 0.20$ in each case), the difference is large enough (averaging 22.5% over the temperature range) to suggest that the phenomenon may be real. If so, these findings seem to contrast with the results of whole-animal gravimetric WLR determinations (Toolson, 1987) in which WLRs of dead individuals were significantly lower than those of live individuals at 30°C. The explanation for this possible disparity is unclear and future experiments will measure TWF across the cuticle of various regions, especially those lacking the large pores.

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REFERENCES

- BERRIDGE, M. J. (1970). Osmoregulation in terrestrial arthropods. In *Chemical Zoology* (ed. M. Florkin & B. T. Scheer), pp. 287–320. New York: Academic Press.
- EDNEY, E. B. (1977). *Water Balance in Land Arthropods*. Berlin: Springer-Verlag.
- HADLEY, N. F., MACHIN, J. & QUINLAN, M. C. (1986). Cricket cuticle water relations: permeability and passive determinants of cuticular water content. *Physiol. Zool.* **59**, 84–94.
- HEATH, J. E. & WILKIN, P. J. (1970). Temperature responses of the desert cicada, *Diceroprocta apache* (Homoptera: Cicadidae). *Physiol. Zool.* **43**, 145–154.

- MACHIN, J., LAMPERT, G. J. & O'DONNELL, M. J. (1985). Component permeabilities and water contents in *Periplaneta* integument: role of the epidermis re-examined. *J. exp. Biol.* **117**, 155–169.
- NEVILLE, A. C. (1975). *Biology of the Arthropod Cuticle*. Berlin: Springer-Verlag.
- RIDDLE, W. A. (1981). Cuticle water activity and water content of beetles and scorpions from xeric and mesic habitats. *Comp. Biochem. Physiol.* **68A**, 231–235.
- 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. (1987). Water profligacy as an adaptation to hot deserts: water loss rates and evaporative cooling in the Sonoran Desert cicada, *Diceroprocta apache* (Homoptera: Cicadidae). *Physiol. Zool.* (in press).
- TOOLSON, E. C. & HADLEY, N. F. (1979). Seasonal effects on cuticular permeability and epicuticular lipid composition in *Centruroides sculpturatus* Ewing 1928 (Scorpiones: Buthidae). *J. comp. Physiol.* **129**, 319–325.
- WINSTON, P. W. & BEAMENT, J. W. L. (1969). An active reduction of water level in insect cuticle. *J. exp. Biol.* **50**, 541–546.