THE EFFECT OF RESPIRATORY PATTERN ON WATER LOSS IN DESICCATION-RESISTANT DROSOPHILA MELANOGASTER

ADRIENNE E. WILLIAMS* AND TIMOTHY J. BRADLEY‡
Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA
*Present address: 1593 Lavender Way, Redding, CA 96003, USA
‡Author for correspondence (e-mail: tbradley@uci.edu)

Accepted 18 August; published on WWW 8 October 1998

Summary

We measured CO₂ and H₂O release from individual fruit flies from five populations of Drosophila melanogaster selected for resistance to desiccation (D flies). Our previous work found that these flies survive for an extended period in dry air, have an increase in the peak height and frequency of CO₂ release, as measured by the standard error of a linear regression (SER) of CO₂ release for the entire survival period, and have reduced water loss rates \( \tilde{V}_\text{H₂O} \) compared with their control or ancestor populations. In the present study, we examined the following respiratory characteristics: \( \tilde{V}_\text{CO₂} \), \( \tilde{V}_\text{H₂O} \), the SER of CO₂ release and the ratio of \( \tilde{V}_\text{CO₂} \) to \( \tilde{V}_\text{H₂O} \) in the D flies. Correlations between these characters were calculated in order to determine the effect of respiratory pattern on water loss. We found that, within the D flies, neither periodic release of CO₂ nor an increased SER for CO₂ release was associated with reduced water loss. In addition, an increased SER was positively correlated with both an increased water loss rate and a decreased survival time. Therefore, although selection for desiccation resistance leads to both an increased SER and a decreased rate of water loss in the D flies, the increased SER does not significantly reduce respiratory water loss.

Key words: Drosophila melanogaster, desiccation resistance, discontinuous gas exchange cycle, water loss.

Introduction

Because of their small size and large surface area to volume ratio, terrestrial insects face potentially high mass-specific rates of water loss. Physiological studies have demonstrated that specific characteristics of their cuticle, behavior, respiratory system and excretory system limit the rates of water loss (Bradley, 1985; Edney, 1977; Hadley, 1994).

In the case of the respiratory system, water loss is minimized by releasing as little water vapor as possible while still achieving the necessary oxygen uptake and carbon dioxide release. Many insects and other arthropods are known to open and close their spiracles in a clearly periodic pattern. These include blood-sucking bugs (Punt, 1944), moth pupae (Levy and Schneiderman, 1966a,b,c), cockroaches (Kestler, 1971), beetles (Lighton, 1988, 1991), grasshoppers (Hadley and Quinlan, 1993; Quinlan and Hadley, 1993), ants (Lighton and Berrigan, 1995), mites (Lighton and Duncan, 1995), ticks (Lighton and Fielden, 1996a) and solphugids (Lighton and Fielden, 1996b). It has been argued that this discontinuous gas exchange cycle (DGC) serves to reduce respiratory water loss (Schneiderman, 1960; Edney, 1977). When the spiracles are closed, carbon dioxide levels build up within the insect and water vapor is retained. When the spiracles subsequently open, carbon dioxide release, relative to water vapor loss, is maximized.

This hypothesis of water savings has been difficult to falsify, because arthropods that breathe using a DGC tend to use it consistently and without large changes in pattern (Lighton, 1996). This prevents us from determining whether a periodic DGC saves more water than some other pattern of spiracular control (e.g. a constant minimized opening or constant flutter).

Previous work with Drosophila melanogaster has found that selection for resistance to desiccation has resulted in populations of flies which show a decrease in water loss rate and an increase in survival time in dry air compared with their controls (Gibbs et al. 1997; Williams et al. 1997). Flies from these populations also show an increase in the height and periodicity of peaks of CO₂ release for the full survival period as measured by the standard error of a linear regression (SER) through the values of CO₂ release. In the present study, we wished to determine whether there is a direct relationship between respiratory pattern and reduced rates of water loss. To do this, we measured \( \tilde{V}_\text{CO₂} \) and \( \tilde{V}_\text{H₂O} \) concurrently from individual D. melanogaster that had been selected for resistance to desiccation (D flies). We examined four respiratory characteristics in detail: \( \tilde{V}_\text{CO₂} \), \( \tilde{V}_\text{H₂O} \), the SER of CO₂ release and the ratio \( \tilde{V}_\text{CO₂}/\tilde{V}_\text{H₂O} \). These characteristics are compared in flies that demonstrate a periodic release of CO₂ and flies that show non-periodic release of CO₂. We also examine how these variables are affected when an individual changes from a non-periodic to a periodic pattern of release of CO₂.
Materials and methods

Fly culture regime

The 50 D flies used in this experiment were part of a larger comparison of three selection treatments of Drosophila melanogaster described by Williams et al. (1998). The raw data on \( V_{CO_2} \) and \( V_{H_2O} \) discussed here were also used in the treatment comparison study, but were analyzed differently. The remaining methods for the selection and handling of the flies and the techniques used to measure and analyze respiratory variables are described by Williams et al. (1998). In the present study, we used in one comparison an additional analysis, namely the standard error of a linear regression through the water release values (water SER).

Statistics

When comparing groups of flies that demonstrated periodic release of CO\(_2\), we performed analyses only on the 18 min recordings that showed the periodic release. Comparisons using these recordings were performed using one-way analyses of variance (ANOVAs) in Excel. We looked for correlations among respiratory characters in all 50 flies by calculating fly means for each variable: \( V_{CO_2} \), \( V_{H_2O} \), the SER of CO\(_2\) release and the \( V_{CO_2}/V_{H_2O} \) ratio. Each respiratory character was measured every 2 h and averaged for each individual fly to provide these fly means. Correlations and associated significance values were calculated using SPSS.

Terminology

The classic pattern of the DGC consists of an open phase in which the spiracles are fully open, a flutter phase in which they open and close rapidly, resulting in a slight increase in \( V_{CO_2} \), and a closed phase in which CO\(_2\) release is indistinguishable from zero. The pattern of CO\(_2\) release displayed by our populations of Drosophila melanogaster is generally different from that of a classic DGC. In our populations, CO\(_2\) release does not drop to zero levels in most of our recordings. This non-zero release of CO\(_2\) is punctuated by bursts of release of larger amounts of CO\(_2\) that vary in peak height and frequency. We have used the standard error of the mean of the linear regression (the SER) drawn through the trace of CO\(_2\) release as a means of objectively quantifying the degree of variability in the pattern of CO\(_2\) release.

Because the pattern of CO\(_2\) release in Drosophila melanogaster is so different from that normally considered to be a DGC, we do not generally refer to this pattern as a DGC. Instead, we discuss the effect of selection on the SER of CO\(_2\) release. We did record patterns from two flies that showed CO\(_2\) release dropping to zero between periodic bursts (see Results). This indicates to us that D. melanogaster is capable of a classic DGC, but that it is rarely used.

We have found that the SER of CO\(_2\) release is variable, and we describe these differences as follows. A large SER indicates large peaks of CO\(_2\) release, either in regular periodic bursts or in bursts that are not visibly periodic. We use the term ‘periodic release of CO\(_2\)’ to describe a pattern that is visibly periodic with a measurable frequency. Neither a large SER nor periodic release of CO\(_2\) need be associated with values of CO\(_2\) release dropping to zero between bursts.

Results

Periodic release of CO\(_2\) and water

Among the populations of flies we have examined, periodic release of CO\(_2\) occurs only in the D flies and is not seen in their control or ancestor populations. Periodic CO\(_2\) release is rather rare even within this group, occurring in only 13 of the 50 D flies examined and in only three of the five populations. We found that peaks in the water loss trace were visible only in conjunction with periodic peaks of CO\(_2\) release. Fig. 1 provides examples of periodic release from four flies, with the concurrent \( V_{H_2O} \) recordings. Interestingly, periodic release of CO\(_2\) did not guarantee periodic water loss; only six of the 13 flies demonstrating periodic CO\(_2\) release showed visible peaks of water loss. In Fig. 1A,C, the peaks of water loss are visible; in Fig. 1B,D, they are not. Clearly, bursts of CO\(_2\) release are not necessarily correlated with bursts of water loss in these four flies.

We wished to determine what additional differences might exist between flies in which periodic water release occurred simultaneously with periodic CO\(_2\) release and flies in which this did not occur. In seeking these differences, we measured nine characteristics in the CO\(_2\) and water vapor recordings: \( V_{CO_2} \), \( V_{H_2O} \), the SER of \( V_{CO_2} \) and \( V_{H_2O} \), the frequency of peaks of CO\(_2\) release, baseline levels of \( V_{CO_2} \) and \( V_{H_2O} \), the volume of CO\(_2\) found within the peaks and the percentage of total \( V_{CO_2} \) found within the peaks. Table 1 summarizes these variables in the 18 min recordings from the 13 D flies that showed periodic CO\(_2\) release. While most of these variables were significantly different between the two groups, those describing peak and baseline values of CO\(_2\) are of most interest. When the majority of CO\(_2\) is released within peaks, the interburst levels of CO\(_2\) and H\(_2\)O are relatively low. As can be seen in Fig. 1A,C, this low H\(_2\)O baseline results in enough contrast between interburst and burst water vapor release for peaks to be seen.

Fig. 2 indicates that the peak height and frequency of CO\(_2\) release is associated with the peak height and frequency of water vapor loss \((r^2=0.64, P<0.01)\). When the CO\(_2\) SER increases in flies that show periodic CO\(_2\) and H\(_2\)O release, the H\(_2\)O SER also increases. At low CO\(_2\) SER values (below 3\( \mu \)mol CO\(_2\) h\(^{-1}\)), the H\(_2\)O SER in all flies was similar to that obtained from an empty chamber. The H\(_2\)O SER in flies with periodic H\(_2\)O release was 0.013\( \mu \)mol CO\(_2\) h\(^{-1}\), in flies without periodic H\(_2\)O release it was 0.011\( \mu \)mol CO\(_2\) h\(^{-1}\) and in flies without periodic CO\(_2\) release it was 0.010\( \mu \)mol CO\(_2\) h\(^{-1}\). An empty chamber gives an H\(_2\)O SER of 0.010\( \mu \)mol CO\(_2\) h\(^{-1}\) \((P=0.24)\).

From the recordings that show peaks of water loss corresponding with peaks of CO\(_2\) release, it is possible to estimate the fraction of evaporative water loss represented by respiratory as opposed to cuticular loss. Such calculations are valid only if there is evidence that the spiracles are completely closed between bursts of CO\(_2\) release. Using recordings from
two D flies in which CO₂ release was indistinguishable from zero between bursts, we found that 27% and 23.8% of the total water vapor was lost within the peaks of water release associated with open spiracles. This limited evidence leads us to estimate that approximately 25% of evaporative water loss in the desiccation-resistant D. melanogaster is respiratory.

Table 1. An analysis of possible differences in respiratory variables between flies showing a periodic release of CO₂ concurrent with a periodic release of water and flies without a cuncurrent periodic release of water

<table>
<thead>
<tr>
<th>Respiratory variable</th>
<th>Flies with periodic water release</th>
<th>Flies without periodic water release</th>
<th>Statistical significance, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline $\dot{V}_{H_2O}$ (nl H₂O h⁻¹)</td>
<td>0.63±0.09</td>
<td>1.2±0.17</td>
<td>0.0122</td>
</tr>
<tr>
<td>Baseline $\dot{V}_{CO_2}$ (µl CO₂ h⁻¹)</td>
<td>0.085±0.040</td>
<td>0.32±0.044</td>
<td>0.0012</td>
</tr>
<tr>
<td>Volume of CO₂ released within peaks (µl CO₂ h⁻¹)</td>
<td>0.097±0.011</td>
<td>0.049±0.010</td>
<td>0.0161</td>
</tr>
<tr>
<td>Percentage of total $\dot{V}_{CO_2}$ released within peaks</td>
<td>86.5±5.1</td>
<td>51.8±5.1</td>
<td>0.0006</td>
</tr>
<tr>
<td>Interval between peaks (s)</td>
<td>97.2±21.4</td>
<td>48.4±8.05</td>
<td>0.0448</td>
</tr>
<tr>
<td>CO₂ SER (µl CO₂ h⁻¹)</td>
<td>0.70±0.07</td>
<td>0.44±0.04</td>
<td>0.0093</td>
</tr>
<tr>
<td>H₂O SER (µl H₂O h⁻¹)</td>
<td>0.41±0.05</td>
<td>0.21±0.01</td>
<td>0.0015</td>
</tr>
<tr>
<td>$\dot{V}_{CO_2}$ (µl CO₂ h⁻¹)</td>
<td>0.58±0.06</td>
<td>0.66±0.040</td>
<td>0.2499</td>
</tr>
<tr>
<td>$\dot{V}_{H_2O}$ (nl H₂O h⁻¹)</td>
<td>0.93±0.1</td>
<td>1.2±0.2</td>
<td>0.1396</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M.
Mean values between the two groups were compared using an analysis of variance.
SER, standard error of the regression of $\dot{V}_{CO_2}$ or $\dot{V}_{H_2O}$. 
Effect of a periodic or strong SER on water savings

As discussed above, of 13 D flies that showed periodic CO₂ release, six showed measurable periodic water vapor loss while seven did not. A comparison between these two groups of flies showed no significant difference in \( \dot{V}_{\text{CO}_2} \) (\( P=0.25 \)) or \( \dot{V}_{\text{H}_2\text{O}} \) (\( P=0.14 \), see Table 1). We randomly selected 13 additional flies from the 37 D flies that showed no periodic release of CO₂ and used recordings that were matched for the degree of desiccation (determined by the time spent in dry air) to those of the 13 flies showing periodic release. This analysis enabled us to compare statistically a group of D flies that exhibit periodic release of CO₂ with a group without periodic release (Table 2). This comparison indicated that flies without periodic release of CO₂ had a significantly reduced CO₂ SER (\( P<0.001 \)) and H₂O SER (\( P=0.017 \)), but no difference in \( \dot{V}_{\text{CO}_2} \) (\( P=0.25 \)) relative to flies showing periodic release. Most importantly, there was no significant difference in \( \dot{V}_{\text{H}_2\text{O}} \) between these two groups (\( P=0.19 \)), indicating that periodic CO₂ release is not related to a reduction in respiratory water loss. In addition, for flies that did show periodic CO₂ release, there was no significant difference in \( \dot{V}_{\text{H}_2\text{O}} \) between individuals showing periodic H₂O release and those in which this did not occur (Table 2).

Individual D flies can exhibit marked changes in respiratory pattern from non-periodic to periodic (see, for example, Fig. 1B). Fig. 3 presents data for three flies in which the respiratory pattern changed from non-periodic to periodic. Note that the mean CO₂ SER values are highly elevated during bouts of periodic CO₂ release. No concomitant reduction in \( \dot{V}_{\text{H}_2\text{O}} \) is observed in these measurement periods. A correlational analysis of the CO₂ SER versus \( \dot{V}_{\text{H}_2\text{O}} \) data presented in Fig. 3 revealed no correlation between these variables for each of the three flies (\( P=0.07, P=0.35, P=0.10 \)).

To determine whether the strength of the CO₂ SER is associated with water savings, we measured correlations between survival time, \( \dot{V}_{\text{CO}_2} \), \( \dot{V}_{\text{H}_2\text{O}} \), the CO₂ SER and the \( \dot{V}_{\text{CO}_2}/\dot{V}_{\text{H}_2\text{O}} \) ratio in the 50 D flies from this experiment. Increased survival time is significantly correlated with increased \( \dot{V}_{\text{CO}_2} \) (\( r=0.34, P=0.009 \)), decreased \( \dot{V}_{\text{H}_2\text{O}} \) (\( r=-0.46, P<0.001 \) and an increased \( \dot{V}_{\text{CO}_2}/\dot{V}_{\text{H}_2\text{O}} \) ratio (\( r=0.61, P<0.001 \)). Surprisingly, however, survival time is negatively correlated with the SER of CO₂ release (\( r=-0.31, P=0.013 \)) (Fig. 4).

We also found that \( \dot{V}_{\text{H}_2\text{O}} \) is positively correlated with the CO₂ SER (\( r=0.24, P=0.050 \), and not negatively correlated, as one might expect. Fig. 4 shows the relationships between CO₂ SER and both water loss rate and survival time.

**Discussion**

Previous studies have demonstrated that, following selection for desiccation resistance, D flies show reduced rates of water loss and increased survival time in dry air (Graves et al. 1992; Gibbs et al. 1997). In addition, D flies show marked changes in their respiratory patterns compared with their controls and ancestral populations, as shown by significant increases in the SER of the CO₂ release pattern (Williams et al. 1997, 1998).
Respiratory pattern and water loss in Drosophila melanogaster

In the present study, we have taken advantage of the marked variations in respiratory pattern displayed among the D flies. Some flies show periodic release of CO2 while others show only an increase in the SER of CO2 release. Some flies show marked changes in respiratory pattern within a 20 min recording period or between recordings. No previous study of the effects of periodic release of CO2 on water loss rates has been able to examine a single insect as it modified its respiratory behavior over a short period and without experimental manipulation (Lighton, 1996). Analysis of the variation within a single recording also reduces the uncertainty associated with comparisons between insects or treatments.

A periodic release of CO2 is not associated with a reduced water loss rate

The D flies show a variety of patterns of CO2 and H2O release. We can utilise this physiological diversity to address whether periodic release of CO2 is associated with decreased rates of water loss compared with the patterns of non-periodic CO2 release recorded at other times. We used the relevant time periods from the 13 flies that exhibited periodic release of CO2 and matched these recordings with those from 13 flies that never showed periodic release. \( \dot{V}_{\text{H}_2\text{O}} \) from flies with periodic release of CO2 was not significantly different from those without. In addition, there was no difference in \( \dot{V}_{\text{CO}_2} \) between the groups, which precludes the functioning of periodic release of CO2 to allow a greater propagation of CO2 to be released with the same amount of water loss.

The pattern of CO2 release in many insects studied to date includes closed-spiracle phases, in which levels of CO2 are low or near zero. We found that only half the flies that showed periodic release had low enough levels of CO2 and therefore of water release during the interburst period to allow us to see concurrent peaks of water loss. The rarity of the periodic H2O release pattern (only recorded in six flies out of 50) suggests that tight closure of the spiracles between open phases is not a critical trait in desiccation resistance. There was no significant difference in the rates of water loss between the six flies with low interburst levels of CO2 and the seven flies with high levels. Also, \( \dot{V}_{\text{CO}_2} \) was not significantly different between these two groups. It is thus unlikely that a greater need to release CO2 resulted in the spiracles being more widely open in the group with a high interburst release.

![Fig. 4. Relationship between the standard error of the regression of \( \dot{V}_{\text{CO}_2} \) (SER) and \( \dot{V}_{\text{H}_2\text{O}} \) and survival time in 50 D flies. The correlations are significant (P<0.05) but not strong (r=0.24 for \( \dot{V}_{\text{H}_2\text{O}} \), r=−0.31 for survival time).](image)

Table 2. Effect of periodic release of CO2 on respiratory variables

<table>
<thead>
<tr>
<th>Respiratory variable</th>
<th>Flies with periodic CO2 release</th>
<th>No periodic H2O release (N=7)</th>
<th>No periodic CO2 release (N=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With periodic H2O release (N=6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO2 SER (( \mu \text{l CO}_2 \text{ h}^{-1} ))</td>
<td>0.70±0.07</td>
<td>0.44±0.04**</td>
<td>0.13±0.017**</td>
</tr>
<tr>
<td>H2O SER (( \text{nl H}_2\text{O h}^{-1} ))</td>
<td>0.41±0.05</td>
<td>0.21±0.008**</td>
<td>0.21±0.008*</td>
</tr>
<tr>
<td>( \dot{V}_{\text{CO}_2} ) (( \mu \text{l CO}_2 \text{ h}^{-1} ))</td>
<td>0.58±0.06</td>
<td>0.66±0.040</td>
<td>0.70±0.05</td>
</tr>
<tr>
<td>( \dot{V}_{\text{H}_2\text{O}} ) (( \text{nl H}_2\text{O h}^{-1} ))</td>
<td>0.93±0.1</td>
<td>1.2±0.2</td>
<td>1.4±0.3</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M.
Asterisks indicate a significant difference (ANOVA) between flies with periodic H2O release and flies without periodic H2O release, or between flies with and without periodic CO2 release; **P<0.01, *P<0.05.
SER, standard error of the regression of \( \dot{V}_{\text{CO}_2} \) or \( \dot{V}_{\text{H}_2\text{O}} \).
We also examined recordings of individual D flies that exhibited isolated bouts of periodicity within a pattern of non-periodic CO₂ release. The water loss rates of these flies could be followed in concert with the changes in respiratory pattern. There was no evidence that periodic CO₂ release caused any reduction in the rate of water loss in these flies. In fact, an increase in $V_{H₂O}$ often occurred within 2 h of the bout of periodicity, although the increase might occur before or after the pattern of CO₂ release changed (Fig. 3).

These studies have permitted us to examine rates of water loss associated with a periodic release of CO₂ among several flies and within a single fly. We conclude from these results that bouts of periodic CO₂ release do not reduce water loss in these insects.

**Increased CO₂ SER is not associated with reduced water loss rate**

The majority of insects and other arthropods examined for respiratory pattern exhibit a periodic DGC, but this pattern may or may not contain all the elements of the ‘classic’ cycle of open, closed and flutter phases. Our five populations of D flies have been selected for resistance to desiccation and show a change in respiratory pattern compared with their controls. This change is demonstrated by a change in the CO₂ SER, but not always by the introduction of a periodic pattern of CO₂ release. All five populations show an increased CO₂ SER and decreased $V_{H₂O}$, while periodicity was recorded in only three of the five populations. Might it be the magnitude of the CO₂ SER that establishes water savings and not its periodicity? We measured correlations between survival time, $V_{CO₂}$, $V_{H₂O}$, CO₂ SER and the $V_{CO₂}/V_{H₂O}$ ratio in the 50 D flies from this experiment.

The correlations between CO₂ SER and both $V_{H₂O}$ and survival time were not in the direction that we would expect if an elevated CO₂ SER were to increase desiccation resistance. The peak height and frequency of CO₂ release are negatively correlated with survival time and positively correlated with water loss rate. These findings indicate that not only does an increased SER of CO₂ release tend not to reduce water loss or lengthen survival in the D flies but that it may in fact be detrimental to the fly.

We conclude then that an increased SER is of no help in reducing water loss. If the spiracles must be open to some extent, it is not apparent that opening and closing the spiracles in an exaggerated or periodic DGC saves more water than do other options such as constant flutter or keeping the spiracles open a consistently small amount.

We must add that our experimental system prevents us from drawing conclusions that would be universal among all insect species. Because *Drosophila melanogaster* do not generally show a clear closed-spiracle phase, we cannot reliably separate respiratory and cuticular water loss. We are forced to infer changes in respiratory water loss by examining respiratory plus cuticular water loss. Also, the models of discontinuous respiration ascribe some water savings to the flutter phase (Levy and Schneiderman, 1966c), when CO₂ release drops to zero and there is an inward bulk flow of air. Because we have no evidence of inward convection of air in our flies, water savings during this portion of a DGC are not likely.

What our experimental model does test is whether the respiratory pattern that is seen in these flies saves water. Our data indicate that, while the SER of CO₂ release has increased in response to selection for resistance to desiccation, it does not seem to reduce water loss.

**The source of increased desiccation resistance in the D flies**

In the present study, we have shown that no correlation can be demonstrated between a highly periodic pattern of CO₂ release or an increase in CO₂ SER and a reduction in respiratory water loss. What then is the physiological basis for reduced water loss and increased desiccation resistance in the D flies?

Hoffman and Parsons (1991) suggested that a reduction in metabolic rate might be a mechanism for resistance to environmental stresses in general and desiccation stress in particular. However, our results and those of other researchers argue against this as a physiological mechanism for resisting desiccation in our populations of flies (Djawdan *et al.* 1997; Williams *et al.* 1998).

Other studies have found differences in the D flies compared with their controls (C flies). Gibbs *et al.* (1997) have shown that the D flies have an increased somatic water content and decreased rates of water loss compared with the C flies. Williams *et al.* (1998) indicate that the D flies lose water at approximately two-thirds the rate of the C flies and that their total loss of body water is twice that of the C flies. In other work, D females also showed statistically significant reductions in excretory water loss compared with C females, but these represented a small proportion (approximately 10%) of total water loss rates (Gibbs *et al.* 1997).

Differences in metabolic storage may also help to explain increased desiccation tolerance in the D flies. These flies have, in the course of selection for desiccation resistance, increased their glycogen levels and reduced their somatic lipid levels (Graves *et al.* 1992; Gibbs *et al.* 1997; Djawdan *et al.* 1996). Gibbs *et al.* (1997) estimate that approximately 50% of the water in D flies is associated with stored glycogen. Because our work does not indicate that a larger CO₂ SER or more periodic release of CO₂ saves water, we are left with the conclusion that factors other than respiration strongly affect water loss.

If increased desiccation resistance in D flies is due principally to increased water storage and reduced cuticular water loss, why are changes in CO₂ SER correlated with selection for desiccation resistance? One possibility is that the changes in respiratory pattern are correlated with changes in locomotory behavior. D flies appear to be more quiescent than C and O flies. Studies using other insect species have found that insects are more likely to show a DGC when inactive. It may be that the increases in CO₂ SER observed in the D flies are associated with behavioral changes in the flies. We are currently conducting studies to examine this hypothesis.
The authors would like to thank M. R. Rose for the use of selected lines of \textit{D. melanogaster}, and A. Gibbs for the use of his equipment. A. Bennett, A. Gibbs, J. Harrison and R. K. Josephson contributed valuable comments on earlier manuscripts. This work was supported by NSF grant IBN 9723404.

References


