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EVIDENCE FOR HYDRATION-DEPENDENT CLOSING OF PORE STRUCTURES IN THE CUTICLE OF *PERIPLANETA AMERICANA*

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Summary

Integumental electrical resistances were measured on the antenna, pronotum, forewing, second abdominal tergite, fifth abdominal sternite and the femur of the third leg in restrained male and female *Periplaneta americana*. The same measurements, excepting those on antenna and wing, were made on last-instar nymphs. Electrical contact was made through two externally applied glass tubes filled with cockroach Ringer with a combined contact area of about 2 mm^2. Resistances corresponding to current flows through two thicknesses of integument were measured using a current-clamping amplifier. Calculated electrical conductances tended to be higher in the antennae, pronotum and abdominal tergites than in the legs and wings. Conductances of the pronotum and abdominal tergites were significantly higher ($P<0.001$) in males than in females. The literature suggests that male abdominal tergites are the site of sex pheromone production. In nymphs as well as adults, the conductances of all areas, except the antennae of males, decreased following dehydration and a decline in animal water content. In most cases the magnitude of the decrease was tightly correlated with initial hydrated conductance. The data suggest that variations in regional conductances in hydrated animals are principally due to differences in dermal gland density. We argue that the decrease in conductance following dehydration is evidence of a mechanism closing dermal gland openings in times of water stress.

Introduction

In order to explain different hydration-related rates of whole-animal weight loss, as well as the effects of injecting homogenates of the brain and other parts of the nervous system, Noble-Nesbitt and Al-Shukur (1987, 1988*a*,*b*) have proposed that the water permeability of the cuticle of *Periplaneta americana* is under hormonal control. Noble-Nesbitt (1990) has argued that the mechanism represents an energy-saving means of losing water during times of excess while conserving it at other times. However, it seems to us unlikely that cockroaches would have evolved a mechanism for increasing cuticle permeability in order to eliminate excess water. With the aid of techniques that separately identify respiratory and cuticular water fluxes, Machin *et al.* (1991) have shown that the

Key words: cockroach integument, cuticle permeability, dermal glands, pheromones, *Periplaneta americana*.

water loss from the spiracles in hydrated cockroaches can be varied and exceeds loss through the cuticle by more than a factor of three. In contrast, reducing cuticular permeability during times of water shortage would have an adaptive advantage because in water-stressed individuals, which invariably remain inactive, cuticular water fluxes are more than twice as great as loss through the spiracles.

Water permeability measurements of the pronotum alone using tritiated water, without the confounding effects of respiratory fluxes, have unambiguously confirmed that hydration-related permeability changes can occur in *Periplaneta americana* cuticle (Machin *et al.* 1992). Furthermore, significant increases in pronotal permeability of dehydrated cockroaches results from injections of brain homogenates from hydrated individuals. Croghan and Noble-Nesbitt (1990) discussed a possible mechanism involving a permeability change of integumental layers arranged in series. However, based on the absence of change in cuticular water content predicted by Machin and Lampert's (1987) modelling, Machin *et al.* (1992) argue that parallel conducting pathways, occupying a relatively small proportion of the total area, are more likely to be involved. They further suggest that pheromonal release through the cuticle might be a source of variation in cuticular water permeability. The idea of rapidly changing water permeabilities resulting from water loss through pore structures is not new in insects; for example, prominent perforations in the thorax of the desert cicada *Diceroprocta apache* are activated for evaporative cooling by elevated environmental temperatures (Hadley *et al.* 1989, 1991). If significant evaporative water loss were to occur through cuticular perforation in *Periplaneta americana*, a means of reducing such activities in times of water stress, perhaps by pore-closing mechanisms, would prolong survival.

It is difficult to obtain information on the abundance of pores associated with insect sense organs expressed per unit area of integument. Suffice it to say that the chemosensory bristles found on *Periplaneta* have one or more perforations, while mechanosensory hairs do not. Sensory organs such as the antennae and cerci have large numbers of perforated sensilla on them (Seelinger and Tobin, 1982); however, the pore abundance on other parts of the cuticle remains uncertain. Information on pheromone-producing structures is equally sparse. Sex pheromone production by female *Periplaneta americana* has been well established by behavioural (Hawkins and Rust, 1977; Schafer, 1977; Silverman, 1977; Tobin *et al.* 1981; Appel and Rust, 1983; Seelinger, 1985), chemical (Adams *et al.* 1979; Still, 1979) and neurological studies (Nishino *et al.* 1983). Although the gut seems to be the main source of pheromone (Sass, 1983), methods of pheromone extraction involving the faeces, filter papers placed in the animal's shelter, or treatment of the animals themselves, provide only ambiguous information about the site of production. The behaviour of attracted males and surface washing experiments led Seelinger and Schuderer (1985) to conclude that female *Periplaneta americana* release male attractants over their entire surfaces. There is morphological evidence that the males of at least some cockroach species release pheromones through the cuticle (Sreng, 1984). For instance, in males of the cockroach *Nauphoeta cinerea*, subcuticular glands are associated with abdominal tergites 2–8 and sternites 3–7 (Sreng, 1985). In a study of dermal glands, which pre-dates modern advances in pheromone biology, Kramer and

Wigglesworth's (1950) observations suggest that male *Periplaneta americana*, at least, have tergal glands.

Two rather different observations have encouraged us to explore the use of electrical techniques in the investigation of hydration-dependent changes in the water permeability of *Periplaneta americana* cuticle. The first establishes a connection between electrical conductances converted from Scheie and Smyth's (1967, 1968) pronotal resistance values and the water permeability of this structure. Both show rises of the same order of magnitude following excision (Machin *et al.* 1992). The second establishes a connection between resistance and cuticle porosity. Scheie and Smyth (1968) attribute regional variations in resistance to differences in the density of pore structures perforating the cuticle. Although they claim their work does not distinguish between the effects of dermal glands and pore canals, they do establish a rough correlation between resistance and dermal gland density.

Materials and methods

Animals

Experimental animals, adults of both sexes and last-instar nymphs, were selected randomly from a culture of *Periplaneta americana* L., maintained at 24–27 °C and 43–45 % relative humidity and continuously supplied with dry laboratory chow and water. Hydrated animals were caught directly from the culture; dehydrated animals were held in small jars (75–100 ml volume), with gauze-covered lids, over silica gel for 4–6 days prior to experimentation.

Water content, measured using the method described in Machin *et al*. (1991), varied according to the stage of the animal, hydrated adults having a mean water content of 0.691±0.004 of wet mass compared with 0.658±0.012 for hydrated nymphs. Dehydrated adults had a mean water content of 0.604±0.007 of wet mass and nymphs 0.578±0.011. Because adults and nymphs were from the same culture, it was reasonable to assume them to be in the same physiological state of hydration. Thus, in comparisons of the effects of dehydration on nymphs and adults, the use of water content as a criterion for physiological state was abandoned in favour of the percentage of initial hydrated body water, calculated from the same live and dry mass data. Despite the differences in water content, routine dehydration procedures produced more or less the same change in initial body water content: adults lost 12.53 %, nymphs 12.15 %.

Electrical techniques

To determine the resistance of the cuticle, we used a modification of the current-clamping amplifier described by de Kramer and van der Molen (1979). In this device, the preparation is inserted into the feedback loop between the output and negative input of an operational amplifier. The current through the preparation is thereby held at the same value as the input current to the amplifier, determined by an input voltage and an input resistance. With this clamped current, the output voltage of the operational amplifier is directly proportional to the resistance of the preparation. This technique is simpler and faster than the bridge method and provides direct readings of resistance. It also avoids

problems where the input resistance of a more standard measuring instrument affects the reading; in this case, the preparation *is* the 'input resistance'.

For the amplifier, we typically used an input resistance of 1 MΩ and an input signal of 50 mV (root mean square, rms), giving a current through the cuticle of 50 nA. With electrodes of about 1 mm in diameter, this represents a current density of about 0.6 μA cm^{-2}, considerably less than the 30 μA cm^{-2} found by Scheie and Smyth (1967) to be the upper limit for Ohm's law to hold in their preparation.

Ideally, pure resistance measurements should be taken using a d.c. signal, particularly since the integument has reactive as well as resistive properties (Scheie and Smyth, 1967). Early experiments using a d.c. signal have shown resistance readings to be very unreliable. This was partly due to a small d.c. voltage present between the inside and the outside. Substituting Ringer for the NaCl solution in the electrodes reduced this voltage to a few millivolts but did not eliminate it entirely; it was neither consistent nor stable, and thus could not be 'nulled' out. To avoid this problem, and possible polarisation of the electrodes, we substituted low-frequency a.c. signals and used the peak-to-peak voltage excursion (or the rms value) as a measure of voltage and, hence, resistance. Although some preliminary experiments were performed at 10 Hz, early measurements indicated appreciable capacitance effects and the lowest practical frequency of 0.1 Hz was adopted.

Electrical measurements

Resistance of adult cockroaches was measured on one antenna, the pronotum, forewing, second abdominal tergite, fifth abdominal sternite and the femur of one of the third walking legs. The same regions were measured in last-instar nymphs except for the antenna and wings. Because of the number of measurements made, we used two external electrodes to reduce stress. Scheie and Smyth (1968) justified this technique by demonstrating that the resistance measured in this way was twice that measured between an internal and an external electrode placed across the cuticle.

Prior to making resistance measurements, all animals were immobilized by chilling for approximately 1 h at 1 °C. During the measuring process they were confined, dorsal side up, inside a rim of sticky wax on a 90 mm×30 mm×3 mm piece of Plexiglas and held firmly in place with a cover of thin Plexiglas (55 mm×21 mm×1 mm) with strategically positioned holes to allow insertion of the electrodes. To help prevent injury, a small piece of foam plastic was placed underneath the animal. Measurements on the ventral surface were made after re-chilling and re-positioning the animal. The animals were allowed approximately 15 min to return to room temperature before any measurements were made.

Electrical connection to the preparation was made externally with electrodes consisting of 1 mm internal diameter glass tubes filled with cockroach Ringer (Machin *et al.* 1985). Electrodes were connected to the amplifier with chloridised silver wire. They were held in a micromanipulator and placed perpendicular to the cuticle surface; in this position, the Ringer contacted the cuticle without spreading. Lifting the electrodes slightly away from the surface made the region of contact between Ringer and cuticle more visible, as did adding Methylene Blue dye to the Ringer in the electrodes. The diameter of the liquid–cuticular interface was measured with an ocular micrometer in the stereo-microscope used for placing the electrodes.

Antennal measurements

A modification of the technique was required to obtain measurements of antennal resistance. Being so much smaller in cross-sectional area than the body, the electrolyte-filled core might be expected to have a significant additional resistance, varying along the antenna because of its taper. A small block of Plexiglas covered with dental wax was placed anterior to the mounted animal and the measurements were made with notched electrodes that held the antenna in place on the wax block. The wax provided a suitably hydrophobic surface, ensuring that the electrolyte formed a discrete drop which did not spread along the length of the antenna.

Antennal core resistances were first determined by placing an antenna severed from the animal at its base on a sheet of dental wax. One electrode made contact at the antenna base *via* a drop of Ringer, while another electrode made contact *via* a second drop of Ringer through the severed antennal tip. Resistance measurements were also made at each stage after progressively removing a few millimetres from the distal end. The resistance of each severed section was obtained by subtraction and its length and average diameter were measured with a calibrated ocular micrometer. Calculation of sectional resistivities revealed rather constant values ($178\pm8\,\Omega\,\mathrm{cm}$), except for the extreme antennal base and tip. The two electrodes used to measure intact antennae were, therefore, placed somewhere along their mid sections, and integumental resistances were calculated in the usual way after subtracting the estimated antennal core resistance between the fluid drops under each electrode. The area of contact was assumed to be equivalent to the cylindrical surface area of cuticle enclosed by each fluid drop.

Isolation and crowding experiment

Scheie and Smyth (1968) showed that long-term isolation (up to 200 days following the final moult) resulted in a marked increase in pronotal resistance, whereas a group of 10 males held together showed no such change. Although their data suggest that no significant change would occur in much shorter periods of isolation, we measured pronotal, fifth abdominal tergite and second sternite resistances every few days for 2 weeks in a group of three males and three females kept together in a roughly cubic 2 l glass container and in the same number of isolated males and females. All were provided with food and water *ad libitum*. No significant difference was found between the two groups for any of the regions measured; using a two-tailed *t*-test $P>0.05$, in all cases.

Gravimetric measurements

Cuticular water losses were compared in two nymphs and three adult males by means of continuous mass recordings for 170 h in dry air. The experiment was performed only with male adults because of potential complexities of oothecal production by females in such long-term experiments. Nymphs were carefully removed from the culture as soon as they had moulted; both nymphs and adults were kept isolated with food and water for approximately 4 days before experimentation. To avoid unduly stressing the animals, individuals from both groups were allowed to explore and spontaneously enter the weighing chamber, a metal gauze cage, and then weighed in the manner described by

Machin *et al.* (1991). The digitized data output from the microbalance was collected and analyzed using a data acquisition and analysis program written by one of us (J.J.B.S.).

Results

Regional differences in integumental conductance

In order to be compatible with permeability, resistance values have been converted to conductance. Table 1 shows that electrical conductances of the integument of hydrated adult cockroaches varied considerably from region to region, but with a consistent pattern. The highest values were observed in the pronotum and abdominal tergites of males and in the antennae of females. The lowest values were in the femur of the legs. Significant differences between the sexes were observed in the conductances of the pronotum and abdominal tergites ($P<0.001$). Antennal conductances were highly variable; for instance, although mean male and female values differed by a factor of three, they were not significantly different ($P>0.2$). Abdominal tergite conductances of males and females differed from those of the nymphs ($P<0.02$), as did those of the legs ($P<0.02$).

Effects of dehydration

Fig. 1 demonstrates that all regions measured, in adults as well as in nymphs, show a decrease in conductance following a period of dehydration. Furthermore, the magnitude of the conductance change increased proportionately with integumental conductance

Table 1. *Regional conductances of hydrated nymphs and male and female adults of* Periplaneta americana

	Conductance (μS cm^{-2})		
Region	Nymph	Adult male	Adult female
Pronotum	96.7±16.2 (12)	123.0±12.0 (11)	68.2±8.0 (20)
Second abdominal tergite	74.4±9.7 (12)	154.8±28.9 (11)	46.3±5.2 (19)
Fifth abdominal sternite	54.8±5.5 (12)	49.0±3.3 (11)	57.9±7.5 (21)
Leg	72.5±11.8 (12)	32.8±3.8 (11)	38.6±5.1 (22)
Wing		55.9±8.2 (10)	43.0±4.4 (33)
Antenna		64.6±4.2 (2)	186.7±85.2 (5)

Values are mean ± S.E.M. (*N*).

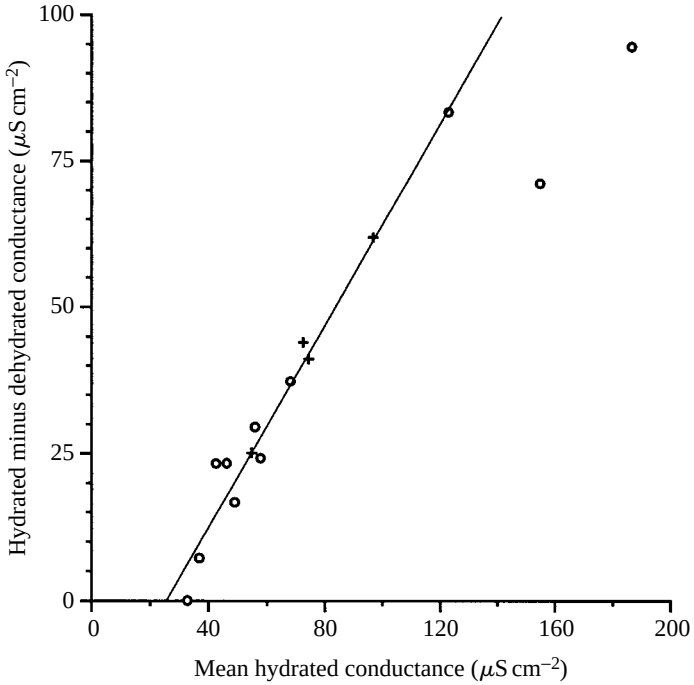

Fig. 1. Correlation between the mean hydrated conductance of different regions of the integument and the decrease in conductance following dehydration. Nymphs, crosses; adults (both males and females), open circles. Two points did not fit on the line and were omitted in calculating the regression equation. The higher point represents female antennae and the lower one, male abdominal tergites. The equation for the regression line is $y=-21.9+0.862x$. Without the outliers, $r^2=0.959$.

when hydrated. Except for the points for female antennae and male abdominal tergites, the two outliers, it can be seen that the correlation is very good ($r^2=0.959$). The regression line cuts the hydrated conductance axis (abscissa) at 25.4 μS cm^{-2} hydrated conductance. The outlying points indicate conductance decreases following dehydration of only 63 % and 76 % of the change indicated by the regression, in male tergites and female antennae, respectively. The conductance changes observed in antennae of male cockroaches greatly differ from the trends described above in showing a large conductance increase to 558±458 μS cm^{-2}, following dehydration.

Differences between nymphal and adult male cuticular water fluxes

Fig. 2 shows representative results obtained from an adult male cockroach (initial hydrated mass 1.12 g) and a nymph (0.58 g). The data for the adult are the same as those presented in Machin *et al.* (1991), standardized for comparing with the smaller nymph at equivalent hydration levels. The adult data chosen for the figure showed the same pattern of change with hydration as that of the other two males subjected to the same protocol. The nymphal data were similarly representative of the two individuals investigated. It can be seen that the adult lost a greater accumulative proportion of its body water (6.8 *versus*

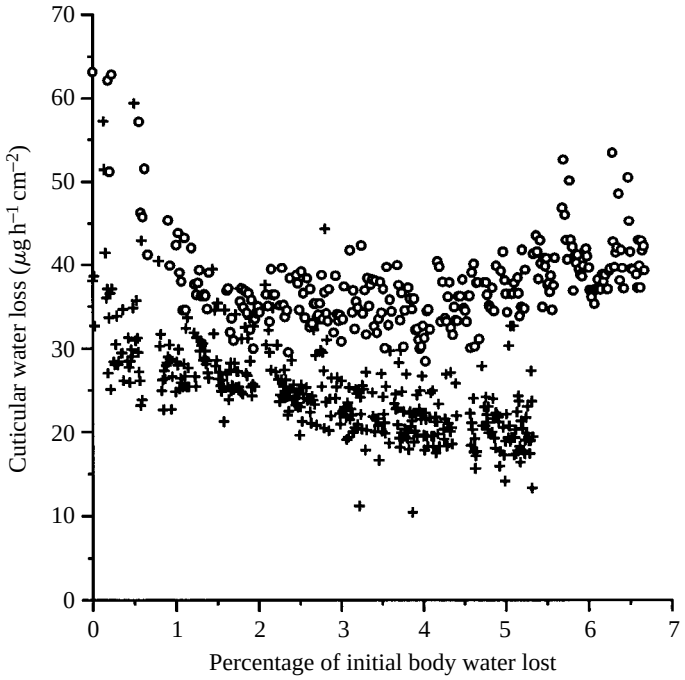

Fig. 2. Comparison of nymphal (crosses) and adult male (open circles) integumental water fluxes with accumulative body water loss, during 170 h of exposure to dry air at 20 °C. The fluxes were calculated from mass losses during spiracular constriction-flutter phases of each animal's respiratory cycle.

5.4 %) in 170 h. Losses are much lower than in the routine dehydration (see Materials and methods), indicative of the much reduced handling stress. A minimum of stress is necessary to produce the cyclic respiratory patterns that enable cuticular fluxes to be measured. The graph also shows different patterns of cuticular flux change as dehydration proceeded in the two animals. Whereas both showed an initial rapid decline, a further slower linear decline throughout the experiment occurred in the nymph. However, after falling to minimum values after a loss of 3 % of body water, adult loss rates began slowly to rise. The records of both animals showed intermittent bursts of elevated loss.

Discussion

Scheie and Smyth (1968) argued that since the waxy epicuticle is an effective barrier to electric current, integumental resistance must be a function of the size and density of open pore structures penetrating the cuticle. They supported their argument with a comparison of their own measurements of pronotal, fifth abdominal tergite and sternite dermal gland densities with resistances. However, no quantitative analysis was attempted. Fig. 3 shows that the correlation between hydrated conductances obtained in our study and dermal gland density data is good (r^2=0.832). We substituted second abdominal tergite gland

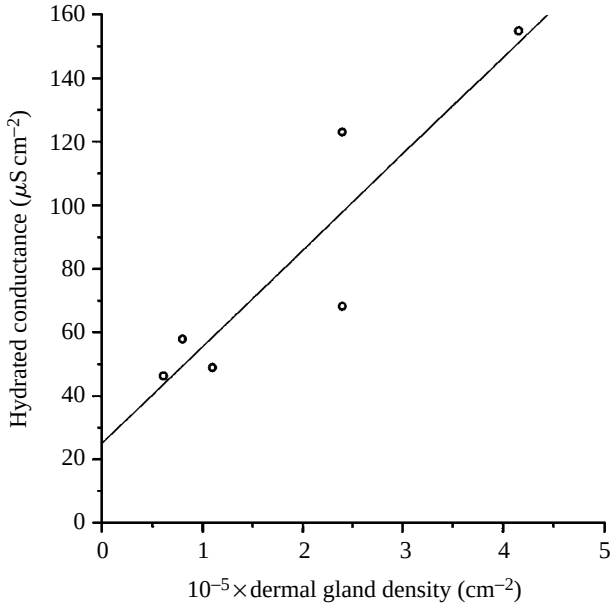

Fig. 3. Correlation between hydrated conductances (our values) and dermal gland densities of male and female pronota, fifth abdominal sternites (taken from Scheie and Smyth, 1968) and second abdominal tergites (taken from Kramer and Wigglesworth, 1950). All points are used to calculate the regression line (r^2=0.832).

densities estimated from Fig. 1 in Kramer and Wigglesworth (1950) because Scheie and Smyth (1968) quote values for the fifth abdominal tergite. It can also be seen that a residual conductance of 25.11 μS cm^{-2} (intercept on the ordinate) is not accounted for by dermal glands.

For some regions, there is a ready explanation for the observed differences in integumental conductance. Porous-walled chemosensory sensilla are abundant on the antennae (Seelinger and Tobin, 1982) and evidently account for the high conductance of these organs. In contrast, they appear to be absent on the central portion of the femur of the legs. Campaniform sensilla, whose cuticular diaphragms could be more conductive, are restricted to the leg joints (Seelinger and Tobin, 1982). There is good evidence, discussed in the Introduction, that the abdominal tergites of male *Periplaneta americana* are sites of sex pheromone release, which can be linked to the high conductance of this region. Similar, but not as extreme, sexual differences were observed in the pronotum; however, we have been unable to find any direct evidence of pheromonal involvement associated with this structure in the literature. The variability of regional conductances in nymphs also raises the possibility that pheromones are released by their integuments.

All surveyed regions of the integument in both nymphs and adults, except the antennae of males, show a conductance decrease following dehydration. A decrease in the number of conducting pore structures or a reduction in average pore conductance, in the area being measured, perhaps by the activation of a closing or constricting mechanism, seems

to be the only explanation. Indeed, the correlation of the magnitude of the change with hydrated conductance (the larger the number of open pores in a given area, the larger the possible change on closing), further supports the proposal that regional electrical conductances are determined by the density of pore structures perforating the cuticle, together with their dimensions. The data for both sexes, as well as for nymphs, indicate that the capacity to respond to dehydration ceases at minimal hydrated conductances in the region of $25\,\mu S\,cm^{-2}$. This observation, in conjunction with an almost identical residual conductance in Fig. 3, suggests that all surveyed areas include conductive structures that are not dermal glands and that do not respond to dehydration. The consistency of the intercept data suggests that the residual conductance is the property of structures occurring in all regions of the integument and with much the same properties throughout. This consistency would seem to eliminate all porous structures but the pore canals. Our data suggest that this pathway accounts for as much as 76.5 % of the total conductance of the legs of male cockroach (at least of their femurs) or as little as 16.2 % of the total conductance of highly conductive regions such as the abdominal tergites of males. The excellent fit of most of the conductance decreases in most regions of adults and nymphs to the same regression line suggests a similar quantitative response to dehydration. Furthermore, plausible explanations for the points that fail to fit the regressions, antennae and male abdominal tergites, can be advanced. Antennal cuticle is perforated with sensilla, not dermal gland canals, and the response to dehydration is predictably different and more complex. The structure or physiological responses of dermal glands involved in pheromone production and release could also be different in male abdominal tergites compared with those in other regions.

Dehydration has been shown (Machin *et al.* 1991) to produce a decrease in overall cuticular water permeability of adult *Periplaneta americana*. This study has shown that nymphs also follow this pattern, although the decrease in cuticular water flux in response to dehydration is greater than that in the adult male. This difference seems to be the principal reason why adult males lost a greater proportion of their body water in 170 h, since surface to mass differences can be discounted. Although the wings add significantly to the surface area of the adults, the unfavourable surface to mass ratio of the nymph due to its smaller size compensated for the effect of the wings, producing a surface to mass ratio of $14.53\,cm^2\,g^{-1}$, compared with $13.08\,cm^2\,g^{-1}$ for the adult male. Although the overall permeability changes favoured the nymph, hydrated conductance data suggest that not all regions of the nymph begin by being less waterproof than the adult male. Leg and abdominal sternite conductances are actually greater in hydrated nymphs. However, the pronotum and abdominal tergites of hydrated males have the higher conductances. The latter, in particular, has double the conductance of the nymph abdominal tergite and represents a comparatively large proportion of the surface. Taking the analysis beyond this point becomes impossibly complicated because areas with the highest conductance when hydrated show the greatest change on dehydration.

The precise relationship between electrical conductance and water permeability remains unknown. By showing that electrical conductance decreases with dehydration in most areas of the integument of both *Periplaneta americana* nymphs and adults, this study has established a general correlation between conductance and water permeability.

It is, therefore, reasonable to suggest that the pore-closing mechanisms revealed by electrical measurement represent an adaptive integumental response reducing water loss in times of water stress. We do not mean to imply that all trans-integumental water flux passes through open dermal gland ducts, simply that a significant proportion of the flow is through porous structures and that their closing significantly reduces water loss. This study provides an explanation of how *Periplaneta americana* cuticle can quickly change permeability to water by invoking a relatively simple response in known cuticular structures, rather than resorting to complex properties of the cuticle for which there is no direct evidence (Croghan and Noble-Nesbitt, 1990). Furthermore, the general understanding of how the non-porous cuticle acts as a water barrier remains unaltered. The work has provided an explanation of cuticle permeability change that can be understood in terms of its adaptive advantage and evolution. It has also confirmed the potential value of electrical techniques in investigations of the physiology of the insect integument and in insect behaviour and communication. For instance, our results suggest that mating and other chemosensory-based activities in *Periplaneta americana* may be disturbed during periods of water stress.

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