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The water conserving physiological compromise of desert insects

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Abstract. Insects living in arid tropical areas may spend long periods without access to free water, and at the end of the dry season they may be severely dehydrated. To survive under such conditions insects have developed a highly restrictive water economy, and tenebrionid beetles from arid tropical areas may lose water at a rate which is a hundred-fold lower than those of insects from humid habitats.

In most insects the dominant route of evaporative water loss is across the cuticle. In dry habitat tenebrionid beetles cuticular water permeability has been reduced so much that the water loss accompanying the exchange of respiratory gases across the spiracles has become the major water loss component. A further significant reduction in water loss must involve a reduction of the spiracular water loss. The dry habitat tenebrionid beetles seem to have utilized this opportunity in that they have metabolic rates which are markedly lower than those of most other insects.

The low metabolism must imply a corresponding reduction in cellular production of ATP, which is the energy source for cellular ionic pumps. Cellular extrusion of sodium is estimated to consume a substantial fraction of the ATP. Reduced ATP production will therefore also cause a reduced cellular sodium pumping and thus a reduced energy gradient of sodium across cell membranes. This in turn reduces the sodium coupled cellular accumulation of amino acids which requires energy from the sodium gradient. This gives rise to the relatively low extracellular concentrations of sodium and high concentrations of amino acids displayed by these insects. In most animals extracellular amino acid concentrations of this magnitude would have led to a substantial urinary loss of amino acids. However, since desert insects possess an exceptionally efficient rectal system for reabsorption of water and solutes from the urine, a large quantity of amino acids can be returned to the haemolymph from the urine in these animals. Thus, the unique capacity of desert tenebrionids to reabsorb water and solutes from their urine appears to be an important condition also for the low transpiratory water loss of these insects.

INTRODUCTION

Insects living in tropical deserts or dry savanna have access to free water only during the relatively short rainy seasons. During the long dry periods between the rainy seasons the insects have no access to dietary water. As an adaptation to meet this situation, many insects living in dry habitats have a great capacity to restrict their water loss (Eddy, 1985). Studies over the last decade have revealed the nature of some of the underlying mechanisms. Insects are also notorious for their unusual extracellular concentrations of inorganic ions and free amino acids. The low concentrations of sodium and the high concentrations of free amino acids in the haemolymph of many insects differ from the situation in all other animals, and each of these features would cause very serious physiological problems in vertebrates and other animals. It is not known what allows insects to survive in spite of having this unusual composition of the extracellular fluid or what is the adaptive significance of these unusual concentrations. Several of these peculiar physiological features seem to be harmonized to form an integrated system that favours survival of insects in dry habitats.

Adaptation of living organisms to an extreme environment is usually associated with modifications of biological processes and features so that the organisms can conform with the special conditions that prevail in that particular environment. The best known and best understood modifications are those which are related directly to the particular stress factors. However, the modification of one process to meet a particular life-threatening environmental condition may create new problems. The internal conditions and processses of organisms are adjusted to be in functional harmony with each other. A dramatic change in one parameter or process may thus affect the functioning of other processes and create a need for further functional modifications. Consequently, adaptation to a given extreme environmental condition may require a complex set of functional modifications, which in combination create a new viable compromise of functional features. A complex adaptation of this kind seems to be the basis of the ability of certain insects to survive under the extreme conditions that prevailing arid tropical areas.

NATURAL STRESS OF DRY HABITATS

Environments which have a mean annual precipitation of < 600 mm p.a. are termed arid or semi-arid (Cloudsley-Thompson, 1991; Sømme, 1995). In most arid or semi-arid areas of tropical regions precipitation is usually restricted to a few weeks or months each year. The rainy seasons are separated by relatively long dry seasons. Some years the rains may fail to occur, causing the dry season to become correspondingly longer. Long term survival of a species requires that the organisms adapt to the worst and not to average conditions, and hence, dry habitat insects must be able to survive for more than half a year without access to food and dietary water.

Many species of tenebrionid beetles, which feed on detritus, pass the dry season as adults, which may display a remarkably restrictive water economy. In spite of these adaptations, the beetles may suffer a substantial water loss and display very low water contents at the end of the dry season (K.E. Zachariassen, unpublished observations). Sometimes there is also substantial lethality, apparently due to dehydration. Hence, there is obviously a strong selection pressure for efficient physiological mechanisms to limit water loss in such species.

CHANGES IN DIFFERENT FEATURES

Several families of dry habitat beetles have adapted to the extreme conditions of arid tropical areas by having undergone a number of dramatic physiological changes, which in sum seem to make the beetles fit to survive for long periods without access to dietary water. The nature of these changes and their adaptive significance will be outlined in the following.

Reduced cuticular water loss

While most insects appear to have a relatively leaky body surface and thus transcuticularwater loss is the main component of evaporative water loss (Edney, 1977), several groups of beetles from arid environments seem to have reduced their cuticular water permeability so much that respiratory water loss has become the major component.

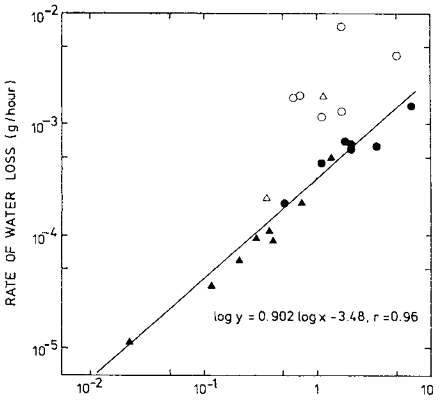

Fig. 1. Relationship between rates of water loss and metabolic rates of species of carabids (circles) and tenebrionids (triangles) from arid (closed symbols) and humid habitats (open symbols). The solid line is the calculated linear regression line of values of arid habitat beetles. From Zachariassen et al. (1987).

Evidence for this has come from several series of experiments. Zachariassen et al. (1987) found that for dry habitat tenebrionid and carabid beetles the rates of evaporative water loss were a function of the metabolic rate. The values of equally large species of these groups of beetles differed substantially, but when the rates of water loss of various species are plotted as a function of their rates of oxygen consumption in a double logarithmic plot, the values of all dry habitat species fall on the same straight line (Fig. 1). The slope of the line is close to 1.0, implying that the rate of water loss and metabolic rate are almost proportionally related to each other. A proportionality between water loss and oxygen consumption is what should be expected if water loss is predominantly via the respiratory pathway.

Further evidence was obtained by Zachariassen & Maloiy (1989), who measured evaporative water loss of dry habitat tenebrionids before and after covering the body surface with water impermeable vaseline, which would reduce transcuticular water loss to zero. The application of vaseline caused no reduction in water loss (Fig. 2), suggesting that even without vaseline the transcuticular water loss was close to zero.

Finally, Zachariassen (1991) observed how independent variation of the relative atmospheric humidity around the head and pronotum, around the abdomen, and inside the subelytral space affected the rates of water loss of a dry habitat tenebrionid beetle. The results revealed that variations in the humidity of air in contact with spiracles (pronotum and subelytral space) had a strong effect on water loss, whereas the humidity of the air

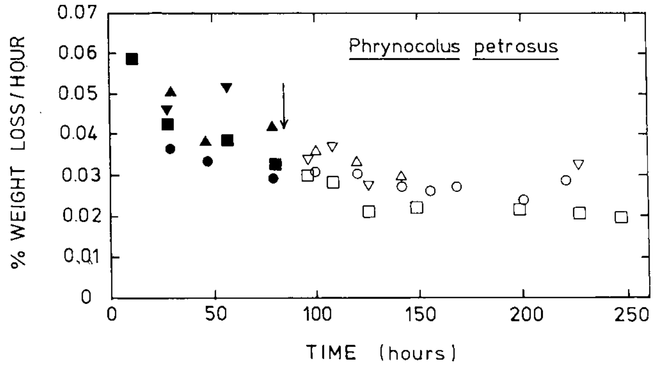

Fig. 2. Rates of evaporative water loss of *Phrynocolus petrosus* tenebrionid beetles before (closed symbols) and after (open symbols) covering the body surface with water impermeable vaseline grease. Each type of symbol represents one individual. From Zachariassen & Maloiy (1989).

surrounding the abdomen, which has a large surface area but no spiracular openings, had only a small effect. The results suggest that < 20% of the evaporative water loss is transcuticular.

The contention that dry habitat tenebrionids have respiratory water loss as the dominant component explains the important role, which was pointed out by Cloudsley-Thompson (1964), of the subelytral space to restrict evaporative water loss. From the experimentally determined rates of water loss, Zachariassen (1991) calculated the mean relative humidity in the subelytral space of the tenebrionid *Phrynocolus petrosus* to be about 95%, conforming well with the idea that the subelytral space reduces water loss by giving rise to a high relative humidity in the air surrounding the spiracles. However, only if evaporative water loss is predominantly spiracular, can the high humidity of a closed subelytral space cause a significant reduction in organismal water loss.

Reduced metabolism

Assuming that a reduction of cuticular water permeability has made respiratory water loss the major water loss component, a further significant reduction in water loss must take place at the expense of respiratory water loss. In accordance with the results in Fig. 1, this can be accomplished if the organisms reduce their metabolic rates. This seems indeed to have happened for the most dehydration resistant dry habitat beetles. As shown in Fig. 3, the respiratory rates of dry habitat tenebrionids are only 1/5 to 1/10 the rates of equally large carabid beetles. The fact that the rates of evaporative water loss of tenebrionids is reduced accordingly (Fig. 1), suggests that the reduction in metabolic rates may be adaptive for low rates of water loss.

Unusual extracellular ionic composition

The oxidative metabolic processes result in the formation of ATP, which is the energy source for a variety of energy requiring cellular processes. The single most important ATP requiring process is the cellular extrusion of sodium by the membrane bound sodium/potassium ATP-ase, the so-called sodium pump. Florey (1966) estimated that cellular sodium pumping consumes up to 70% of the total ATP turnover in resting frog muscles. Other authors (e.g. Ganong, 1987) present values around 30%. Most investigators agree that a very substantial part of cellular ATP turnover is spent in transmembrane sodium pumping.

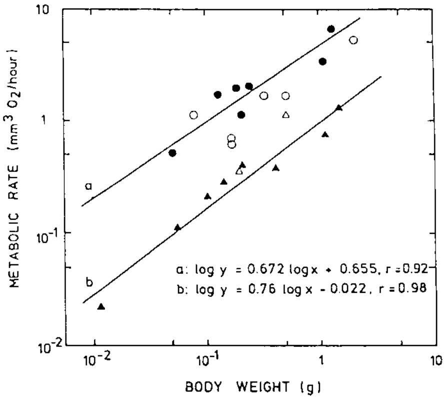

Fig. 3. Respiratory rates of carabid (circles) and tenebrionid (triangles) beetles from arid (closed symbols) and humid (open symbols) habitats, plotted as a function of body mass. The lines are calculated linear regression lines for dry habitat carabids and tenebrionids respectively. From Zachariassen et al. (1987).

The active sodium extrusion from the cells gives rise to a high concentration quotient of sodium across the cell membranes, and in combination with the electrical membrane potential the concentration quotient of sodium gives rise to a high electrochemical potential difference ("energy gradient") of sodium across the cell membranes. The extracellular sodium concentration is about ten-fold higher than intracellular values, and in marine mussels the sodium energy gradient is reported to range from 8 to 12 kJ/mol (Børseth et al., 1992). The energy gradient of sodium is the energy source of several important energy requiring cellular processes.

It should be expected that the low metabolic rates displayed by tenebrionids would reduce their capacity to transport sodium out of the cells and to build up a high energy gradient of sodium across their cell membranes. No data have been published regarding the transmembrane electrochemical potential difference of sodium in insects. However, the values of extracellular sodium concentrations may be taken as indicative of the value of the sodium gradient in that high extracellular concentrations are likely to be associated with high energy gradients and vice versa.

Vertebrates and most invertebrates have extracellular sodium concentrations from 140 mmolal or higher (Schmidt-Nielsen, 1990). This is also the value of desert carabids and many other insects (Zachariassen et al., 1987). However, desert tenebrionids have considerably lower extracellular sodium concentrations (Zachariassen et al., 1987). Extracellular sodium concentrations and metabolic rates of various species of desert carabids end tenebrionids are plotted together in Fig. 4. From the data in Fig. 4 it appears that the metabolic rates of beetles are strongly correlated with extracellular sodium concentrations, the low metabolic rates of the tenebrionids being associated with low extracellular sodium concentrations, while the high metabolic rates of the carabids are associated with high sodium concentrations in the extracellular fluid.

Thus, it appears that the reduced respiratory rates of desert tenebrionids have developed at the expense of the ability of the beetles to maintain high extracellular sodium concentrations and thus probably high energy gradients of sodium.

High extracellular levels of free amino acids

A characteristic feature of animal cells is their strong capacity to accumulate free amino acids from the extracellular medium. Typically there is a concentration quotient of 100 or 1000 between intracellular and extracellular concentrations (Aunaas & Zachariassen, 1994). The accumulation is seen in all animal cells, even in the cells of primitive marine animals, many of which accumulate free amino acids directly from the seawater, where concentrations are in the micromolar range (Manahan et al., 1982). In many marine organisms energy gained from oxidation of de-aminated amino acids accumulated from seawater makes up a substantial fraction of the energy budget (Manahan et al., 1982). In addition to being used as an energy source and for synthesis of proteins, free amino acids are important as intracellular osmolytes (Bricteux-Gregoire et al., 1962), substrates and products of anaerobic

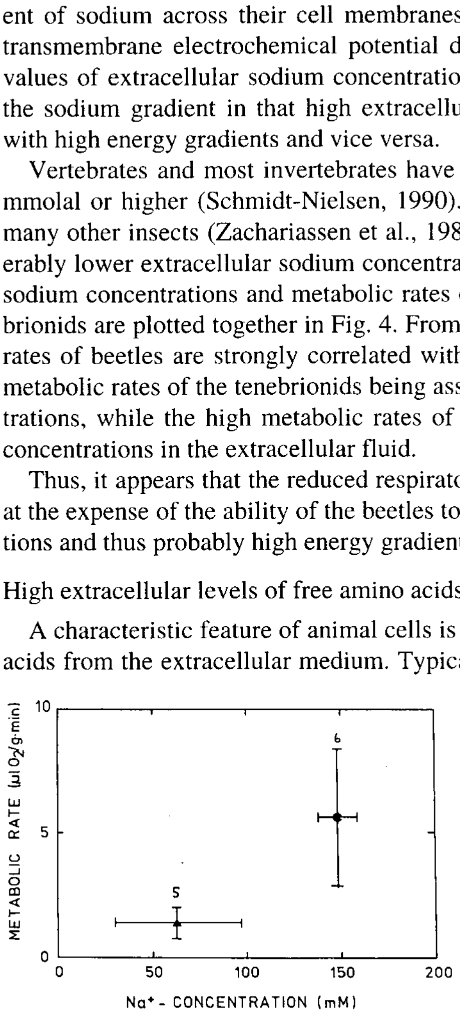

Fig. 4. Relationship between metabolic rate and extracellular sodium concentration of tropical carabid (circle) and tenebrionid (triangle) beetles. Bars represent standard deviations, and number of species measured are given on the top of the vertical bars. From Zachariassen et al. (1987).

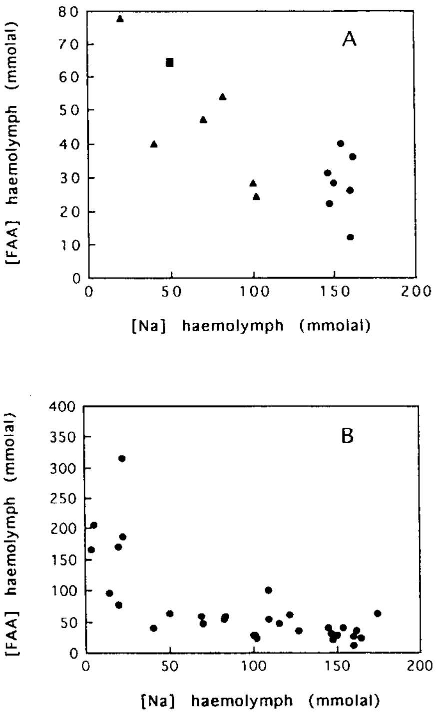

Fig. 5. Relationship between extracellular concentrations of sodium and free amino acids in various species of insects. A. Data for carabid (circles), cerambycid (squares) and tenebrionid (triangles) beetles. B. Data for a number of other insect species, taken from Sutcliffe (1963). Each point represents one species.

metabolic pathways (West et al., 1966) and as regulators of various cellular processes (West et al., 1966). Thus, free amino acids seem to have a variety of important functions in animal cells.

The cellular accumulation of free amino acids takes place by means of secondary active transport, coupled to a passive influx of sodium, and thus by the use of energy from the transmembrane sodium gradient (Eddy, 1985). A common membrane bound transport molecule has binding sites for both sodium and a free amino acid, and it cannot pass the lipid phase of the cell membranes unless both binding sites are occupied. Both solutes may be released on the inside. Sodium will be transported actively out by the sodium pump, while the amino acids will become accumulated intracellularly until the energy gradients of the amino acids match that of sodium.

While the total extracellular amino acid concentrations of most animal groups are around 1 mmolal, many insects have total amino acid concentrations of 50–100 mmolal in the haemolymph. This unusual feature of insect may be related to their unusual extracellular concentrations of sodium. In insects with a low extracellular sodium concentration and a correspondingly low sodium gradient the capacity to transport amino acids into the cells is likely to be low, and hence, they are also likely to have low amino acid concentration quotients across the cell membranes. This implies that in insects with low transmembrane sodium gradients a relatively large fraction of the amino acids should be expected to remain in the extracellular fluid, i.e. extracellular amino acid concentration should be relatively high. The results in Fig. 5A and B are in accordance with this prediction, in that insects with low extracellular sodium concentrations seem to have the highest extracellular concentrations of free amino acids and vice versa.

For vertebrates such high amino acid concentrations in blood plasma would make it impossible for the kidneys to reabsorb the amino acids effectively from the glomerular filtrate, and the organisms would suffer an intolerable loss of valuable amino acids. In insects this does not seem to represent a problem, probably because of their efficient manner to reabsorb water and solutes from the urine.

Efficient system to reabsorb water from the urine

Insects use uric acid as an excretory product for nitrogen (Schmidt-Nielsen, 1990). Uric acid has a very low solubility in water, implying that already at low concentrations it will precipitate and become osmotically inactive (Schmidt-Nielsen, 1990). Insects have taken advantage of this property of uric acid by allowing it to precipitate when water is reabsorbed from the rectum, which seems to have the same water reabsorbing function in the urine production of insects as the collecting ducts have in mammals. Instead of becoming osmotically concentrated, insect urine becomes a gradually thicker paste-like substance, which remains low in osmotic activity even when a large amount of uric acid is present in a small volume of urine. The avoidance of high osmotic activity, which would tend to bind water osmotically in the urine, allows insects to perform an exceptionally comprehensive reabsorption of water from the urine. The urine of many dry habitat tenebrionids is excreted as pellets with a very low water content.

The efficient reabsorption of water also facilitates the reabsorption of solutes by creating concentration gradients for passive diffusion back to the haemolymph across the rectal epithelium. This allows insects to reabsorb essentially all free amino acids from their urine, in spite of having the same high concentrations in the fluid of the Malpighian tubuli as in the haemolymph.

THE WATER CONSERVING PHYSIOLOGICAL COMPROMISE OF DRY HABITAT INSECTS

Dry habitat insects seem to have taken advantage of their unusual physiological features to develop an exceptional capacity to limit evaporative water loss. Due to their efficient reabsorption of urinary water from the rectum they can avoid a comprehensive urinary loss of free amino acids in spite of having high concentrations in the extracellular fluid. Hence, they do not need to reduce extracellular amino acid concentration by accumulating them in the intracellular compartments. This reduces the influx of sodium to intracellular fluid compartments, and it may also allow them to have a lower electrochemical potential difference of sodium across cell membranes than most other animals. This in turn leads to a reduced energy expenditure for sodium pumping and thus to reduced metabolic rates. Eventually this causes a proportional reduction in respiratory water loss, and since the transcuticular water loss in these insects is small, it also leads to a nearly proportional reduction in the total transpiratory water loss.

The reduced metabolic rate of dry habitat tenebrionids also implies that their stored energy resources last longer. This is important because food availability also is very limited throughout the dry season. Thus, in the dry season the beetles depend predominantly on stored energy, and their survival depends on their capacity to make the stored food resources last for a sufficiently long time.

The survival strategy of dry habitat carabids differs from that of the tenebrionids. Like the tenebrionids the carabids have reduced their cuticular water permeability so much that respiratory water loss has become the dominant component of transpiratory water loss. Evidence for this has been obtained by Zachariassen et al. (1987) (Fig. 1) and by Zachariassen & Maloiy (1989), who covered the body surface of dry habitat carabids with water impermeable vaseline in the same way as they did with tenebrionids. As for tenebrionids (Fig. 2), treatment with vaseline did not affect the rates of water loss of the carabids to any noticeable extent.

However, in contrast to the tenebrionids the carabids do not display reduced metabolic rates. Accordingly, they do not display low extracellular sodium concentrations and high extracellular concentrations of free amino acids which characterize dry habitat tenebrionids. The extracellular sodium concentrations of carabids are like those of vertebrates, and although their amino acid concentrations are somewhat higher than those of vertebrates, they are substantially lower than the values for tenebrionids (Fig. 5A). The carabids also produce a less concentrated urine and lose body water substantially faster than the tenebrionids. To compensate for their high rates of water loss the dry habitat carabids are active predators throughout the dry season, preying upon any small organism (ants, termites etc.) they may come across. Since these prey organisms contain 60–70% water, the predatory behaviour of the carabids implies that they have access to water and energy through the entire dry season.

To succeed as predators, carabids must be fast runners and also have the physical strength required to capture and kill the prey organisms. A high metabolic rate and a high extracellular sodium concentration may be a condition for the high activity level. In contrast, the dry habitat tenebrionids generally appear to be very sluggish and slow. Thus, the strategy of the carabids appears to be to limit transpiratory water loss by reducing the transcuticular water loss component substantially. As predators they have access to food and water throughout the dry season, but to succeed they must have a high activity, and they have not undergone the dramatic changes in metabolic rate and solute concentrations displayed by the tenebrionids.

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