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Breathe softly, beetle: Continuous gas exchange, water loss and the role of the subelytral space in the tenebrionid beetle, *Eleodes obscura*

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Abstract

Flightless, diurnal tenebrionid beetles are commonly found in deserts. They possess a curious morphological adaptation, the subelytral cavity (an air space beneath the fused elytra) the function of which is not completely understood. In the tenebrionid beetle *Eleodes obscura*, we measured abdominal movements within the subelytral cavity, and the activity of the pygidial cleft (which seals or unseals the subelytral cavity), simultaneously with total CO_2 release rate and water loss rate. First, we found that *E. obscura* has the lowest cuticular permeability measured in flow-through respirometry in an insect ($0.90\,\mu g\ H_2O\,cm^{-2}\,Torr^{-1}\,h^{-1}$). Second, it does not exhibit a discontinuous gas exchange cycle. Third, we describe the temporal coupling between gas exchange, water loss, subelytral space volume, and the capacity of the subelytral space to exchange gases with its surroundings as indicated by pygidial cleft state. Fourth, we suggest possible mechanisms that may reduce respiratory water loss rates in *E. obscura.* Finally, we suggest that *E. obscura* cannot exchange respiratory gases discontinuously because of a morphological constraint (small tracheal or spiracular conductance). This ‘‘conductance constraint hypothesis’’ may help to explain the otherwise puzzling phylogenetic patterns of continuous vs. discontinuous gas exchange observed in tracheate arthropods.

Keywords: Metabolic rate; Open-flow respirometry; Cuticular permeability; Respiratory water loss; Discontinuous gas exchange cycle

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1. Introduction

Deserts are among the most hostile habitats on earth. Factors such as extreme temperatures, low humidity, lack of shadows, strong winds and the inaccessibility of free water for months or even years enhance dehydration stress and may lead to death by desiccation. Under these extreme conditions, small arthropods and particularly tenebrionid beetles are a conspicuous component of the fauna, and not infrequently the only animal to be seen during the day. Yet their high body surface to mass ratios enhance water loss and desiccation. To achieve this impressive resistance to desiccation, these small animals possess several behavioral, morphological and physiological adaptations (Cloudsley-Thompson, 1975, 2001). High desiccation tolerance is one example of a physiological adaptation, and its characteristics include the ability to lose a high proportion of body water reserves without adverse effects; indeed, hemolymph water content can reach values close to zero (Pedersen and Zachariassen, 2002; Zachariassen and Einarson, 1993; Zachariassen and Pedersen, 2002). Also important in this regard are reduction of cuticular water loss by epicuticular waxes (McClain et al., 1985) and lipid/hydrocarbon cuticular composition (Hadley and Schultz, 1987; Hadley and Savill, 1989). Excretory water loss is reduced by the cryptonephridial complex (Wigglesworth, 1972), which reabsorbs almost all of the water that would otherwise be excreted. Finally, a reduction in respiratory water loss (RWL) can also help to minimize total water loss, for

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example by the evolution of the discontinuous gas exchange cycles (DGC; reviews in Miller, 1981; Kestler, 1985; Lighton, 1994, 1996, 1998; Chown and Nicolson, 2004; White et al., 2007), although the DGC's role in saving water is discussed and other possible hypotheses of its origin have been suggested (see Chown et al., 2006). Reducing RWL is especially important in insects with very low cuticular permeability (CP), such as harvester ants (Lighton et al., 1993) or beetles (Zachariassen et al., 1987; Chown and Davis, 2003). However, in the case of either continuous or discontinuous gas exchange, the fact that morphological adaptations, such as invagination of the gas exchange systems or the development of occludable spiracles, leads to considerable water retention, is unquestionable (Mellanby, 1935; Kestler, 1985; Schmidt and Wagele, 2001). The subelytral cavity may, in a sense, add a second level of control to the spiracles of beetles so equipped, perhaps allowing the spiracles to exchange gases without losing excessive water.

The subelytral cavity is an air-filled space between the fused elytra and the abdomen. The abdominal spiracles open into this cavity, which is connected to the environment by a single aperture above the anus, the pygidial cleft. It is believed that this arrangement helps to lower the rate of respiratory transpiration (Ahearn, 1970), but other functions for the subelytral cavity have also been suggested (Cloudsley-Thompson, 1975; Slobodchikoff and Wismann, 1981). At the beginning of the 20th century, Buxton (1923) formed his hypothesis of winglessness as adaptation to excessive wind. Although he did not posit a water-conserving function for the cavity, he perceptively noted that *"The extremely close fit between the margins of the elytra and the sternite is remarkable: it would appear to hinder the ventilation of the subelytral space into which the spiracles open."* (Buxton, 1923). Years later, Dizer (1955) showed that removal of the elytra produced a substantial increase of water loss in beetles with hermetic subelytral cavities and little increase in winged species (i.e., intact beetles with a subelytral cavity not hermetically sealed). Further studies by Cloudsley-Thompson (1964) and Ahearn and Hadley (1969) confirmed these results. The latter authors hypothesized that water loss is reduced because the spiracles open into a chamber that is humidified by expired air from the tracheal system instead of the drier atmosphere outside the subelytral cavity. The air inside the subelytral cavity, in addition to reducing transpiratory water loss, may also serve as a temperature "buffer zone" which delays heat transfer to the beetle's internal structures, allowing longer exposure to insolation, despite its black coloration (Hadley, 1970). Cloudsley-Thompson (1964), however, found no support for the thermal hypothesis. Another possible function, which is not mutually exclusive with the water saving and thermal hypotheses, is the one first stated by Fiori (1977) and later elaborated by Slobodchikoff and Wismann (1981), although without citing Fiori's (1977) previous study. According to those authors, the subelytral cavity serves as a space for abdominal volume expansion to store large quantities of detrital food, which is of low nutritional value or to store drinking water, which is a great advantage for organism living in deserts, where water is available for short periods of times only after occasional rains (Slobodchikoff and Wismann, 1981). The latter hypothesis received support from observations of elytral cavity depth changes as a function of beetle's hydration status (Slobodchikoff and Wismann, 1981). Finally Draney (1993) proposed that the cavity *per se* is an architectural constraint resulting from the need for abdominal expansion within the heavily sclerotized, fused elytra that evolved primarily as a water conservation adaptation.

After some early work on gas exchange in beetles with sealed subelytral cavities (Ahearn, 1970; Bartholomew et al., 1985; Lighton, 1987, 1988a, 1991a), a burst of information about the dynamics of gas exchange in flightless beetles has recently been published by Duncan and collaborators (Duncan, 2003; Duncan and Byrne, 2000, 2002, 2005; Duncan et al., 2002). Previous results suggested a unidirectional airflow within the tracheal system of the tenebrionid beetle, *Eleodes armata* (air entered the tracheal system through the thoracic spiracles and was exhaled through the abdominal spiracles inside the subelytral cavity; Ahearn, 1970). On the contrary, Duncan and Byrne (2000) found in the Namib Desert tenebrionid beetle *Onymacris multistriata* a tidal airflow mainly through the mesothoracic spiracles. They also report the remarkable use of only one mesothoracic spiracle, at least while at rest, in the dung beetle *Circellium bacchus* (Scarabaeidae) (Duncan and Byrne, 2000, 2002).

Although much work has been done concerning the possible functions of the subelytral cavity, it is striking that to our knowledge, no study has yet directly observed the dynamics of the subelytral cavity (except for measurements of the relation between abdominal pulsations, changes in the pressure of the subelytral air and CO_2 expelled (Lighton, 1988a and a more recent preliminary work by Lighton and Schilman, 2005). In other words, simultaneous observations of the movements of the abdominal tergites (changes of subelytral cavity volume), movements of the pygidial cleft, CO_2 release, and water loss in any non-flying beetle is lacking. Thus, we performed these experiments on the darkling beetle *Eleodes obscura* using reflective-mode fiber optic probes together with flow-through respirometry. In addition, we measured the metabolic rates (MR) and water loss rates of intact beetles, and separated their respiratory and cuticular components by means of regression analysis (Gibbs and Johnson, 2004).

2. Materials and methods

2.1. Animals

E. obscura were collected in the Sonoran Desert of Mazatzal Mountains, Maricopa County, Arizona. They were kept in communal plastic containers