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ROUTES OF TRANSPIRATORY WATER LOSS IN A DRY-HABITAT TENEBRIONID BEETLE

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Summary

Routes of evaporative water loss in the tenebrionid beetle *Phrynocolus petrosus* Gerstaecker from dry savanna in East Africa were investigated. The humidity of the air surrounding the abdomen, the air surrounding the head and pronotum and the air inside the subelytral cavity was varied independently, and the effects on organismal rate of water loss were observed.

The rate of organismal water loss dropped when the humidity around the head and pronotum and inside the subelytral cavity increased. Saturation of these air compartments, which both exchange respiratory gases with the tracheae through the spiracles, reduced the organismal rate of water loss by more than 80%, even when the large abdominal surface was surrounded by dry air. The results indicate that the transcuticular water loss makes up only about 20% of the total transpiratory water loss in these beetles, i.e. transcuticular water permeability is very low.

The results also indicate that the average air humidity inside the subelytral cavity of normal intact beetles is close to saturation. Water loss from the subelytral chamber is reduced accordingly, and appears to make up less than 10% of the total transpiratory water loss. The water loss over the pronotal spiracles amounts to about 70%, and is thus the dominant component of transpiratory water loss in these beetles.

Introduction

Arid tropical regions support a rich insect fauna. Free water is available only occasionally, and most of the time the insects depend on water contained in the food and water produced by metabolism (Edney, 1977). Many insects in these areas display a restrictive water balance. This is achieved by producing dry faeces and by reducing transpiratory water loss to a minimum (Edney, 1977; Zachariassen *et al.* 1987).

There are two components of transpiratory water loss: the transcuticular component, which takes place across the cuticle of the outer body wall, and the respiratory component, which accompanies the exchange of respiratory gases through the spiracles. Wigglesworth (1965) claimed that in many insects cuticular

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water loss is so low that the respiratory component becomes the major component of transpiratory water loss. However, on the basis of more recent experimental evidence, several authors (Ahearn, 1970; Hadley, 1970) seem to agree that in resting insects the respiratory water loss is small compared with the transcuticular component. The transcuticular water loss is assumed to be the dominant component even in dry-habitat insects with a restrictive water balance (Edney, 1977). Other authors have obtained results indicating that respiratory water loss is of somewhat greater importance. By using a gravimetric technique, Cooper (1983) found that respiratory water loss makes up about 36 % of total evaporative water loss in *Eleodes armata* beetles. By using a technique with tritiated water, Nicolson *et al.* (1984) found that in the African desert tenebrionid beetle *Onymacris plana* the respiratory water loss is about 70 % of the total evaporative water loss.

It has also become common among investigators to calculate rates of organismal water loss in relation to body surface area and to use the resulting values (also related to saturation deficit) as a measure of cuticular water permeability (Edney, 1977; Punzo and Huff, 1989). Edney (1977) presented estimated values for cuticular water permeability from a great number of insect species, including several dry-habitat tenebrionid beetles. All values had been calculated by relating the rates of *total* transpiratory water loss to body surface area. The use of the total organismal water loss in these calculations implies that the respiratory water loss is negligible in comparison with the transcuticular component.

Evidence obtained by Zachariassen *et al.* (1987) and Zachariassen and Maloiy (1989) indicates that, in tenebrionid and carabid beetles from arid habitats in East Africa, the respiratory water loss is the dominant component of water loss. However, the evidence was to a great extent indirect and, to draw firm conclusions, it is necessary to have more direct observations of the relative importance of the various avenues of transpiratory water loss.

The present experiments were designed to determine the relative importance of the different avenues of transpiratory water loss from a dry-habitat tenebrionid beetle. The technique also allows comparisons to be made between the water loss component through subelytral spiracles and the component through the pronotal spiracles. Furthermore, the experiments provide data for determination of the average relative humidity of the air in the subelytral chamber.

Materials and methods

The experiments were carried out with tenebrionid beetles of the species *Phrynocolus petrosus*, which were collected on dry savanna in the vicinity of Isiolo, Kenya. The water balance of the species is highly restrictive, and specimens can survive without food and water in a dry atmosphere at room temperature for at least 3 months (Zachariassen *et al.* 1987).

The beetles were 18–20 mm long and weighed 1.5–2.0 g at the time of collection. They were transported to the laboratory inside small plastic boxes, and kept at room temperature (about 23 °C) for up to 2 months before they were used in the

experiments. They were given water and fed pieces of fresh potato. Prior to the experiments the beetles were starved for 3 days to prevent faecal mass loss from influencing the body mass measurements.

The relative magnitudes of the different routes of water loss were investigated by varying independently the humidity of the air surrounding the head and pronotum (with the pronotal spiracles), and the humidity of the air in the subelytral space (with the abdominal spiracles), and observing the effects on the rate of organismal water loss, determined as the rate of loss of body mass. The air surrounding the abdomen was dry throughout the experiments.

The magnitude of a given route of water loss can be determined by observing the reduction in the rate of organismal water loss following an increase of the humidity of the ambient air of that route from normal to saturated. However, the use of saturated air may lead to condensation of water on body surfaces, a phenomenon that would disturb the mass loss determinations. This phenomenon is particularly problematic if it occurs in the subelytral space, where condensation of water cannot be detected visually. For this reason, the present experiments were carried out by varying the air humidity in the subelytral chamber over a wide range of sub-saturated values, and the effect of full saturation was determined by extrapolation.

The experiments were carried out using the apparatus illustrated in Fig. 1. The apparatus consists of a Plexiglas tube, closed at both ends by Plexiglas lids in close contact with the inner wall of the tube by means of gas-proof O-ring fittings.

A Plexiglas ring with an external O-ring, which fits exactly the inner wall of the Plexiglas tube, divides the inner chamber into two separate compartments. The beetle was mounted in the central part of the Plexiglas ring, attached to a 'collar' made of 0.1 mm thick celluloid. The celluloid collar was fixed to the Plexiglas ring by means of a double set of equidiametric O-rings, as shown in Fig. 1. The central perforation of the celluloid collar was shaped to fit the cross section of the frontal part of the abdomen, and the collar was glued to the beetle with epoxy resin, so that no gas could leak between the collar and the beetle.

Mounted in this way, the anterior part of the beetle, i.e. head and pronotum with pronotal spiracles and forelegs, was exposed to one of the air compartments (the anterior chamber) and the abdomen with middle legs and hindlegs was exposed to the other (the posterior chamber). The Plexiglas ring, the 'collar', and the beetle formed a waterproof barrier, preventing vapour from leaking from one compartment to the other. The epoxy did not harm the beetles, which were in apparently good shape even months after the termination of the experiments.

Air at different relative humidities could be led independently to the different chambers *via* thin tubes of stainless steel, leading through the Plexiglas lids. To control the air humidity in the subelytral chamber, two thin (i.d.=0.5 mm) tubes of stainless steel (the elytral tubes) were inserted through the elytra and fixed to the outer surface of the elytra with small droplets of epoxy. By means of flexible polyethylene tubes running through the posterior chamber, the elytral tubes were connected to similar tubes leading through the posterior Plexiglas lid. The polyethylene tubes were shaped as illustrated in Fig. 1 in order to obtain sufficient

elasticity to connect them to the steel tubes running through the posterior lid after the beetle had been mounted inside the chamber. Leakage of air between the posterior and the subelytral chambers was prevented by sealing the natural opening of the subelytral chamber with epoxy.

The efficiency of the sealing of the chambers was tested by injecting a small volume of air from a syringe to the respective chambers (one chamber at a time) and observing the air pressure on a mercury manometer connected to the chamber (Fig. 1). A persistent elevation of the air pressure after the injection indicated that the sealing was efficient.

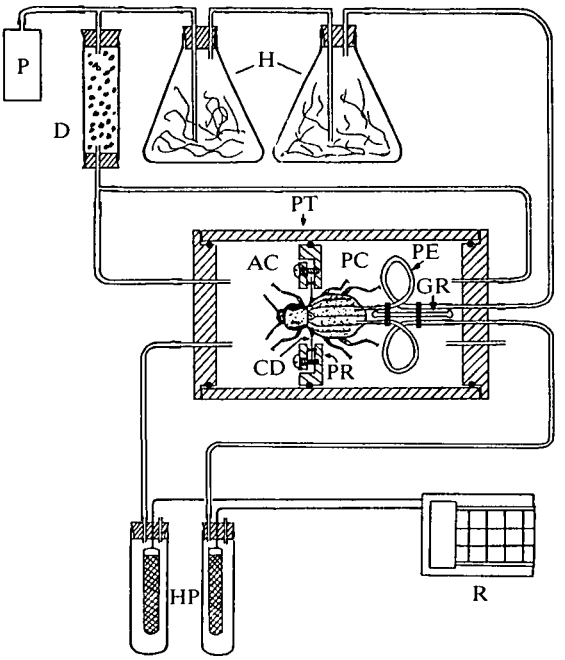

Fig. 1. The apparatus used to measure water loss components over the abdominal surface, head and pronotum with pronotal spiracles, and the subelytral space with subelytral spiracles. The apparatus consists of a Plexiglas tube (PT) with inner diameter 7 cm. The inner space in the tube is closed at both ends by Plexiglas lids, across which air can pass *via* thin tubes of stainless steel. A Plexiglas ring (PR) is placed inside the chamber so that it divides the chamber into an anterior chamber (AC) and a posterior chamber (PC). The beetle is glued to a perforated celluloid disc (CD), which is mounted in the central opening of the Plexiglas ring. Air may enter and leave the subelytral space through thin tubes of stainless steel, running through the elytra. The transelytral tubes are connected to steel tubes running through the posterior lid *via* two flexible polyethylene tubes (PE). Each polyethylene tube forms a loop which is kept in position around a supporting glass rod (GR) by means of two loose plastic straps, allowing the tubes to slide over the rod when they are being mounted to the steel tubes of the posterior lid. P, air pump; H, Erlenmeyer flasks containing wet filter paper to humidify the air; D, Column of silica gel to dry the air; HP, humidity probes to monitor the relative humidity of air leaving the compartments; R, line recorder to register the output from the humidity probes.

The air was dried (relative humidity <5 %) by running it slowly through a column of silica gel of diameter 3.5 cm and length 17.5 cm (Fig. 1). High relative humidities were obtained by running the air slowly through two Erlenmeyer flasks containing strips of moistened filter paper (Fig. 1). Intermediate air humidities were obtained by using only one Erlenmeyer flask and reducing the number of filter paper strips in it.

The air flow was established by means of an aquarium pump, as illustrated in Fig. 1. The air flow through the anterior and posterior chambers ranged from 5 to 10 ml min^{-1}, whereas the flow through the subelytral chamber was 2–4 ml min^{-1}. The dimensions of the tubes leading gas to and from the chambers were adjusted so that the air pressure inside the chambers remained low. This was particularly important for the subelytral chamber.

The air humidity was monitored by running the air leaving the chambers through small test tubes containing a Vaisala HMP 42U humidity probe (Fig. 1). The probes produced a voltage output which was proportional to the relative humidity. The probes had been calibrated by the manufacturer for relative humidities from 10 to 100 %. Although the calibration was guaranteed by the manufacturer, a rough control was made by measuring the voltage output of the probes in air in equilibrium with silica gel and in air saturated with water vapour. The tests confirmed that the calibrations were correct.

Even small temperature changes affect relative humidity and, to avoid thermal gradients, the saturation chamber and humidity probes were situated close to the experimental chamber, and lamps and other heat sources were kept away from the apparatus.

The voltage output from the humidity probes was recorded on a Pharmacia two-channel line recorder, which allows the voltage to be determined with an uncertainty of about ±1 %. Only the air from the anterior and subelytral chambers was monitored continuously on the recorder. However, the humidity of the air in the posterior chamber was measured several times a day to check that it remained dry.

The rate of water loss was determined as the percentage loss of body mass per hour. The beetle was weighed before it was glued to the collar, and the combined mass of beetle and collar determined after the epoxy had hardened. To determine the mass change of the beetle during the experiments, the beetle with its collar was dismounted and weighed, and the mass was subtracted from the last previously determined value. Depending on the rate of water loss, the weighings were carried out at intervals ranging from 3 to 12 h. Rates of water loss were calculated for each period. The weighings were carried out on a Mettler balance, accurate to ±0.1 mg. All experiments were carried out at room temperature (23–25 °C).

Before the elytral tubes were mounted and the opening of the subelytral cavity sealed, the rate of water loss of intact beetles mounted to the collar was determined with dry air (relative humidity <5 %) in both the anterior and the posterior chamber.

To determine the magnitudes of the water loss components over the anterior

part of the body including the pronotal spiracles, in the subelytral chamber with the abdominal spiracles, and over the abdominal surface, two series of water loss measurements were made with each beetle. While the air in the posterior chamber (which surrounds the abdomen) was kept dry in both series, the air in the anterior chamber was kept either dry (series A) or saturated (series B). In each series the relative humidity of the air in the subelytral chamber was varied between zero and 98 %. The humidity of the anterior chamber was changed randomly between dry and saturated throughout the experimental period, so that the measurements of both series were distributed over the entire period.

Statistics

The statistical significance of differences in slopes was tested using Student's *t*-test, as described by Bailey (1959). The significance of differences between the elevations of the regression lines was tested using Student's *t*-test according to formulae presented by Zar (1984).

Results

Since the absolute rates of water loss differ substantially between individuals, the results from each beetle are presented separately.

Fig. 2 shows the rates of organismal water loss of six *Phrynocolus petrosus* beetles with the head and pronotum surrounded by dry (line A) and vapour-saturated air (line B) plotted as a function of the saturation deficit of the air in the subelytral chamber. The abdomen was surrounded by dry air in both series of experiments. The rates of water loss of the same beetles, mounted in the apparatus with dry air in both outer chambers, and measured before the elytra were perforated with tubes (intact beetle), are represented by the broken horizontal lines in Fig. 2.

The slopes of the regression lines A and B do not differ significantly from each other in any of the beetles ($P>0.2$), but they differ significantly from the slope of a hypothetical horizontal line ($P<0.001$). Thus, a reduction of the relative humidity of the air in the subelytral chamber from saturation to zero caused the rate of organismal water loss to increase significantly, and apparently with the same slope, both when the air in the anterior chamber was dry and when it was saturated with vapour.

For all beetles, the elevation of line A differs significantly ($P<0.01$) from that of line B for the same beetle. Thus, an increase in the relative humidity in the anterior chamber from zero to saturation caused a significant and quite substantial reduction in the rates of organismal water loss.

The results imply that the relative humidity of the air in the anterior and the subelytral chambers strongly influenced the rate of water loss of *Phrynocolus petrosus* beetles. Saturation of the air in both these chambers at the same time (represented by ordinate intersection point of line B=a_B) reduced the rate of organismal water loss to about 16 % of the organismal water loss of the intact

beetle in dry air. This substantial reduction occurred in spite of the fact that the large abdominal surface, which makes up approximately 80 % of the total body surface, was still surrounded by dry air.

The values in Fig. 2 allow quantitative estimates to be made of the magnitude of the various water loss components. The ordinate intersection point of line B (a_B) represents the rate of water loss when the air in the subelytral space and the frontal chamber is saturated, i.e. the rate of water loss over the abdominal cuticle in dry air. By taking into consideration the fact that the abdominal surface makes up about 80 % of the body surface, and assuming that the cuticle of the head and

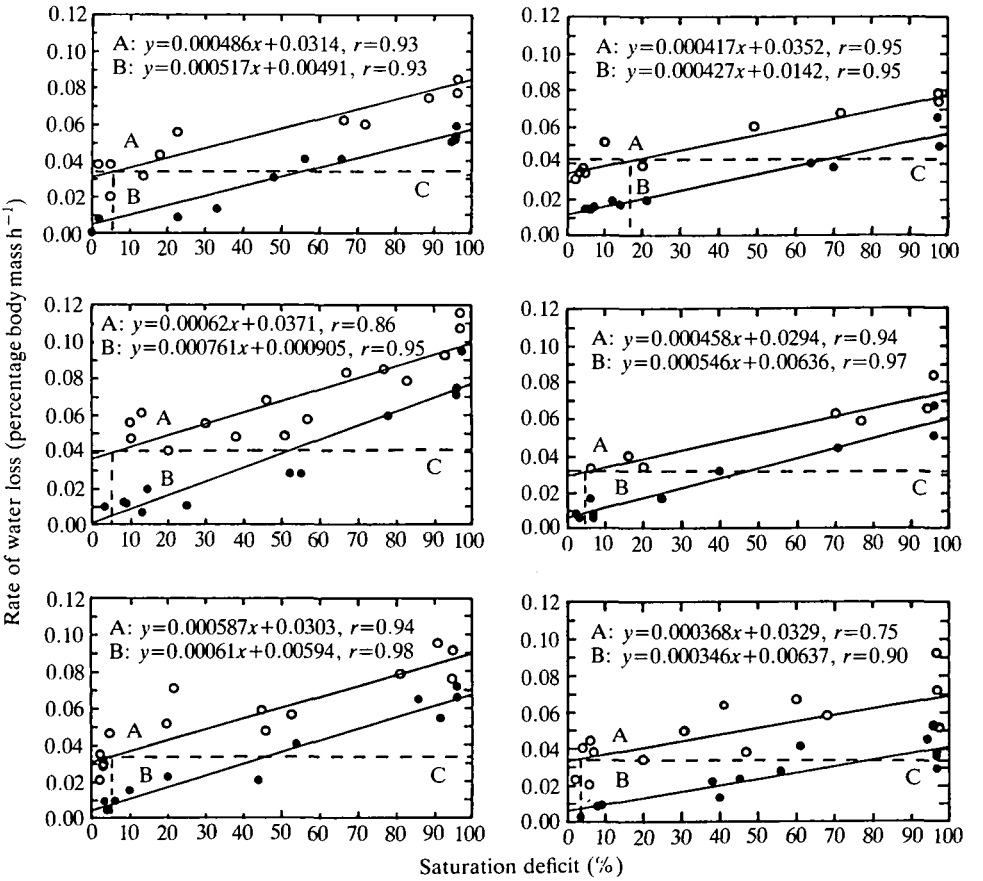

Fig. 2. Rates of water loss of *Phrynocolus petrosus* beetles with head and pronotum exposed to dry air (open circles, line A) and vapour-saturated air (closed circles, line B), plotted as a function of the saturation deficit of the air in the subelytral space. The rate of water loss is calculated as a percentage of the initial body mass. The air surrounding the abdomen was dry during both series of experiments. Horizontal broken curve (line C), mean rate of water loss measured in intact beetles (elytra not perforated) with the entire body surface exposed to dry air. Vertical broken line, projection onto the abscissa of the intersection between lines A and C, showing the saturation deficit of subelytral air of intact beetles.

Table 1. *Distribution of evaporative water loss over different routes in six* Phrynocolus petrosus *beetles in dry air*

Beetle number	a_C (% h^{-1})	a_{CUT} (% h^{-1})	Relative a_{CUT} (% of a_C)	a_{SE} (% h^{-1})	Relative a_{SE} (% of a_C)	a_{PRS} (% h^{-1})	Relative a_{PRS} (% a_C)
1	0.033	0.0061	18.5	0.0016	4.8	0.0253	76.6
2	0.041	0.0011	2.7	0.0039	9.5	0.0360	87.7
3	0.034	0.0074	21.8	0.0037	10.9	0.0229	67.3
4	0.043	0.0176	40.9	0.0078	18.1	0.0176	40.9
5	0.032	0.0080	25.0	0.0026	8.1	0.0215	67.2
6	0.034	0.0080	23.5	0.0009	3.2	0.0249	73.2
Mean±S.D.			22.1±11.2		9.1±4.8		68.8±14.3

*a_C, rate of water loss of intact beetles.

a_{CUT}, rate of cuticular water loss, estimated by dividing b_A values in Fig. 2 by 0.8, which is the fraction of the total cuticular area made up by the abdominal cuticular surface.

a_{SE}, rate of subelytral water loss, estimated by subtracting the rate of water loss of beetles in dry air with a saturated subelytral chamber (a_A) from the rate of water loss of the intact beetle (b_C).

a_{PRS}, rate of water loss over pronotal spiracles, estimated by subtracting the rate of cuticular water loss from the head and pronotum ($a_A \times 0.2/0.8$) from the total rate of water loss from the head and pronotum (difference in elevation between lines A and B, $a_A - a_B$).

pronotum has the same water permeability as the abdominal cuticle, the total cuticular water loss can be estimated as $a_B/0.8$.

The rate of subelytral water loss can be estimated as the difference $a_C - a_A$, where a_C is the rate of water loss of the intact beetles (i.e. the ordinate value of the broken line) and a_A is the rate of water loss when the beetles are surrounded by dry air, but the air in the subelytral cavity is saturated (i.e. the ordinate intersection point of line A).

The rate of water loss from the pronotal spiracles can be estimated from $a_A - a_B - (a_B/0.8) \times 0.2$, where $a_A - a_B$ is the total water loss from the head and pronotum and $(a_B/0.8) \times 0.2$ is the cuticular water loss from the same body segments.

The values of the various components of water loss, calculated from the data in Fig. 2, are presented in Table 1. The data in Table 1 reveal that substantial differences exist between individual beetles with regard to the magnitude of the various routes of water loss. The data indicate that, on average, transcuticular water loss makes up about 22% of the total organismal water loss, whereas subelytral water loss makes up only 9%. By far the greatest component is that *via* the pronotal spiracles, which makes up about 70% of the total organismal water loss.

The saturation deficit of the air in the subelytral space of the intact beetles was determined as the abscissa value of the intersection between line A and the horizontal broken line (line C), which represents the organismal rate of water loss of the intact beetles. The results are presented in Table 2. The mean saturation

Table 2. *Saturation deficit in the subelytral cavity of* Phrynocolus petrosus *beetles, estimated from the intersection points between lines A and C in Fig. 2*

Beetle number	Saturation deficit (%)
1	5.5
2	5.5
3	5.0
4	16.5
5	4.5
6	3.0
Mean±S.D.	6.7±4.5

deficit was 6.7±4.5%, i.e. the air in the subelytral chamber had to be almost saturated to give a rate of organismal water loss equal to that of the intact beetles.

The opening of the spiracles is regulated by the tension of CO_2 in the trachea, i.e. high CO_2 tensions are the physiological stimulus for opening of the spiracles (Wigglesworth, 1965). High CO_2 tensions in the air surrounding the spiracles are also likely to lead to increased frequency of spiracular opening and thus to increased spiracular water loss. The fact that the air led through the subelytral cavity in the present experiments had the same low CO_2 tension as ambient air may have caused the rates of subelytral water loss during the present experiments to be lower than those occurring in normal beetles with an intact opening between the subelytral cavity and the ambient air.

To investigate to what extent the low CO_2 tension used during the experiments may have affected the subelytral water loss, the frontal and abdominal components of water loss were measured for another group of beetles with an intact subelytral cavity (elytra not perforated and the opening between the subelytral cavity and the ambient air intact), and the values were compared with corresponding values calculated from the results in Fig. 2. Beetles with intact subelytral systems were mounted inside the apparatus shown in Fig. 1, and the rates of water loss were measured when the air in the frontal chamber was dry (a_I) and when it was saturated (a_{ABD}). The fraction of abdominal water loss was determined as a_{ABD}/a_I. The corresponding fraction for the experimental data in Table 1 was calculated as $(a_B+a_C-a_A)/a_C$, where a_B is the cuticular water loss across the abdominal cuticle and a_C-a_A is the subelytral water loss. The measured fractions of abdominal water loss are shown in Table 3. According to Table 3, the fraction of abdominal water loss of the intact beetles is 0.291±0.045, whereas the mean corresponding fraction of the experimental beetles dealt with in Table 1 is 0.27±0.119. Because of the substantial variation between specimens within each group, the difference is not statistically significant.

Discussion

Although the absolute and relative rates of water loss obtained from the six *Phrynocolus petrosus* beetles differ, they display the same general pattern. A

Table 3. *Rates of total and abdominal water loss in intact* Phrynocolus petrosus *beetles*

Beetle number	a_I (% h^{-1})	a_{ABD} (% h^{-1})	a_{ABD}/a_I
I	0.043	0.0101	0.23
II	0.031	0.0094	0.30
III	0.049	0.0138	0.28
IV	0.055	0.0203	0.37
V	0.044	0.0122	0.28
Mean±S.D.			0.291±0.045

a_I, rate of water loss from the whole intact beetle in dry air.
a_{ABD}, rate of water loss from the abdomen.

closer examination of the data provides information regarding the relative magnitudes and other features of the routes of transpiratory water loss.

The results (Fig. 2, Table 1) reveal that the transcuticular water loss makes up only about 20% of the total evaporative water loss from the beetles. Results indicating that the transcuticular water loss of *P. petrosus* is small compared to the respiratory component have previously been obtained by Zachariassen *et al.* (1987) and by Zachariassen and Maloiy (1989). The latter study revealed that sealing the abdominal surface with a water-impermeable layer of Vaseline did not noticeably affect organismal water loss.

The results make it possible to estimate the cuticular water flux in relation to cuticular surface area and saturation deficit. The average body mass of the experimental beetles was 1.5 g, and the average rate of water loss 0.03% body mass h^{-1}, of which 20% is transcuticular. The surface area of an average beetle (including the surface of the legs and antennae) is estimated to be about 7 cm^2. Assuming that the saturation deficit is 2670 Pa (at 23°C), the surface- and saturation-deficit-related rate of cuticular water loss becomes 0.0048 g h^{-1} cm^{-2} Pa^{-1}. This value is among the lowest ever reported for insects (Edney, 1971; Nicolson *et al.* 1984). By using tritiated water, Nicolson *et al.* (1984) found the cuticular water permeability of the desert tenebrionid *Onymacris plana* within the same temperature range to be 0.0056 g h^{-1} cm^{-2} Pa^{-1}, i.e. a slightly higher value than that obtained in the present study.

The small magnitude of the transcuticular water loss component to the dry air in the posterior chamber also implies that the water loss component over the joints and coxae of the middle legs and hindlegs is very small. Thus, even the flexible structures of the joints and coxae appear to form efficient barriers against evaporative water loss.

The results in Fig. 2 and Table 1 reveal, furthermore, that the subelytral water loss makes up only about 9% of the total organismal water loss, and that the small magnitude of the subelytral water loss is the result of a high relative humidity in the air in the subelytral cavity. The results in Fig. 2 and Table 2 indicate that the

average air humidity in the subelytral space of intact *P. petrosus* beetles is above 90 %, i.e. it is almost saturated. Since the rate of evaporation is proportional to the saturation deficit, this high humidity will contribute strongly to keeping the subelytral water loss low.

The data in Fig. 2 also reveal that when the subelytral spiracles are surrounded by dry air, the organismal rate of water loss is about twice that of the intact beetles in dry air. Thus, the low subelytral water loss caused by the high subelytral air humidity has a substantial water saving effect even at the organismal level. This conclusion is in agreement with the findings of Cloudsley-Thompson (1964) and Ahearn and Hadley (1969), who presented experimental evidence indicating that the subelytral space of desert beetles acts to reduce organismal water loss.

The fact that the subelytral air humidity has such a marked effect on organismal water loss implies that the subelytral apparatus may also be an efficient system for regulating water loss. If they ever need to compensate for over-hydration, the beetles may increase the ventilation of the subelytral chamber, and thus reduce the air humidity in this compartment. This would cause a substantial increase in organismal water loss.

The small magnitudes of the transcuticular and subelytral water losses in *P. petrosus* beetles leave the water loss *via* the pronotal spiracles as the dominating water loss component. The data presented in Table 1 reveal that the average value of this component is about 70 % of the total evaporative water loss.

The relative magnitudes of the different routes of water loss found in the present study differ from the results reported for tenebrionid beetles by other authors. By using gravimetric techniques, Ahearn (1970) and Cooper (1983) studied routes of water loss in *Eleodes armata* from the southwest of the United States. Nicolson *et al.* (1984) combined the tritiated water techniques with gravimetry to investigate the avenues of water loss from *Onymacris plana* in the Namib desert. These investigators found that gravimetrically determined total water loss of *O. plana* beetles was more than 80 % higher than the sum of transcuticular and subelytral water loss determined by means of tritiated water. They considered that water loss from pronotal spiracles was likely to be the basis of the discrepancy. The results of these studies are summarized in Table 4, together with the results of the present study.

Comparison of the values from the different species reveals that the relative magnitudes of subelytral and transcuticular water loss observed for *P. petrosus* in the present study are considerably lower than the values reported for *E. armata* and *O. plana*. Accordingly, *P. petrosus* has a substantially greater component of water loss from pronotal spiracles than the other species. The relatively small component of transcuticular water loss of *P. petrosus* indicates that this species has a lower cuticular water permeability than the other investigated species, a feature that is likely to reflect a more restrictive water balance in *P. petrosus*. Data obtained by Ahearn and Hadley (1969) and Edney (1977) indicate that this is actually the case. Ahearn and Hadley (1969) found that the rate of water loss of *E. armata* in dry air at 27 °C was 0.254 % h^{-1}, whereas Edney (1977) reported that the

Table 4. *Relative magnitude of routes of water loss in three species of tenebrionid beetles*

Route	Percentage of total water loss rate			
	Phrynocolus petrosus	*Eleodes armata*		*Onymacris plana*
Pronotal spiracles	69	5*	11†	45‡
Subelytral	9	25*	25‡	25‡
Transcuticular	22	70*	64†	30‡

* From Ahearn (1970); † from Cooper (1983); ‡ from Nicolson *et al.* (1984).
Data for *P. petrosus* are from the present study.

corresponding value for *O. plana* at 24 °C was 0.052 % h^{-1}. The average rates at 24 °C of the six specimens of *P. petrosus* in the present study was 0.028 % h^{-1}. The moderate differences in body mass and temperature are not sufficient to explain these differences in rates of water loss.

The other striking difference between the present results and those obtained from other species is the low rate of subelytral water loss of *P. petrosus*. One factor contributing to the low subelytral water loss obtained by the present method could be that the technique used in the present study probably involves artificially low subelytral tensions of CO_2. However, the results in Table 3 indicate that the fraction of water lost from the abdomen of the experimental beetles is not significantly lower than that of intact beetles. If a difference exists, the fraction of water lost from the abdomen in the experimental beetles is only slightly smaller than that of intact specimens. Thus, the effect of low CO_2 tension in the subelytral cavity does not seem to be of great importance in limiting water loss. Also, the high relative humidity of the subelytral cavity of intact beetles serves to minimize the effect of subelytral CO_2 tension on subelytral as well as organismal rates of water loss, since it reduces the subelytral water loss.

The high relative humidity in the subelytral chamber is likely to be caused by slow diffusive water loss from the subelytral chamber to the environment. The passage from the subelytral chamber to the ambient air is closed most of the time, leaving only short periods during which vapour can diffuse out of the chamber. By prolonging the periods when the subelytral chamber is closed so much that the subelytral air stays saturated most of the time, the exchange of respiratory gases between the abdominal tracheae and the subelytral chamber will be accompanied by only a small net water loss from the tracheae.

Since transcuticular water loss appears to make up only a minor fraction of the total water loss of this species, it is inappropriate to calculate cuticular water permeability on the basis of total organismal water loss. The present results indicate that values for cuticular water permeability of dry-habitat beetles based on total organismal water loss may be overestimated by a factor of up to 5. Thus,

other methods should be developed to express the features of the water balance of dry-habitat insects.

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