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AGGREGATE PROTECTION AGAINST DEHYDRATION IN ADULT FEMALES OF THE CAVE CRICKET, *HADENOECUS CUMBERLANDICUS* (ORTHOPTERA, RHAPHIDOPHORIDAE)

JAY A. YODER, HORTON H. HOBBS III, AND MATTHEW C. HAZELTON
Department of Biology, Wittenberg University, Springfield, OH 45501 USA, jyoder@wittenberg.edu

The role of aggregation in water conservation in adult female cave crickets, Hadenoecus cumberlandicus, *in Laurel Cave (Carter Co., KY) was investigated. Grouped crickets retained water more effectively (water loss rates were lower) as densities increased from 1, 5, 10, and 20 crickets per cluster. Dry air currents (flow rate 43 mL/min) that passed over an aggregation of 20 eliminated the group effect with regard to water loss, suggesting that the mechanism operates by raising the relative humidity inside the cluster. Rapid water loss rate characterizes the water balance profile and is reflected by high activation energies for water loss and low quantities of cuticular lipid. There was no evidence for water vapor uptake. Natural gains and losses are high in* H. cumberlandicus, *and this agrees with their preference for the deep cave environment. Conversely, water turnover is lower for another trogloxenic cricket,* Ceuthophilus stygius, *that is less cave-adapted.*

Roosting aggregations of >200 cave crickets, *Hadenoecus cumberlandicus* Hubbell and Norton, are abundant year-round in the interior of Laurel Cave, a small, cold cave in eastern (Carter Co.) Kentucky (KY), USA (Pfeffer *et al*. 1981). The upper level of this cave, where these crickets reside, is unusual in that it is cold and relatively dry, with little, if any, free water in the cave (excluding ceiling condensation dampness) throughout most of the year, even after heavy rains. Greatest abundance and size of aggregations of *H. cumberlandicus* occur during the driest times of year, mainly in winter. Presumably, the aggregations begin as clusters of nymphs hatched from eggs laid on the cave floor. Nymphs move from the ground to more moisture-rich microhabitats associated with the ceiling and walls, roosting at those sites between above-ground foraging bouts during development into adults (Peck 1976). Positioning high above the cave floor puts them in the more moist, slower moving air of the cave ceiling, and, in certain parts of their geographical range, helps to prevent attack by ground-dwelling predatory beetles (Peck 1976). Cave crickets are trogloxenic (complete life history in caves but must feed in surface habitats; [Hobbs 1992]) and cave-adapted (elongated antennae and legs, reduced eyes and pigmentation, with a thin, translucent cuticle) (Peck 1976). They are active mostly at night, emerging from the cave to forage and then returning to roost, digest food, and defecate (Poulson *et al*. 1995). During winter, outside foraging activity of *H. cumberlandicus* is not as great, they move further into the dark zone of the cave, seeking moisture on the ceiling and recessed wall and ceiling depressions, and remain together for many months (H. Hobbs & M. Hazelton, unpub. observations).

Formation of aggregations has a profound impact on many insects by enhancing water conservation. As group size increases, loss component of water budget decreases. The typical mechanism operates by generating a higher relative humidity inside the cluster (Yoder & Smith 1997). Such behavioral regulation of water loss by forming groups is especially important for arthropods because their surface area is great relative to their volume, creating an extreme potential water loss problem (Hadley 1994). We hypothesized that grouped cave crickets may retain water more effectively than isolated individuals. This was examined by comparing water loss rates for female adult cave crickets, *H. cumberlandicus*, in groups of different sizes. In addition, water content, dehydration tolerance, critical transition temperature, activation energies for water loss, and quantities of cuticular lipid were determined. The possibility for water gain by atmospheric water vapor absorption also was examined.

To understand better the influence of cave-adaptation on water balance characteristics, a water balance profile was constructed for another Laurel Cave trogloxene, the camel cricket, *Ceuthophilus stygius* (Scudder). This cricket also occurs in fairly dense aggregations on the ceiling and in small hibernating groups in the dark zone in winter (Studier & Lavoie 1990). Unlike *H. cumberlandicus*, *C. stygius* favors entrance areas in Laurel Cave rather than the deep cave. Every few days during the warmer months *C. stygius* forages outside the cave. *H. cumberlandicus*, with its significantly larger, distensible crop for storing food (Peck 1976), may avoid the risk of dehydration and predation during surface foraging for up to several weeks.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE

Cave crickets, *H. cumberlandicus*, and camel crickets, *C. stygius*, were collected from the upper level of Laurel Cave, Carter Co., KY, USA, in late November. Crickets were held in large plastic coolers and supplied with moist leaf litter and paper towels until return to the laboratory. Since only a parthenogenetic population of *H. cumberlandicus* resides in Laurel Cave (Hubbell & Norton 1978) only female adult crickets, distinguished by a sclerotized ovipositor, were used in the experiment within 24 h of collection. An aspirator and forceps were used to transfer crickets.

BASIC OBSERVATIONS

Temperature was held at 20 ±1°C to permit comparison with other water balance literature (Hadley 1994); other temperatures were controlled by environment cabinets (± 0.5°C). Specimens were weighed using an electrobalance (CAHN 25, Ventron Co.; Cerritos, CA; precision of 0.2µg SD and accuracy of ±6µg at 1 mg). Crickets were weighed and returned to test conditions < 1 min. Relative humidities (% RH) were generated in hermetically sealed glass desiccators (8000 cc, L X W X H) with glycerol-distilled water mixtures (Johnson 1940) or saturated salt solutions containing an excess of solid salt (Winston & Bates 1960). Crickets were kept separated from mixtures by a perforated porcelain plate and could roost upside down in the chambers. Calcium sulfate (Drierite) provided 0% RH (Toolson 1978). Test atmospheres were measured with a hygrometer (±3.0% RH; Thomas Scientific, Philadelphia, PA).

Wharton's (1985) standard methods were used to determine water balance characteristics, and profile analysis follows interpretations by Hadley (1994). Thus, % RH was expressed as a water vapor activity (a_v, a_v = % RH/100) and activity of the insect's body water (a_w) = 0.99 (Wharton 1985). Pretreatment consisted of 6 h starvation at 0.93 a_v and then 0.33 a_v until 4-6% body mass had been lost, so that mass changes reflect changes in water levels of the insects. Specimens were weighed individually within 1 min, without enclosure and without anesthesia, and were permitted to walk directly onto the weighing pan of the balance.

WATER LOSS RATES

To determine the body water loss that could be tolerated, crickets were weighed, placed at 0.0 a_v and reweighed at 15 min intervals. Mass measurement when crickets were unable to right themselves and crawl one body length was defined as the critical mass (= dehydration tolerance limit). Amount of water loss, from initial to critical mass, was expressed as a percentage of initial mass (Yoder & Barcelona 1995).

Under 0.0 a_v conditions, water loss is a function of exponential decay (Equation 1; Wharton 1985),

$$(1) \quad m_t = m_0 e^{-k_t}$$

or $\ln m_t / m_0 = -k_t$, where m_0 is the initial water mass, m_t is the water mass at any time, t, and k is the percentage of water lost in time, t, elapsed between m_0 and m_t (Wharton 1985). The slope of a regression on a semi-logarithmic plot, thus, ln (m_t / m_0) *vs*. time, is the rate of water loss (integumental plus respiratory water loss) and is expressed as %/h. Water mass (m) is the difference between initial and dry mass, and is expressed as a percentage of initial mass (= % body water content). The weighing interval for rate determinations was 1 h. Dry mass was determined according to Hadley (1994). Briefly, insects were killed by freezing and placed at 0.0 a_v and 90°C until mass remained constant plus an extra full day of drying (total 3-4 days).

GROUP EFFECTS

To explore whether there is a group effect with regard to water loss, water loss rates (0.0 a_v; eqn. 1) were determined for individuals in groups of different sizes (1, 5, 10 and 20). Groups of crickets were housed directly within relative humidity chambers. Individuals for monitoring were designated with a small spot of white paint (Pactra, Van Nuys, CA) placed on the dorsum (Yoder & Grojean 1997). Removal of the cricket for mass determinations caused no observable disturbance to the cricket grouping. Paint had no effect on mass changes (data not shown). Mechanistic determination of the group effect was tested by passing dry air currents (flow rate 43mL/min; Wharton & Knülle 1966) over the cluster (Yoder & Smith 1997).

ACTIVATION ENERGIES

Passive water loss rates (k) were derived similarly, except specimens were first killed and temperature was varied. Freshly killed insects are required for this experiment (Wharton 1985; Hadley 1994); one group was killed by freezing and another group was killed by HCN vapor (<10 h). Thus, loss is attributed solely to that through the cuticle without a respiratory component (Wharton 1985). Activation energies, E_a, reflecting cuticular permeability (Yoder & Denlinger 1991), were derived from the slope of a regression on an Arrhenius plot (rate *vs*. reciprocal absolute temperature, T) in accordance with standard equations and methodology (Toolson 1978) (Eq. 2 & 3):

$$(2) \quad \ln k = - E_a \boldsymbol{R}^{-1} T^{-1} + \ln A$$

$$(3) \quad E_a = -\{(t_i \ln k_i - t_i n^{-1}) \text{ X } [t^2_i - (t_i)^2 n^{-1}]^{-1}\}\mathbf{R}$$

where $\boldsymbol{R}$ is the gas constant, A is the frequency factor, and t is the temperature over range i with respect to the loss rate (k) of the amount (n) of water. Activation energies were expressed as J/mol. A change in E_a denotes a new temperature range (i), indicated when R >0.95 (Yoder & Spielman 1992), and a critical transition temperature is found at the point of intersection describing the two E_a s. The critical transition temperature is the temperature where a phase change occurs in epicuticular lipids resulting in a particularly rapid water loss.

WATER VAPOR ABSORPTION

To determine whether cave crickets can use water vapor as a primary source of water, pre-weighed crickets were placed at different relative humidities (85%, 93%, and 98% RHs = 0.85, 0.93, and 0.98 a_vs) and reweighed every 2 h. Ability to maintain a relatively constant water mass (m) in unsaturated air (that is, < 0.99 a_w = body water) indicates that loss is balanced by gains from the air. Lowest a_v where water gain from the air occurs is designated as the critical equilibrium activity (Wharton 1985) and shows where water balance (gain = loss) under these conditions is achieved.

CUTICULAR LIPIDS

Cuticular lipid (nonpolar and polar lipids) was extracted by two, 2 min, washes in chloroform:methanol (2:1) and quantified as described for insects by Yoder *et al*. (1992). Prior to extraction, crickets were first killed by freezing and then thawed to room temperature. Extracts (N=15 crickets per extract) were passed through a silica gel column (Waters Assoc.) and eluted with 3 column volumes (2 mL) of solvent. The extract was concentrated to dryness with a stream of N_2 gas, reconstituted with 10µL of solvent, and dried onto preweighed aluminum pans at 0.0 a_v under N_2. Pans were weighed and returned to these storage conditions (0.0 a_v under N_2) until mass remained constant. Amount of lipid is expressed as µg/individual.

DATA ANALYSIS

An analysis of variance (ANOVA) was used to compare data (Wharton 1985; Hadley 1994). For individual cricket tests, data are the mean ± SE of three replicates of 15 individuals each. In the group effect experiment, there were 45 groups tested with one designated, paint-marked, individual per group. Percentage data were arcsin transformed prior to analysis, and characteristics derived from regression lines were analyzed by a test for the equality of slopes of several regressions (Sokal & Rohlf 1981). Statistical analysis was set at P=0.05.

RESULTS

WATER BALANCE PROFILE OF *H. CUMBERLANDICUS*

A group effect with regard to water loss was observed for cave crickets in aggregations (Fig. 1). Compared to isolated specimens (water loss rate = 2.74 ±0.21%/h), rates for group-caged specimens of 20 individuals (1.63 ±0.29%/h) were reduced by approximately 1/2 (F=3.549; df=89; P<0.05). Water loss rates for crickets in test group sizes of 5 (1.84 ±0.33%/h) and 10 (2.08 ±0.17%/h) were between both extremes. In moving air, the water loss rate for an individual in a group size of 20 was 3.09 ±0.27%/h and was not different from a single, isolated individual (water loss rate = 2.74 ±0.21%/h); thus, moving air eliminated the group effect with regard to water loss rates. Initial, individual dry and water mass values were 0.389 ±0.09g, 0.111 ±0.04g and 0.278 ±0.05g, respectively, and the percentage body water content was 71.47 ±0.44% for all cave crickets in the experiment. In all cases, the water mass was a positive correlate of the dry mass (R≥0.98; P<0.001). Crickets were similar in size, shape, and water content in the experiment. We concluded that the nearly two-fold difference in water retention that we note is most likely group-related.

Cave crickets became irreversibly dehydrated once 24.59 ±0.76% body mass (= critical activity point) had been lost. This dehydration tolerance limit is reflected by survivability estimates of <12 h for isolated individuals and >12 h for group-housed individuals in dry air. Passive water loss rates for killed crickets were nearly tripled (6.12 ±0.52%/h) compared to living specimens. These passive water loss rates displayed direct Boltzmann dependence on temperature (R≥0.99; P>0.001; Fig. 2), yielding an E_a for water loss of 9.28 J/mol. A temperature threshold for a particularly rapid water loss was not detected; *i.e.*, no critical transition temperature is present. No abrupt change in water loss occurred due to a temperature-induced phase change in epicuticular lipids; that is, the observed slope is continuous (a single component curve) over a broad (4-60°C) temperature range.

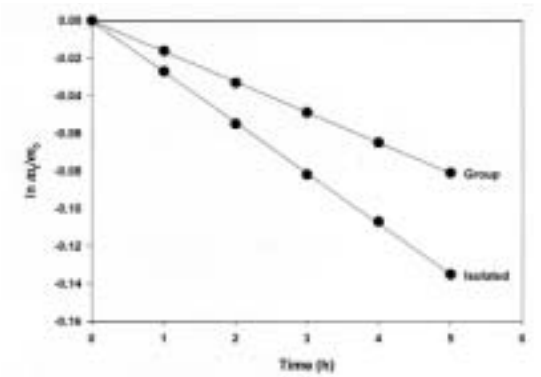

Figure 1. Water retention of aggregated (group size of 20) and isolated (group size of 1) cave crickets, *Hadenoecus cumberlandicus* at 0.0 a_v (a_v = water vapor activity = percentage relative humidity/100) and 20°C. The slope of the line through the plot is the rate of water loss; m_t, water mass at any time t and m_0, initial water mass.

Cave crickets did not absorb water vapor from the air. Water mass declined in all experimental a_v s tested (Fig. 3). More water was retained as a_v increased, implying passive gains by physical adsorption and passive chemisorption of water vapor. No active vapor uptake component appeared to be operating. Body water losses were not countered by gains from the air; *i.e.*, no equilibrium mass (gain = loss) was achieved. In absence of an active mechanism, the activity gradient between the cricket's a_w (= 0.99; Wharton 1985) and that of ambient air (0.99 a_w > 0.98 a_v and below) produces a water loss by simple diffusion. Thus, in this cricket species, the critical equilibrium activity is >1.00 a_v, indicating that water must be imbibed as a liquid.

COMPARATIVE OBSERVATIONS WITH *C. STYGIUS*

Water loss rate for the non-cave-adapted species, *C. stygius*, was 1.59 ±0.32%/h, nearly two times less than *H. cumberlandicus*, with a corresponding lower E_a value of 3.77 ±0.44 J/mol (F=4.427; df=89; P<0.05). This cricket is much larger (initial mass = 1.245 ±0.15 g; dry mass = 0.391 ±0.08 g; and water mass = 0.854 ±0.12 g) than *H. cumberlandicus*, but both have a similar percentage body water content (68.59 ±0.51%; ANOVA; P>0.05). Like *H. cumberlandicus*, dry mass and water mass correlated positively (R≥0.96; P<0.001), the dehydration tolerance limit was 26.07 ±0.83%, no critical transition temperature was detected and the critical equilibrium activity is >1.00 a_v.

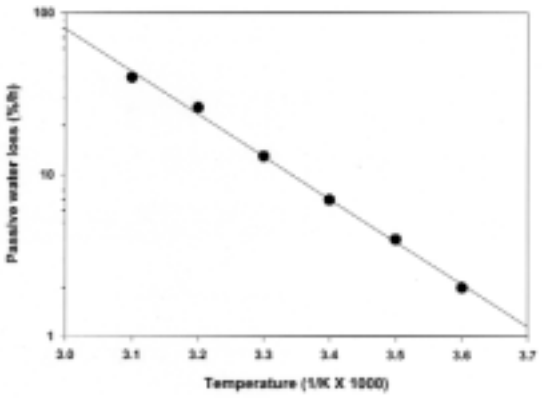

Figure 2 (right - top). Possible presence of a critical transition temperature in freezer-killed adult females of the cave cricket, *Hadenoecus cumberlandicus*, examined by Arrhenius analysis. The continuous slope (E_a) indicates that no critical transition temperature is present.

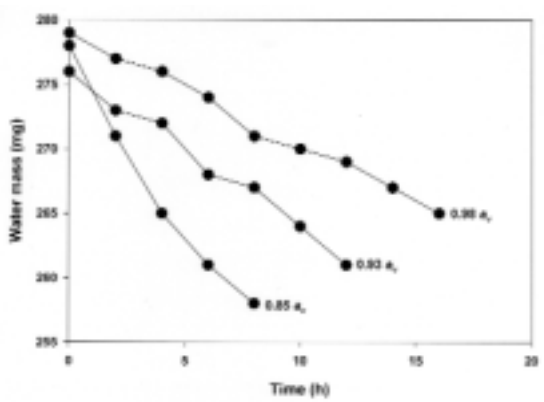

Figure 3 (right - bottom). Water mass loss by cave crickets, *Hadenoecus cumberlandicus*, in unsaturated air at 20°C indicates failure to maintain equilibrium (gain is not equal to loss) by absorbing water vapor from the air. 'a_v', water vapor activity = percentage relative humidity/100.

CUTICULAR LIPID

Quantity of cuticular lipid extracted from the surface of *C. stygius* was 386.41 ±0.64 µg/individual compared to significantly lower amounts of 202.33 ±0.67 µg/individual for *H. cumberlandicus* (F=3.457; df=89; P<0.05). The decreased water turnover associated with *C. stygius* correlated with their ability to survive longer in dry air than *H. cumberlandicus*. The only species-specific differences observed in this study between *C. stygius* and *H. cumberlandicus* were body size, water loss rate, and amount of cuticular lipid. We did not investigate the group effect in *C. stygius*.

DISCUSSION

A group effect is a feature of the water balance profile of the cave cricket, *H. cumberlandicus*. Other water balance characteristics, such as 71% water content, 24% dehydration tolerance limit, and lack of critical transition temperature agree with most insects (Hadley 1994). An especially fast water loss rate is an additional feature of the cave cricket. Compared to *H. cumberlandicus*, the less cave-adapted camel cricket, *C. stygius*, retains water more effectively, in part, because of a greater amount of cuticular wax (decreased integumental water loss), and larger size (smaller surface area to volume ratio). Female adults of *H. cumberlandicus* regulate water loss behaviorally by forming aggregations. The importance of a group effect with regard to enhancing survival is apparent in many arthropod species (*e.g.*, Glass *et al*. 1998; Yoder & Knapp 1999; Yoder & Smith 1997; Yoder & Grojean 1997; Grassé & Chauvin 1944), but is not always linked to water conservation (Yoder *et al*. 1994; Yoder & Hoy 1998). Whether water conservation is facilitated through group formation by the camel cricket, *C. stygius*, is not known. It also remains unclear whether the group effect is restricted to female adults, *H. cumberlandicus*, or to Laurel Cave, and we are presently investigating these questions.

Examination of activation energies (E_as) provides an explanation for the fast water loss rate of *H. cumberlandicus*. E_as for water loss, describing passive water loss over a broad temperature range, have not been previously determined in crickets, nor have E_as been used to examine cave adaptation. Though fluctuations in deep cave temperature rarely occur, the importance of E_a pertains to properties of the cricket cuticle. Lower E_a values are associated with extra cuticular lipid (Yoder & Denlinger 1991; Yoder *et al*. 1992), a barrier that restricts integumental water loss, because fewer molecules of water (*n*; eqn. 3) escape through a less permeable cuticle (Wharton 1985). Conforming to this reasoning, lower E_as of the more waxy camel cricket, *C. stygius*, reveal a cuticular modification compared to the less waxy *H. cumberlandicus*. Indeed, our results indicate that *C. stygius* has nearly double the amount of cuticular lipid as *H. cumberlandicus*. This quantitative difference in cuticular lipid is sufficient to explain the lower E_a of *C. stygius* because more energy is required to move water from the exchangeable water pool (*m*; eqn. 1) through a thicker wax layer. Lower E_a for *C. stygius* suggests that they are more water-tight than *H. cumberlandicus*. Thus, more rapid water loss rate of *H. cumberlandicus* is attributed, in part, to a lower amount of cuticular wax. A less waxy cuticle has been touted to contribute to the extreme sensitivity to drying for cavernicoles (Peck 1976), but this is the first direct evidence that this is the case.

The high relative humidity (usually above 95 a_v in deep cave; H. Hobbs, unpublished observations for Laurel Cave) of the deep cave habitat would appear to be ideal for active water vapor absorption. Use of atmospheric water has not been previously examined in cave crickets. However, cave crickets failed to maintain an equilibrium water content (*i.e.*, gain is not equal to loss) in unsaturated air in the experiment. Arthropods capable of such a feat pump water actively against the 0.99 a_w of their body water and show no losses of water in unsaturated air, sustaining a constant water mass (*m*; eqn. 1) for many days (Wharton 1985). In the absence of an active mechanism, water loss by *H. cumberlandicus* occurs in unsaturated air because

the ambient test $a_v < 0.99\ a_w$ of the cricket, and the water mass depletes by simple diffusion. No gain was detected that could not be explained by wholly passive processes; water was not added to the exchangeable water pool (*m*; eqn. 1), and the amount gained correlated closely with increasing a_v (Wharton 1985). Water must be imbibed as a liquid, because the critical equilibrium activity >1.00 a_v. Thus, the most likely source of water is free water from droplets or by ingesting moist food like most insects (Hadley 1994).

GENERAL INTERPRETATIONS

One benefit of cricket aggregation is enhanced water conservation for survival throughout the year, particularly in Laurel Cave where somewhat dry conditions prevail year-round, and especially during harsh winters or unusually hot and dry summer conditions. Another possible benefit obtained by aggregation formation is a higher rate of completing development by nymphs, because the 'group' operates by generating a higher relative humidity within the aggregation (Yoder & Smith 1997), satisfying an absolute moisture requirement for development (Peck 1976). From a water balance perspective, *H. cumberlandicus* is hydrophilic because of its fast water loss rate according to Hadley (1994). This matches their preference for caves with atmospheres that are generally close to saturation, and, especially, the stable, more humid conditions of the cave ceiling. Laurel Cave is ideal because it is cold, lowering water loss rates of these otherwise 'leaky' crickets by suppressing integumental and respiratory water loss components (this study; Studier *et al*. 1987).

Frequent outside surface foraging trips are detrimental to survival, because cave crickets are extremely sensitive to dehydration and vulnerable to predation. Enhanced water conservation by low temperature and group effects, plus a distensible crop for food and water storage, enable *H. cumberlandicus* to remain inside the protected, cold arena of Laurel Cave for longer periods of time, and, thereby, avoid the high-risk surface environment. In contrast to *H. cumberlandicus*, the less cave adapted water balance profile of the camel cricket, *C. stygius*, correlates with their greater abundance near cave entrances rather than in the less variable deep cave, and their greater outside surface foraging activity (every few days compared to every few weeks for *H. cumberlandicus*; H. Hobbs, unpublished observations), and suggests that low temperature is less crucial for maintaining water balance.

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