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THE CONTROL OF WATER LOSS IN DESERT TENEBRIONID BEETLES

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INTRODUCTION

Among the most conspicuous desert arthropods are beetles of the family Tenebrionidae, which occur in xeric regions of the Old and New World (Cloudsley-Thompson & Chadwick, 1964; Jaeger, 1965). Various aspects of their natural history have been described (Buxton, 1924; Hafez & Makky, 1959; Lawrence, 1959; Cloudsley Thompson, 1963), but such studies have been limited to field observations of adult beetles, descriptions of feeding habits and behaviour, and estimations of relative abundance.

Physiological aspects of water balance in a number of terrestrial arthropods from a variety of habitats have been recently reviewed (Edney, 1957, 1967, 1968; Cloudsley-Thompson, 1964*a*; Bursell, 1964; Wigglesworth, 1965). There has been a paucity of specific studies on the control of water balance in desert Tenebrionidae, although some data are available for African and European species. Cloudsley-Thompson (1956) reported higher rates of transpiration under similar experimental conditions from British as compared with Tunisian beetles, suggesting that the xeric animals were better regulators of their water losses. Other investigations concerned with morphological adaptations for restricting the rates of water loss have provided information on the role of the tenebrionid subelytral cavity. These studies have shown that this cavity is an effective mechanism for reducing transpiration (Dizer, 1955; Marcuzzi, 1960; Koch, 1961; Cloudsley-Thompson, 1964*b*; Ahearn & Hadley, 1969). Possible thermal advantages provided by this structure (Bolwig, 1957) are less clear, although the air space may serve as a thermal boundary layer between elytra and abdomen (Hadley, 1970).

Preliminary investigations on the physiological control of water balance in North American desert tenebrionids have been conducted (Ahearn & Hadley, 1969; Ahearn, 1969). The present study is an extention of these findings and a more detailed analysis of their adaptive significance.

MATERIALS AND METHODS

Beetles (*Eleodes armata* LeConte, *Centrioptera muricata* LeConte and *Cryptoglossa verrucosa* (LeConte)) were collected with pitfall traps at South Mountain Desert Park, Phoenix, Arizona from June 1968 to September 1969 and maintained in the

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laboratory in glass terraria at 23 ± 1 °C on a diet of fresh lettuce and cabbage. All animals, except those examined for faecal water loss, were starved for at least 24 h to clear gut contents before measuring evaporation rates. Water loss, based on weight changes, was measured to 0·1 mg. with a Mettler, single-pan balance. Exposure humidities were maintained with distilled water, saturated salt solutions (Winston & Bates, 1960) or silica gel. Humidity accuracy was checked by a Serdex relative humidity indicator (Bacharack Industrial Instrument Co., Pittsburgh, Pa.) and an Atkins Thermister Psychrometer, Model 3Z02B (Atkins Technical Inc., Gainesville, Florida). The humidity instruments had stated accuracies of ± 2·0%.

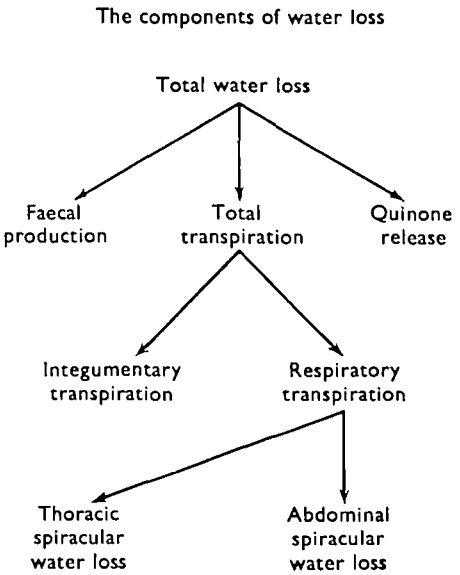

Fig. 1. Components of water loss in desert tenebrionid beetles as determined in the present investigation. Arrows indicate sources of each contributing component.

Beetles were exposed to experimental conditions in individual beakers in an apparatus that provided moving air of controlled temperature and humidity (Ahearn & Hadley, 1969). Air was circulated in the closed system at a rate of 1000 ml/min, and was passed through a humidifier flask containing either distilled water or a saturated salt solution, then through a glass wool trap, before reaching a desiccator containing the beetles. A glass tube of dehydrated silica gel replaced the humidifier when near 0% R.H. was desired. The entire system was placed in a programmed refrigerator-incubator where temperatures were controlled to ± 0·5 °C. Desiccator temperatures were monitored with a Yellow Springs Telethermometer and corresponded well with incubator temperatures.

Transpiration (evaporation), defaecation, and release of defensive abdominal quinone droplets and/or oral fluids were the three principle avenues of water loss in tenebrionid beetles (Fig. 1). Total water loss from living specimens included only components of transpiration and quinone droplet release, since pre-starvation of all animals for 24 h prior to testing eliminated water loss from defaecation. Total transpiration (integumentary and respiratory water loss) was determined by recording weight loss of individuals which did not release any detectable amounts of quinones or

oral fluids during exposure to experimental conditions. Water loss by way of quinone droplet release and/or oral fluid secretion was estimated by taking the mean difference between total water loss and total transpiration in pre-starved beetles.

Because the spiracles of tenebrionid beetles do not open directly to the exterior, but into cavities in the abdomen and thorax (Fig. 2) (Cloudsley-Thompson, 1964*b*), conventional methods of sealing the spiracular openings were not possible without cutting the exoskeleton. When integumentary (cuticular) transpiration alone was measured, beetles were killed in cyanide gas, and nail polish was used to seal the anal and oral regions, and the intersegmental areas dividing the body. This prevented the escape of moisture passing from the respiratory surfaces to the sub-skeletal cavities

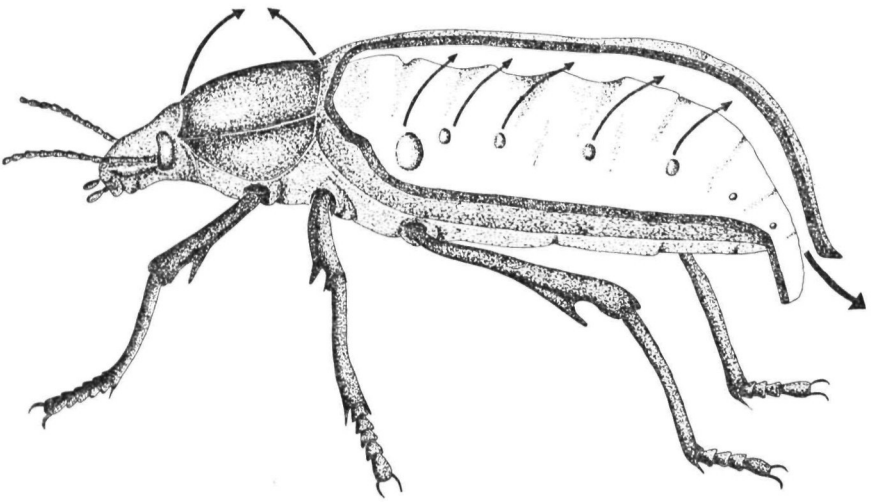

Fig. 2. Diagram of *Eleodes armata* LeConte showing subelytral cavity and probable avenues of respiratory water loss. Arrows from thorax indicate thoracic spiracular water loss passing through intersegmental areas. Spiracular losses derived from the abdomen first pass into the subelytral cavity and then reach the environment through a single orifice above the anus.

and eventually to the environment (Fig. 2). Dissection of freshly killed beetles revealed that thoracic and abdominal air spaces were not continuous so that spiracular losses of water from the thorax took place only at the intersegmental areas. Since the fused elytra of these beetles form a solid case surrounding the abdominal tergites and their spiracles, only one small orifice above the anus had to be sealed in order to prevent the escape of abdominal spiracular moisture. In this manner water loss from abdominal spiracles was separated from respiratory loss through thoracic spiracles. Weight loss from living beetles with the abdomen sealed was considered to be due to integumentary transpiration plus respiratory transpiration from the thoracic spiracles. Water loss from the thoracic spiracles was measured as the mean difference between integumentary transpiration alone and integumentary transpiration plus thoracic respiratory transpiration. The component of water loss from the abdominal spiracles was estimated as the difference between total respiratory losses and the loss due to thoracic respiration.

Oxygen consumption was measured with large (220 ml) Warburg flasks submerged in a constant-temperature water-bath. A 10% solution of KOH (0·5 ml) was placed in the side arm of each flask to absorb CO_2. Humidities inside the Warburg vessels

during an exposure period were determined with General Purpose Humidity Cards (Humidial Co., Colton, Calif.) to a stated accuracy of ±5% R.H. Relative humidities between 40 and 60% were attained when standard procedures for measuring oxygen consumption (Umbreit, Burris & Stauffer 1964) were followed. Humidities less than 10% within the chambers were obtained by placing weighed quantities of $CaSO_4$ in the bottom of each vessel. Circular pieces of aluminum screen inserted into the flasks prevented animals from coming in contact with the dehydrant. Beetle volumes were measured to 0·1 ml by water displacement, while volume of a known weight of $CaSO_4$ was calculated from its density. Volume of each respirometer was calculated, taking into consideration the displacement produced by the KOH, $CaSO_4$, screen, and beetle. Equilibrium time for each exposure temperature was 1 h.

RESULTS

Comparison of water loss in living and freshly killed beetles

Before comparing contributions of various pathways to total water loss it was necessary to determine whether living animals were able to actively restrict their losses of water. Rates of weight loss from living specimens of *Eleodes armata*, *Cryptoglossa verrucosa* and *C. muricata* were compared to weight-loss rates of freshly killed specimens at 35 °C and 0% R.H. over a 3-day period (Fig. 3). In all three species water loss from dead animals far exceeded the loss shown by live animals at the same temperature and humidity. The differences in weight loss between the two groups, however, were not very distinct until they had been exposed for 10 or more hours to test conditions. Water loss in living and dead beetles of all three species at 35 °C and 0% R.H. was linear with respect to time for 10–80 h of exposure. Rates of water loss before 10 h, however, may have been substantially different than rates measured between 10 and 70 h of exposure.

C. verrucosa (both living and dead groups) lost considerably less water over 3 days exposure to 35 °C and 0% R.H. than either group of *E. armata* or *C. muricata*, which indicated better water control in the former species. Better water regulation in *C. verrucosa* was also evident when a comparison was made between the magnitude of difference in water loss of living versus dead groups of each species. The divergence of the two groups over three days was much greater in *C. verrucosa* than *E. armata* or *C. muricata*.

Total water loss

Comparative rates of total water loss of pre-starved specimens of *E. armata*, *C. muricata* and *C. verrucosa* at a series of exposure temperatures and 0% R.H. are shown in Fig. 4. Survival at all temperatures was good except at 45 °C where all animals died during the 12 h exposure. All three species of beetles demonstrated increased total water loss with increased ambient air temperature. Mean total water-loss rates of *E. armata* and *C. muricata* were similar from 25 to 40 °C, but were twice as great as values exhibited at the same temperatures by *C. verrucosa*. At 45 °C mean rates were approximately the same for all three species, indicating that the greatest difference in water loss between species occurred at the temperatures likely to be encountered by these animals under natural conditions. Large amounts of dark brown material were found at the end of the exposure periods lining the insides of many beakers holding the

animals exposed to 40 and 45 °C. The large ranges in water-loss values for each species at these two temperatures were therefore, to a great extent due to the release of variable quantities of quinone and oral fluids. The incidence of this brown material

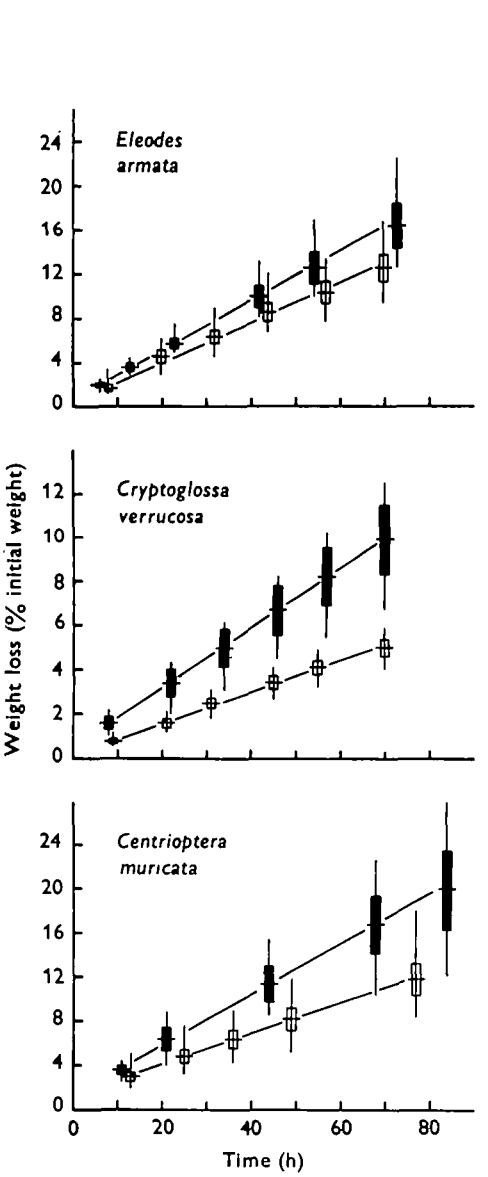

Fig. 3

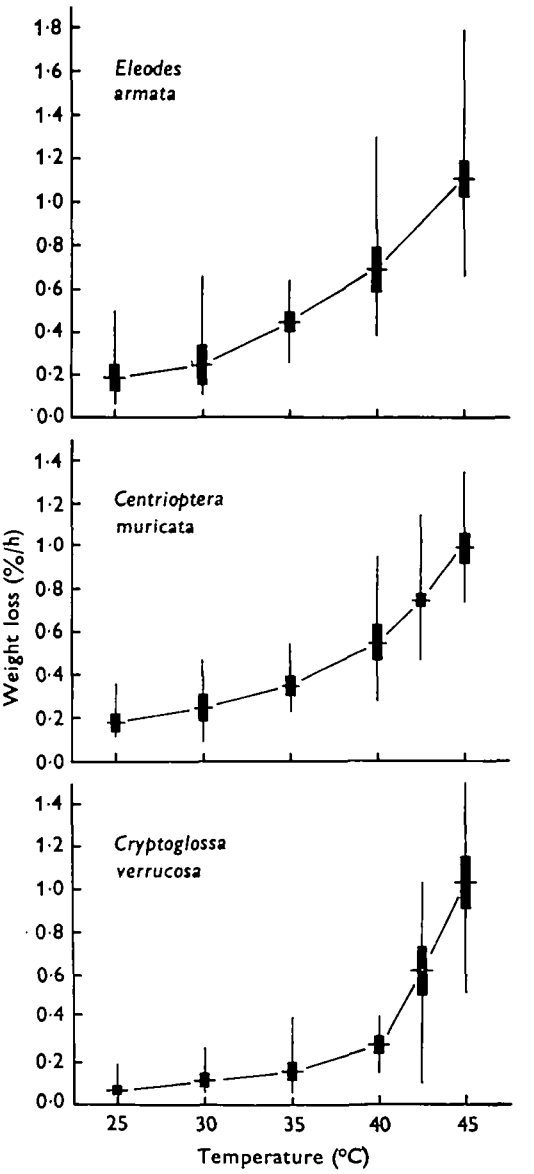

Fig. 4

Fig. 3. Rates of water loss from living and freshly killed specimens of *E. armata*, *C. verrucosa* and *C. muricata* exposed to 35 °C and 0% R.H. over a 3-day period. Horizontal lines represent group means (min. '*n*' = 10), vertical lines ranges, and rectangles 95% confidence limits. ■, Dead animals; □, live animals.

Fig. 4. Comparative rates of total water loss of *E. armata*, *C. muricata* and *C. verrucosa* at progressively higher temperatures and at 0% R.H. All animals were exposed to the experimental conditions for 12 h. Horizontal lines represent group means (min. '*n*' = 14), vertical lines ranges, and blackened rectangles 95% confidence limits.

inside beakers was much less at lower temperatures, although several individuals of *E. armata* released this fluid even at 25 and 30 °C.

Effects of relative humidity on total transpiration

To determine the extent to which rates of transpiration in desert tenebrionid beetles were dependent upon the ambient relative humidity, pre-starved animals of two species, *E. armata* and *C. verrucosa*, were exposed to zero, 40 and 75 % R.H. at 30 °C for 72 h. Attempts to determine rates of transpiration at near-atmospheric saturation (97 % R.H.) failed because at that level both species released copious amounts of quinone and/or oral fluids.

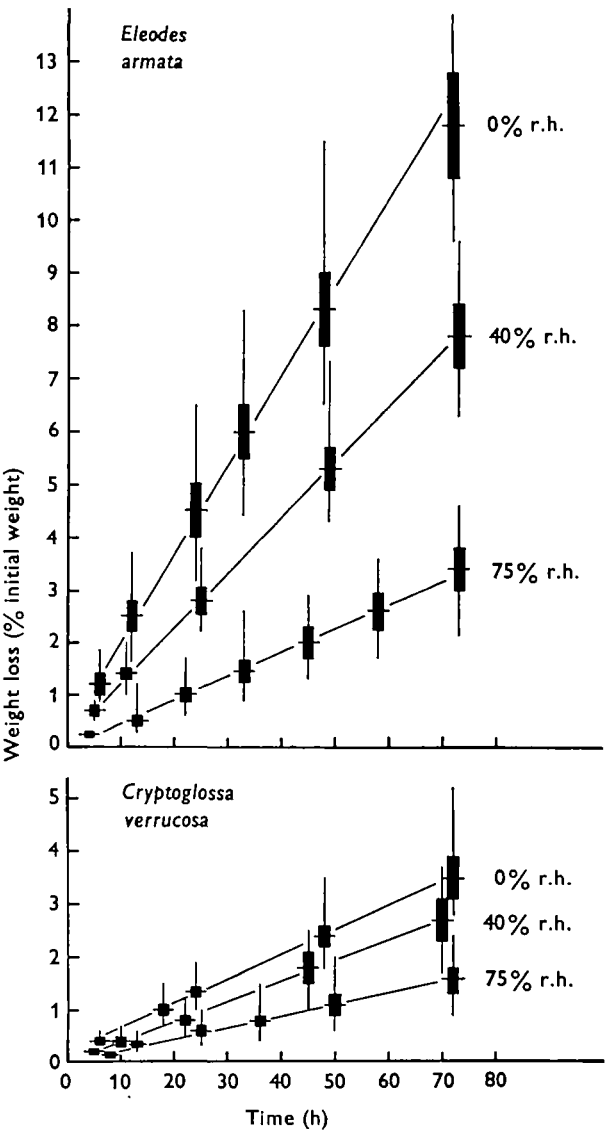

Fig. 5. Rates of total transpiration by *E. armata* and *C. verrucosa* at 30 °C and at zero, 40, and 75 % R.H. over a 3-day exposure. Horizontal lines represent group means (min. '*n*' = 14), vertical lines ranges, and blackened rectangles 95 % confidence limits.

Both species exhibited a linear rate of water loss at all exposure humidities with the greatest losses occurring at the lower humidities (Fig. 5). *E. armata* lost considerably more water at all three humidities than did *C. verrucosa*. Water loss in *E. armata* after 72 h at 0% R.H. was approximately four times the amount lost at 75% R.H.,

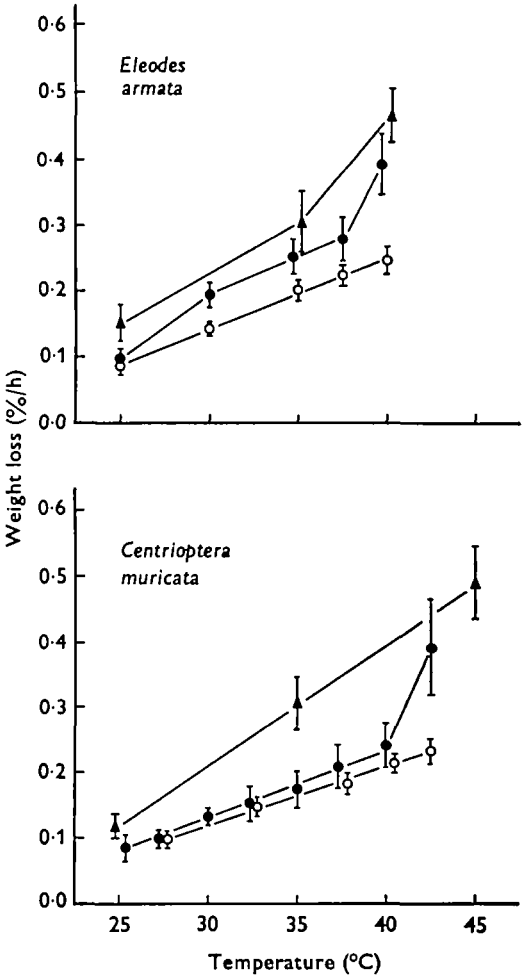

Fig. 6. Rates of transpiration from dead sealed, dead unsealed, and living groups of *E. armata* and *C. muricata* exposed for 6 h to a series of temperatures at near 0% R.H. Groups of beetles were exposed only once to each temperature. Triangles and circles are group means (min. 'n' = 10) and vertical lines represent 95% confidence limits. ▲, Dead animals unsealed; ●, live animals; ○, dead animals sealed.

whereas at these same two humidity extremes *C. verrucosa* exhibited only slightly greater than a two-fold difference in its water loss. Accompanying the increased level of water loss in *E. armata* was greater individual variation in water-loss values. These results suggested that varying levels of ambient relative humidity had a greater effect on the magnitude of transpiration in *E. armata* than in *C. verrucosa*.

Transpiratory water loss

An initial test was conducted to establish that applied nail polish sealed off losses from spiracular openings and, at the same time, had no noticeable injurious effect upon the beetle integument. Freshly killed specimens of *E. armata* and *C. muricata* were divided into sealed and unsealed groups and exposed to temperatures from 25 to 45 °C at 0% R.H. Group 1 (unsealed; min. '*n*' = 10) was measured for integumentary water loss plus evaporation from respiratory surfaces, while Group 2 (sealed; min. '*n*' = 10) was measured for integumentary transpiration alone. Living animals of both species (min. '*n*' = 10) were also exposed to the same temperature and humidity conditions as the two groups of killed specimens. In both species the water loss from unsealed animals (Group 1) exceeded the losses shown by sealed animals (Group 2) at every temperature (Fig. 6), suggesting that the experimental technique used to seal the organisms did not grossly distort the 'normal' level of integumentary permeability and did serve as an effective method of preventing losses of water from the spiracular openings. Rates of water loss exhibited by living animals were intermediate between rates shown by sealed and rates shown by unsealed dead animals. Since respiratory activities cease at death, differences in water loss between living and dead (Group 2) beetles at the same temperatures were due to spiracular losses through respiration. Rates of water loss from living and dead (Group 2) specimens of *C. muricata* at each temperature from 25 to 40 °C were not significantly different ($P > 0{\cdot}05$). In contrast, rates of water loss exhibited by these two groups of *E. armata* were significantly different ($P < 0{\cdot}05$) at each temperature except 25 °C.

Rates of water loss in *E. armata* and *C. muricata*, exhibited by unsealed, freshly killed beetles (Group 1) were slightly higher at each temperature than rates shown by living animals (Fig. 6). These results indicate that there may have been some loss or breakdown in water regulation by these beetles at all exposure temperatures soon after death which led to slightly greater rates of water loss than those which occurred in regulating, live animals. Since unsealed, dead (Group 1) beetles lost more water at all temperatures than living or sealed, dead (Group 2) animals, spiracular control of water loss was probably reduced following death. Cyanide-killed beetles were examined and had several spiracles in partially opened positions. Spiracles maintained in a slightly open position would allow for passage of greater amounts of water than would occur in living animals which regulated their spiracular openings.

Rates of transpiratory water loss from integumentary and respiratory pathways for *E. armata*, *C. verrucosa* and *C. muricata* at a series of temperatures (25 to 42·5 °C) are shown in Fig. 7. Cuticular (integumentary) transpiration was measured in dead, sealed animals, while estimated values of respiratory transpiration were calculated as the mean difference between total and cuticular transpiration at each temperature. All three species exhibited a relatively linear increase in both total and cuticular transpiration with rising temperature at the lower end of the temperature scale. Although rates of cuticular transpiration for each species remained linear throughout the entire range of temperatures, rates of total transpiration increased markedly above previous rates at 40 °C for *E. armata* and 42·5 °C for *C. verrucosa* and *C. muricata*. Rates of total and cuticular transpiration by *C. verrucosa* and *C. muricata* at temperatures from 27·5 to 40 °C were not significantly different ($P > 0{\cdot}05$), but were at 42·5 °C

($P < 0{\cdot}05$). Cuticular transpiration therefore accounted for almost 100% of total transpiration at lower temperatures for these two species. Respiratory transpiration from *C. verrucosa* and *C. muricata* became an important avenue of water loss only at extremely high temperatures. Total and cuticular transpiration from *E. armata* were

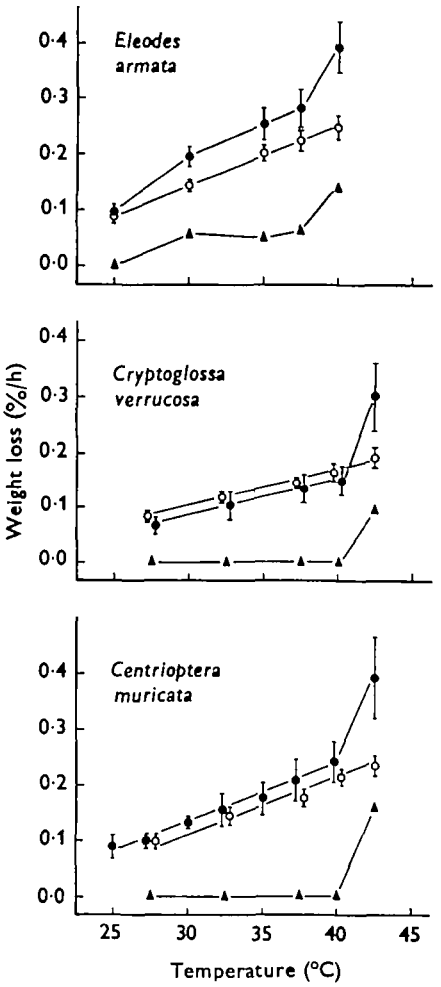

Fig. 7. Comparison of total, cuticular and estimated respiratory transpiration in *E. armata*, *C. verrucosa* and *C. muricata* exposed for six hours to progressively higher temperatures at 0% R.H. Groups of beetles were exposed only once to each temperature. Triangles and circles represent group means (min. 'n' = 10) and vertical lines 95% confidence limits. ●, Total transpiration; ○, cuticular transpiration; ▲, estimated respiratory transpiration.

significantly different ($P < 0{\cdot}05$) at temperatures from 30 to 40 °C, but not at 25 °C ($P > 0{\cdot}05$). *E. armata* demonstrated a measurable rate of respiratory transpiration at all temperatures above 25 °C.

Components of water loss in Eleodes armata

In order to determine the relationships between each source of water loss in tenebrionid beetles, one species was selected for detailed analysis. The contributions of each

water-loss component to total water loss by *E. armata* is presented in Table 1. Every component of total water loss increased in rate as exposure temperatures were elevated from 25 to 40 °C. Total transpiration (51·3–55·7 %) accounted for a slightly higher percentage of total water loss than did quinone-droplet release (48·6–44·3 %) over the temperature range, but the proportions of total losses contributed by each of these two components remained relatively constant at each temperature.

Table 1. *Components of total water loss in* Eleodes armata

(Rates were measured as mean values of % weight loss/hour followed by 95 % confidence limits. The relative humidity was maintained at near 0 % R.H. Minimum sample size per mean was ten beetles. Values in parentheses represent percentage of total water loss.)

Temp. (°C)	Total water loss	Total transpiration	Integumentary transpiration	Est. resp. transp.*	Estimated quinone secretion†
25	0·187 ± 0·062	0·096 ± 0·015 (51·3 %)	0·093 ± 0·012 (49·7 %)	0·003 (< 2·0 %)	0·091 (48·6 %)
35	0·453 ± 0·039	0·253 ± 0·030 (55·9 %)	0·200 ± 0·013 (44·1 %)	0·053 (11·8 %)	0·200 (44·1 %)
40	0·692 ± 0·102	0·385 ± 0·053 (55·7 %)	0·248 ± 0·022 (35·9 %)	0·137 (19·8 %)	0·307 (44·3 %)

* Values are mean differences between total and integumentary transpiration.
† Values are mean differences between total water loss and total transpiration.

Table 2. *Components of transpiratory water loss in* Eleodes armata

(Rates were expressed as mean values of % weight loss/hour followed by 95 % confidence limits. The relative humidity was maintained at near 0 % R.H. Minimum sample size per mean was ten beetles. Values in parentheses represent percentage of total transpiration.)

Temp (°C)	Total transpiration	Integumentary transpiration	Estimated respiratory transpiration*
25·0	0·096 ± 0·015	0·093 ± 0·012 (97·0 %)	0·003 (< 3·0 %)
30·0	0·200 ± 0·018	0·141 ± 0·010 (70·5 %)	0·059 (29·5 %)
35·0	0·253 ± 0·030	0·200 ± 0·013 (79·0 %)	0·053 (21·0 %)
37·5	0·283 ± 0·040	0·222 ± 0·020 (78·4 %)	0·061 (21·6 %)
40·0	0·385 ± 0·053	0·248 ± 0·022 (64·4 %)	0·137 (35·6 %)

* Values are mean differences between total and integumentary transpiration.

Integumentary transpiration was a much greater source of water loss than respiratory transpiration at each temperature. The percentage of total water loss from the integument was approximately 49·7 % at 25 °C, but fell to 35·9 % at 40 °C (Table 1). In contrast, the change in percentage contribution of respiratory water loss from less than 2 % at 25 °C to almost 20 % at 40 °C represented a ten-fold increase. Integumentary water loss from *E. armata* decreased in its contribution to total transpiration as temperatures were raised from 25 to 40 °C, while the percentage of total transpiration associated with respiration increased at the same temperatures (Table 2). At

25 °C almost all transpiration was derived from the integument (97 %), indicating a very low level of respiration and respiratory water loss. At the extreme exposure temperature (40 °C), *E. armata* still lost about two-thirds of its total transpiration through the integument, even though the percentage from respiration at this temperature was more than 10 times greater than its contribution at 25 °C.

Estimations of water loss from thoracic and abdominal spiracles were calculated after exposure of groups (min. '*n*' = 8) of *E. armata* to temperatures from 30 to 40 °C (Table 3). The proportion of respiratory water loss derived from thoracic spiracles increased from 30 to 40 °C, while the percentage contribution from abdominal spiracles decreased over the same temperature range.

Table 3. *Estimation of the respiratory transpiration components in* Eleodes armata *at* 0 % R.H.

(Rates were expressed as mean values (min. '*n*' = 8) of % weight loss/h. Values in parentheses represent percentage of estimated total respiratory transpiration.)

Temp. (°C)	Total trans.	Integ. trans.	Total resp. trans.	Integ. + thoracic spiracular trans.	Thoracic resp. trans.*	Abdominal resp. trans.†
30	0·200	0·141	0·059	0·150	0·009 (15·3 %)	0·050 (84·7 %)
35	0·253	0·200	0·053	0·220	0·020 (37·7 %)	0·033 (62·3 %)
40	0·385	0·248	0·137	0·322	0·074 (54·0 %)	0·063 (46·0 %)

* Values are mean differences between integ. + thoracic spir. trans. and integ. trans.
† Values are mean differences between thoracic resp. trans. and total resp. trans.

Species differences in cuticular transpiration and cuticular transition temperatures

Investigations to determine the extent of waterproofing afforded by cuticular wax layers of arthropods at different temperatures have led to the finding that rates of cuticular water loss may increase abruptly as the ambient temperature is slowly elevated past a certain 'transition temperature'. The rates of cuticular transpiration of three species of desert tenebrionid beetles were examined to determine whether the cuticular transition phenomenon could be demonstrated. Groups (min. '*n*' = 10) of freshly killed beetles (*E. armata*, *C. muricata* and *C. verrucosa*) that had been carefully sealed with nail polish were subjected to temperatures from 25 to 70 °C at 0 % R.H., each group being used for only a single determination. Mean group rates of water loss were determined at each temperature and the results were expressed as % weight loss/mm Hg/h × 1000 to give values which were independent to the changing saturation deficits at different temperatures (Fig. 8). *E. armata* was slightly more permeable and had a lower transition temperature than either *C. muricata* or *C. verrucosa*. The approximate temperature at which integumentary permeability increased for *E. armata* was near 40 °C, whereas the approximate transition temperatures for *C. muricata* and *C. verrucosa* were near 47·5 and 50 °C, respectively.

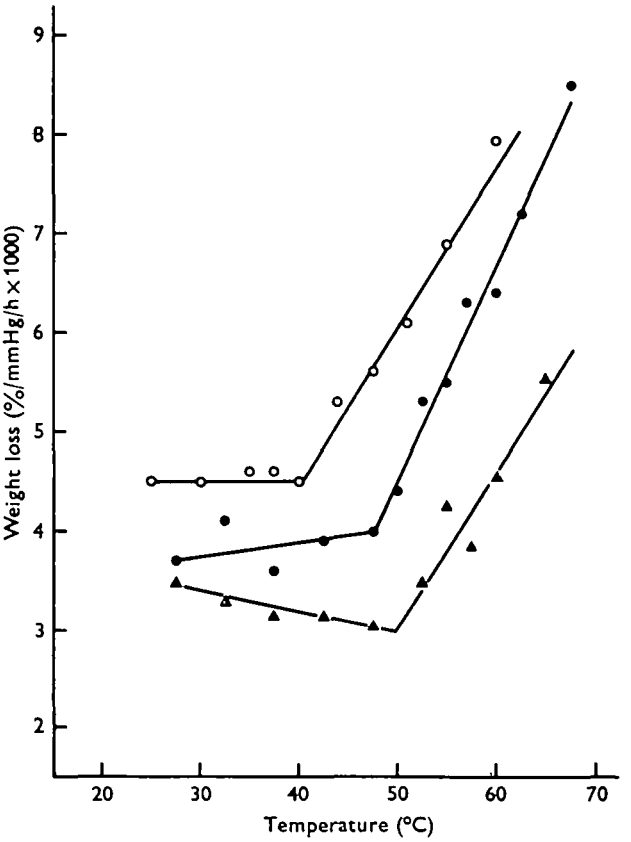

Fig. 8. Comparative rates of cuticular transpiration and cuticular transition temperatures in freshly killed and sealed *E. armata*, *C. muricata* and *C. verrucosa* at progressively higher temperatures and 0% R.H. Groups of beetles were exposed only once to each temperature. Triangles and circles represent group means (min. '*n*' = 10). ○, *E. armata*; ●, *C. muricata*; ▲, *C. verrucosa*.

Faecal water loss

Water loss from defaecation was estimated by comparing weight changes in starved and non-starved groups of *E. armata*, *C. muricata* and *C. verrucosa* exposed for 6 h to 30 °C and 0% R.H. All animals were maintained in beakers during the exposure period so that released faecal pellets by the non-starved groups would be retained. Water loss from beetles in the starved groups, which did not release any detectable quinone droplets or faeces during the exposure, was due to transpiration alone, while weight loss in beetles of the non-starved groups, that did not release quinone droplets, was calculated as being due to transpiration and faecal water loss. The losses of water of these two groups of *E. armata*, *C. muricata* and *C. verrucosa* are shown in Fig. 9. Both the starved and non-starved groups of *E. armata* demonstrated the greatest water loss of all three species of beetles under the experimental conditions. After 6 h of exposure, *C. verrucosa* lost approximately one-third of the transpiratory water as did *E. armata*, while transpiration in *C. muricata* accounted for two-thirds of the value found for *E. armata*. The same relationship existed between the mean values of water loss of the three species in the non-starved groups. The large spread in the con-

fidence limits for water loss in the non-starved groups probably was a result of the variable amounts of defaecation that occurred within these groups, since the size of the limits for transpiration values alone in the starved animals were quite small. Water loss from transpiration plus faecal production (non-starved) in *C. muricata* and *C. verrucosa* was not significantly different ($P > 0{\cdot}05$; group comparison test) than the water loss from transpiration alone (starved) in these same species. Water loss from the two groups of *E. armata* was significantly different ($P < 0{\cdot}05$; group comparison

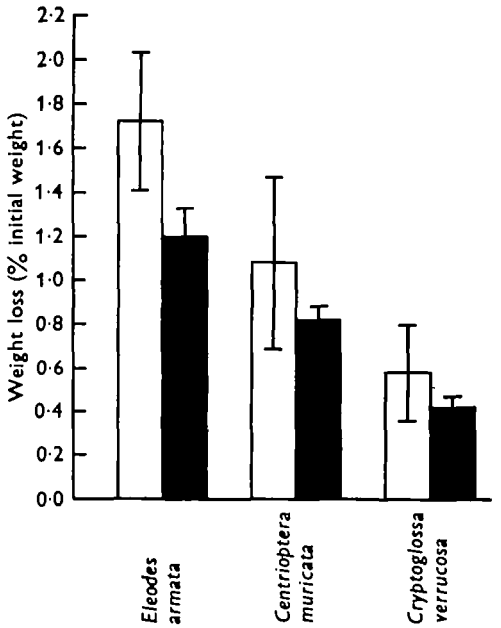

Fig. 9. Estimation of water loss from defaecation in *E. armata*, *C. muricata* and *C. verrucosa* over a 6 h exposure at 30 °C and at 0% R.H. Bars represent group means (min. 'n' = 13) and vertical lines 95% confidence limits. □, Transpiration + faeces; ■, transpiration only.

test). Faecal water loss represented a larger proportion of total water loss in *E. armata* than in either *C. muricata* or *C. verrucosa*; smaller losses of water from faecal production in the latter two species may have been due to a high degree of physiological control of excretion.

Oxygen consumption and estimated respiratory transpiration in Eleodes armata

The oxygen consumption of *E. armata*, *C. muricata* and *C. verrucosa* was measured at a single temperature (35 °C) to determine which species of beetle had the highest respiratory rate. Relative humidities during the exposure periods varied between 40 and 60%. *Eleodes armata* consumed greater volumes of oxygen per unit of body weight per hour than did either of the other two species (Table 4). These data correlate favourably with comparative measurements of estimated respiratory transpiration. *Eleodes armata* was the only species in which the respiratory water loss could be evaluated at 35 °C (Fig. 7), while both *C. muricata* and *C. verrucosa* lost so little water by way of respiration that spiracular transpiration could not be estimated at this

temperature. Because *E. armata* demonstrated both measurable oxygen consumption and respiratory transpiration at 35 °C, a more detailed comparison between the two parameters in this species was conducted.

To make a valid comparison between oxygen consumption and spiracular water loss, the relative humidity in each respirometer was adjusted to near 0% R.H. Oxygen consumption by *E. armata* exhibited an inverse linear relationship with body weight at 32·5 °C and 0% R.H. (Fig. 10). The relatively high r^2 value (0·665) indicated

Table 4. *Comparison of rates of oxygen consumption of three tenebrionid beetles at* 35 °C *and* 40–60% R.H.

(Groups of beetles were exposed to experimental conditions for at least one hour before oxygen consumption was recorded. Oxygen consumption values, recorded at hourly intervals over a 6 h exposure period, are means followed by 95% confidence limits).

Species	Sample size	Rate of oxygen uptake (mm^3 O_2/g/h)
Eleodes armata	66	446·52 ± 29·17
Centrioptera muricata	60	266·50 ± 19·21
Cryptoglossa verrucosa	60	182·72 ± 35·50

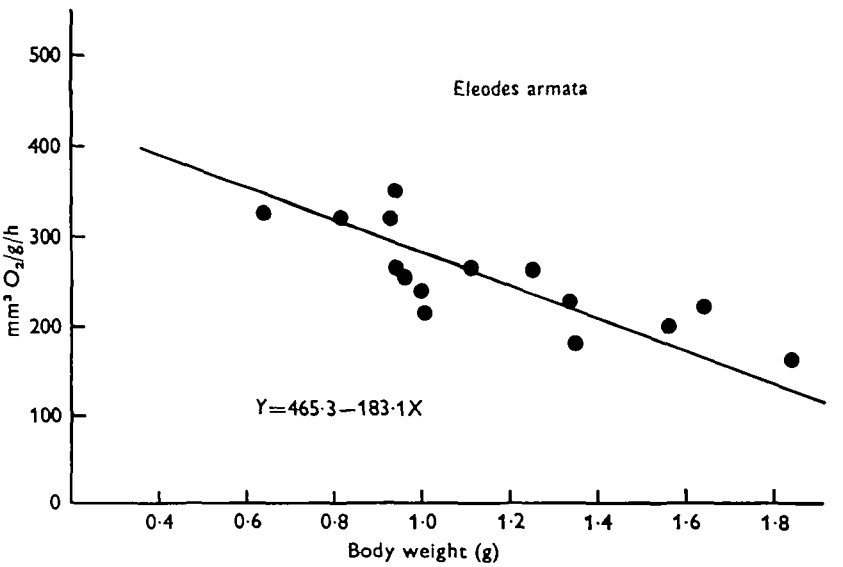

Fig. 10. Regression of oxygen consumption versus body weight in *E. armata* at 32·5 °C and 0% R.H. Each circle represents the mean of three 1 h recordings of oxygen uptake by an individual beetle.

a high correlation between the two parameters. Oxygen consumption varied from approximately 200 mm^3 O_2/gm/h for beetles weighing between 1·4 and 1·8 g to over 300 mm^3 O_2/g/h for beetles weighing less than 1·0 g. In order to reduce the effects of body size on subsequent measurements of oxygen uptake at a series of temperatures, only specimens of *E. armata* weighing near 1·0 g were selected for further investigation.

Groups of *E. armata* (min. '*n*' = 10) were exposed to temperatures from 25 to 40 °C at 0% R.H. to measure temperature-induced changes in respiration. Mean

group values of oyxgen consumption at each temperature were taken every half-hour for a maximum of 7 h following the usual 1 h equilibration period (Fig. 11). Oxygen uptake by *E. armata* increased with rising temperature over the entire exposure range. Nearly constant values of oxygen consumption (approximately 250 mm^3 O_2/g/h) were recorded by animals exposed to 30, 32·5 and 35 °C. From 25 to 37·5 °C the mean group values of oxygen utilization at each temperature showed very little variation from one-half hour reading to the next. In contrast, the beetles exposed

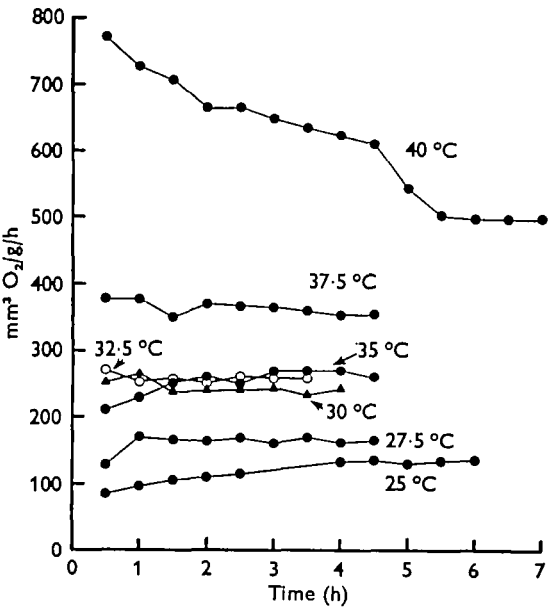

Fig. 11. Comparative rates of oxygen consumption in *E. armata* at temperatures from 25 to 40 °C at 0% at R.H. Values of oxygen consumption at each temperature were recorded every half hour. Circles and triangles represent group means (min. '*n*' = 10).

to 40 °C demonstrated a marked decrease in oxygen consumption during the first five hours of measurement followed by 2 h of constant oxygen uptake. Since the initial oxygen consumption values for the first hour of exposure to 40 °C were nearly 300 mm^3 O_2/g/h higher than values recorded after constancy had been achieved, this temperature probably had imposed an initial thermal stress on the animals which was later partially compensated for during the prolonged exposure.

Mean half-hourly values of oxygen consumption over the entire exposure period at each temperature except 40 °C were summed, and grand means, representing the overall oxygen consumption of the groups at these temperatures, were calculated. A grand mean, representing only the first 6 h, was calculated for animals exposed to 40 °C. The grand means of oxygen consumption of *E. armata* from 25 to 40 °C were compared to the estimated respiratory transpiration values of this species at the same temperatures (Fig. 12A). A striking similarity existed between the general shapes of the two curves. A regression analysis was calculated on data from corresponding temperatures (Fig. 12B). The very high r^2 value (0·955) suggests that water loss from respiration in *E. armata* was directly correlated with oxygen consumption. Therefore, as the

oxygen demand by these animals increased, there was also a direct increase in the amount of water loss by way of respiratory transpiration. These results suggest that the method used in this investigation to estimate respiratory water loss in *E. armata* was probably relatively accurate. If the same degree of accuracy occurred in estimating this source of water loss in *C. muricata* and *C. verrucosa*, then these two species were probably much better spiracular regulators of water loss than was *E. armata*, since it was not possible to obtain a value of respiratory transpiration from either of the former animals at temperatures from 25 to 40 °C (Fig. 7).

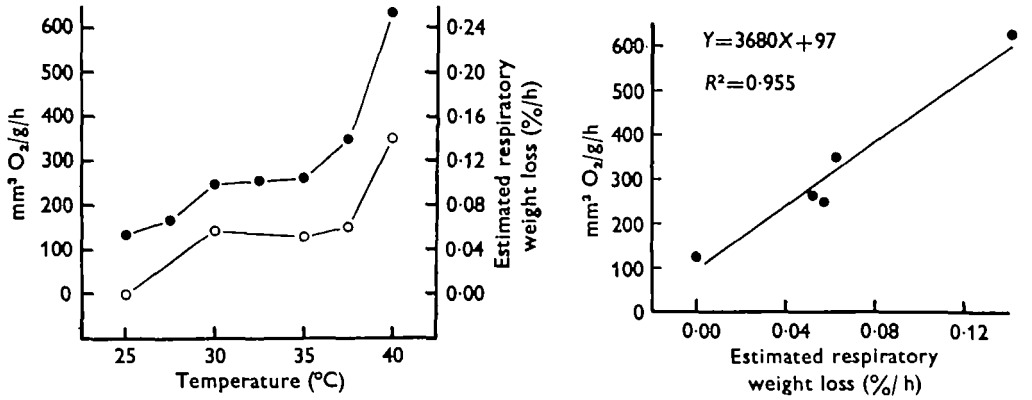

Fig. 12A. Comparison of rates of oxygen consumption (grand means; min. '*n*' = 70) and estimated respiratory transpiration in *E. armata* at a series of temperatures and at 0% R.H. Measurements of estimated respiratory transpiration were obtained from Fig. 7 and represent mean differences between total and cuticular transpiration at each temperature. ●——●, O_2 uptake; O——O, weight loss.

Fig. 12B. Regression of oxygen consumption versus estimated respiratory weight loss in *E. armata*. The circles represent mean values of oxygen consumption and estimated respiratory transpiration (Fig. 12A) recorded at 25, 30, 35, 37·5 and 40 °C and at 0% R.H.

Discontinuous respiration in Eleodes armata

The maintenance of a constant rate of oxygen consumption over a given period of time would result in greater spiracular losses of water than the employment of a cyclic breathing pattern. Measurements of oxygen consumption over very short time intervals (10 min) provided information as to the possible breathing pattern of *E. armata*. Ten individuals of this species, each weighing approximately 1·0 g, were used for measurements of oxygen consumption at 32·5 °C and 0% R.H. There was considerable fluctuation in the volume of oxygen consumed by each beetle over a series of twelve 10 min periods (Fig. 13). In several animals, periods of relatively high oxygen uptake alternated with intervals of lower respiratory activity. No cyclic rhythm associated with spiracular regulation of water loss could definitely be ascribed to these breathing fluctuations, but since a constant level of oxygen consumption was not maintained by any beetle throughout the entire 2 h exposure period, some reduction in water loss through the spiracles may have occurred.

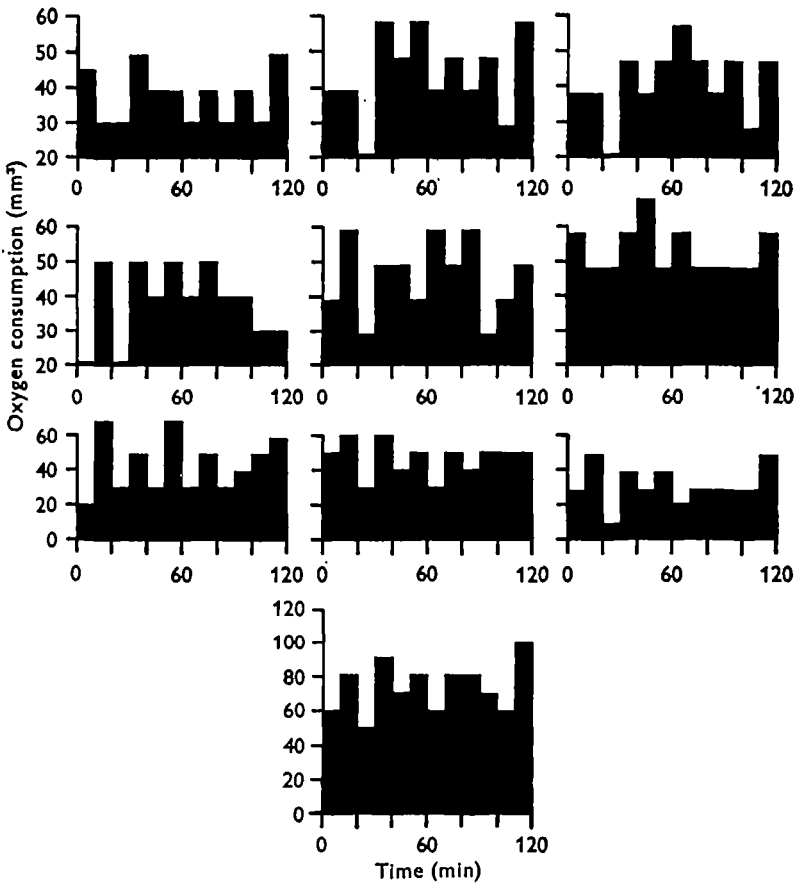

Fig. 13. Rates of oxygen consumption by *E. armata* over short time intervals (10 min) at 32·5 °C and 0% R.H. Each 2 h histogram represents the oxygen consumption of a single beetle.

DISCUSSION

A high correlation exists between rates of transpiration and dryness of the habitat for isopod crustaceans (Warburg, 1965*a–c*; Edney, 1954, 1967, 1968), insects (Cloudsley-Thompson, 1956; Bursell, 1957, 1958; Edney, 1967) and myriapods and arachnids (Cloudsley-Thompson, 1956, 1961, 1967; Ahearn, 1970*a*). These studies suggest that greater rates of water loss are associated with those arthropods inhabiting moist environments. In a 15-month field study conducted near Phoenix, Arizona (Ahearn, 1970*b*), *E. armata* was collected in maximum numbers from pitfall traps during the autumn months (September–October), while *C. muricata* and *C. verrucosa* were most abundantly trapped during the summer (June–July). The autumn appearance of *E. armata*, the most permeable species, may signify a physiological restriction of maximum activity to the less-demanding environmental conditions present during this season. The summer beetles, *C. muricata* and *C. verrucosa*, because of their low transpiration rates, were well adapted to the hot, dry conditions to which they were exposed. Therefore, the relationship between permeability and habitat dryness

appears to hold for South Mountain desert tenebrionid beetles and reflects their comparative physiological adaptations to environmental stress.

Three factors which contributed to total water loss in tenebrionid beetles were: release of quinone droplets and/or oral fluids, defaecation, and respiratory and cuticular transpiration (Figs. 1 and 4). At temperatures of 25, 35 and 40 °C quinone droplet release and/or oral fluid secretion accounted for approximately 50% of total water losses in *E. armata* (Table 1). Contributions to total water loss by way of excreted honeydew droplets have been reported for aphids (Cockbain, 1961). At 25 °C and 57–82% R.H., 34% of total water losses during tethered flight in *Aphis fabae* were attributable to excretion, while the remaining 66% was derived from the combination of respiratory and cuticular transpiration. In nature, because of the severe water deficit which would result, water loss via copious quinone droplet release and/or oral fluid secretion in tenebrionid beetles may only occur during infrequent periods of predator avoidance and/or temporary exposure to unfavourable climatic conditions.

Rates of water loss from defaecation in *E. armata*, *C. muricata* and *C. verrucosa* were low when compared to their respective rates of transpiration (Fig. 9), suggesting that physiological control of faecal water loss may be an important adaptation of these animals to the desert habitat.

Comparisons of transpiration in groups of living and freshly killed arthropods have been made by several investigators with considerable differences of opinion resulting from interpretations of the data. Wigglesworth (1945) found that, provided the spiracles of the experimental organisms were sealed, there was no difference in the rates of evaporation from dead or from living insects. This interpretation was based upon the premise that short periods of exposure had to be used so that the water content of dead and living insects remained similar. In a later paper (Davies & Edney, 1952), rates of transpiration for live spiders (*Lycosa amentata*) at 30 and 40 °C were lower than rates of freshly killed animals with free book-lungs, but were higher at these temperatures than the water loss demonstrated by dead spiders with their book-lungs sealed. They suggested that differences between evaporation rates of living and dead (unblocked book-lungs) animals may have been attributed to 'active inward secretion' of water by epidermal cells of the cuticle in living animals. Little attention was given to a discussion of possible contributions to increased water loss by partially open book-lungs in the dead spiders. Smaller losses from the blocked group of dead spiders than from the intact freshly-killed animals may have resulted from a reduction in book-lung evaporation due to the use of the sealing agent. In a similar study, Winston & Nelson (1965) reported that dead clover mites (*Bryobia praetiosa*) lost considerably more water due to transpiration than did the control groups of living animals at 25 °C and several different humidities. Freshly killed scorpions, *Hadrurus arizonensis*, with book-lungs sealed exhibited greater losses of water at temperatures from 25 to 40 °C and 0% R.H. than untreated living animals (Neil F. Hadley, Arizona State University, personal communication). The latter results indicate that, at least for this species, increased transpiration following death is largely due to a decrease or cessation of active cuticular control.

Loss of the spiracular controlling mechanism upon death in desert tenebrionid beetles may have been largely responsible for greater water loss in the dead, unsealed animals than in the living or dead, sealed groups over short periods of exposure (Fig. 6). Since

cuticular transpiration in sealed, freshly killed animals was less than, or equal to, the losses exhibited by living specimens of both *E. armata* and *C. muricata*, it can be assumed that loss of cuticular transpiratory control was probably of minor significance over these short periods of exposure. As the length of time after death increased, loss of cuticular control may have assumed a far greater role in determining total levels of transpiration and may have accounted for the increasing divergence of transpiratory losses in living and dead beetles over long periods of exposure (Fig. 3).

From the above discussion it appears that a wide variety of changes accompanying death may occur in the water-regulation mechanisms of arthropods. For short periods of exposure some insects may show no significant differences in water loss between living and dead groups. For other arthropods, such as the mites (*Bryobia praetiosa*), spiders (*Lycosa amentata*), scorpions (*H. arizonensis*) and beetles (*E. armata* and *C. muricata*), water loss may be significantly greater in dead than living specimens. This cessation of active water regulation at death may be due to approximately equal decreases in cuticular and spiracular control as in the clover mites (Winston & Nelson, 1965), or it may be predominently derived from losses of cuticular (scorpions; N. F. Hadley, personal communication) or spiracular (tenebrionid beetles) control.

Rates of total transpiration at 30 °C and 0% R.H. for *E. armata*, *C. muricata* and *C. verrucosa* amounted to 0·200, 0·120 and 0·090% initial body weight/h, respectively (Fig. 7). Scorpions, desert arthropods sympatric with tenebrionid beetles in many of the world's deserts, have transpiratory rates almost an order of magnitude less than those exhibited by the beetles in the present study. Cloudsley-Thompson (1956, 1961) found transpiratory losses from African scorpions (*Leiurus quinquestriatus* and *Androctonus australis*) to be 0·030 and 0·032%/h, respectively, at 33 °C and 0% R.H. In a similar study, he demonstrated a water loss of 0·042%/h under these same conditions for the Sonoran scorpion, *Hadrurus hirsutus* (Cloudsley-Thompson, 1967). Other desert arthropods appear to be more permeable than scorpions or beetles and generally exhibit somewhat higher values of water loss under the same environmental conditions. Tarantula spiders (*Eurypelma helluo* and *E. californicum*) demonstrated transpiration rates of 0·600 and 0·500%/h (Herreid, 1969), while ventilating locusts (*Locusta migratoria*) exhibited values near 0·600%/h (Loveridge, 1967). These results suggest that desert tenebrionid beetles occupy an intermediate position among desert arthropods with respect to their control of transpiratory water loss.

Water loss from the cuticular surface was a larger component of total transpiration than was the estimated loss from the spiracles at all exposure temperatures for *E. armata*, *C. muricata* and *C. verrucosa* (Fig. 7 and Table 2). At temperatures below 40 °C respiratory losses of water for each tenebrionid were very small, but at 40 °C for *E. armata* and 42·5 °C for *C. muricata* and *C. verrucosa* spiracular water loss increased so that its contribution amounted to one-third of the total transpiratory losses. These results clearly indicate that temperatures above 37·5 °C interfered with spiracular regulation of water loss, probably because of increased oxygen demand or CO_2 build-up.

Early studies by Ramsay (1935), Beament (1945), Wigglesworth (1945) and Lees (1947) have shown that the main barrier to the outward flow of water through the insect integument lies in the epicuticle. More recently, Beament (1958, 1959, 1961, 1964*a*, *b*) suggested that impermeability is a result of closely packed and oriented polar

lipid molecules near the surface of the epicuticle. Current thought is that the transition phenomenon of the insect cuticle (abrupt increase in the rate of cuticular transpiration at a particular temperature) is a result of a physical change, such as a disorientation of these lipid molecules in the epicuticle, that enhances the passage of water through the lipid layer. Interspecific differences in temperatures at which lipid disorientation occurs are believed to be largely due to the carbon-chain length of the lipids, high transition temperatures being found for cuticles with long epicuticular lipids.

E. armata was slightly more permeable and had a lower cuticular transition temperature (40 °C) than either *C. muricata* (47·5 °C) or *C. verrucosa* (50 °C). These critical temperatures are similar to those of other arid insect species. Loveridge (1968*a*) demonstrated an abrupt increase in cuticular permeability in *Locusta migratoria* at temperatures between 46 and 48 °C. Another grasshopper, *Schistocerca gregaria*, exhibited a transition temperature of 48 °C (Beament, 1959), while *Tenebrio molitor* (Wigglesworth, 1945) and *Rhodnius prolixus* (Beament, 1959) had transition temperatures of 50 and 58 °C respectively. Although the ecological significance of the transition phenomenon is unclear, it appears that arthropods with high transition temperatures have low transpiration rates (Edney, 1967). In agreement with this general relationship, *C. verrucosa*, the beetle with the highest cuticular transition temperature, also had the lowest rate of cuticular water loss, while the most permeable species, *E. armata*, possessed the lowest temperature of cuticular transition.

Oxygen consumption rates of *E. armata*, *C. muricata* and *C. verrucosa* measured at 35 °C and 40–60 % R.H. ranged between 180 and 450 mm^3 $O_2/g/h$ (Table 4) and were low when compared to rates exhibited by mesic arthropods at similar temperatures (Keister & Buck, 1964). If water loss were to parallel oxygen consumption, species with low metabolic rates may lose considerably less water (assuming similar cuticular permeabilities) than animals with higher respiratory activities.

The very high correlation between oxygen consumption and estimated respiratory transpiration in *E. armata* over the temperature range of 25 to 40 °C at 0 % R.H. strongly suggests that the breathing rate of this animal is directly responsible for the observed spiracular water loss (Fig. 12). The physiological mechanisms used by *E. armata* to regulate its rates of oxygen consumption and respiratory transpiration are not known, but may result from spiracular regulation involving unidirectional tracheal air flow, discontinuous breathing patterns and controlled ventilation.

Unidirectional air flow in the tracheal system may help to conserve water reserves under desiccating conditions by having the first few ventilatory cycles clear the tracheal trunks of water vapour so that successive cycles need not expire completely saturated air (Loveridge, 1968*b*). Buck (1962) and Miller (1960*a*, *b*) suggested that a unidirectional air flow through an arthropod would occur if the thoracic spiracles were used for inspiration and some of the abdominal pairs for expiration. In *E. armata* at 30 °C and 0 % R.H. nearly 85 % of total estimated respiratory water loss came from abdominal spiracles (Table 3), suggesting that these respiratory centres may have been the greatest site of expiration associated with unidirectional air flow through its tracheal system. The marked elevation in the estimated proportion of water loss from the thoracic spiracles in *E. armata* with a rise in temperature from 30 to 40 °C, may indicate a change from unidirectional air flow at lower temperatures to active thoracic ventilation in both directions at the higher temperatures.

The short-term inconsistencies in oxygen consumption exhibited by *E. armata* (Fig. 13) suggest that discontinuous respiration, due to periodic ventilation of the tracheal system, may have occurred in this species of beetle and may represent a respiratory adaptation for reducing spiracular water loss. Similar periodic bursts in oxygen uptake have been reported for the carabid beetles, *Hadrocarabus problematicus* (Punt, Parser & Kuchlein, 1957) and *Carabus nemoralis* (Punt, 1956*a*, *b*) and were associated with concurrent cycles of CO_2 release. Hadley & Hill (1969) demonstrated alternating periods of high and low oxygen consumption by the scorpion, *Centruroides sculpturatus* during 5 min intervals at 20 °C, but found that at 40 °C oxygen consumption by this species became more uniform due to metabolic increases under stress. Discontinuous respiration, involving a cyclic spiracle-opening rhythm, could restrict the outward flow of tracheal water vapour to periods when the spiracles were open for respiratory gas exchange. Tissues with high CO_2 tolerance and low O_2 demand may help to facilitate the operation of cyclic respiration and the concurrent reduction in spiracular water loss.

Adaptations exhibited by desert arthropods are elaborations of behaviour, morphology and physiology of similar species inhabiting less extreme terrestrial environments. For survival under arid conditions, desert tenebrionid beetles may restrict their greatest seasonal and diurnal activities to periods of minimum temperature and humidity stress. Relatively low rates of cuticular permeability in tenebrionids are similar to values reported for other xeric arthropods and are considerably less than transpiration rates of species inhabiting more moist environments. In addition, small losses of water through the spiracles in these beetles may signify the presence of highly controlled respiratory mechanisms for water regulation. These respiratory control mechanisms may involve the combination of low metabolic rate, discontinous respiration, and physiological regulation of tracheal air flow. Such adaptations for restricting the flow of body water to the environment have contributed to the successful establishment of tenebrionid beetles in the desert habitat.

SUMMARY

1. Total water loss in tenebrionid beetles was composed of transpiratory losses from the cuticle and spiracles, water associated with defaecation, and water from the release of defensive quinone droplets or oral fluids.

2. Freshly killed specimens of *E. armata*, *C. muricata* and *C. verrucosa* had higher transpiration rates over long and short exposures than did living animals of the same species. These results may reflect the cessation of active water retention by cuticular and spiracular regulation in dead animals.

3. Cuticular transpiration, although low in absolute rate, was a greater source of water loss than respiratory transpiration in *E. armata*, *C. muricata* and *C. verrucosa* at temperatures from 25 to 42·5 °C and at 0% R.H., suggesting that spiracular control of water loss was of considerable importance in maintaining water balance.

4. A marked increase in respiratory transpiration over previous low rates was observed at 40 °C for *E. armata* and at 42·5 °C for *C. muricata* and *C. verrucosa*, and indicated a temperature-induced breakdown in spiracular water regulation due to increased respiratory activities. In contrast, cuticular transpiration maintained a linear rate of increase over the temperature range investigated.

5. A direct relationship existed between oxygen consumption and estimated respiratory transpiration for *E. armata* from 25 to 40 °C and at 0 % R.H.

6. Evidence for the presence of discontinous respiration and unidirectional tracheal air flow in *E. armata* was presented.

7. Cuticular transition temperatures were measured for *E. armata* (40 °C), *C. muricata* (47·5 °C), and *C. verrucosa* (50 °C) with results showing that the autumn species (*E. armata*) possessed a lower cuticular breakdown point than either of the two summer species.

8. Much of the success of tenebrionid beetles in desert habitats is due to the development of highly impermeable cuticles and well regulated spiracular control mechanisms for reducing the loss of body water.

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