The Journal of Experimental Biology 206, 2779-2786

doi:10.1242/jeb.00498

Evolved patterns and rates of water loss and ion regulation in laboratory-selected populations of *Drosophila melanogaster*

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Accepted 15 May 2003

Summary

We have investigated water loss from, and ion regulation within, the hemolymph and tissues of five replicate populations of *Drosophila melanogaster* that have undergone laboratory selection for enhanced desiccation resistance (i.e. the D populations). We compared the patterns and rates of water loss and the ion content of the D populations prior to and during desiccation with those of five replicate control (C) populations. The net rate of water loss in the C flies was ~3-fold greater than that of the D flies during the initial hours of desiccation. After 8 h, both C and D flies had considerable reductions in water loss rate. During 24 h of desiccation, the tissue water content of the D flies was conserved, while the C flies were faced with significant loss of tissue water during the initial 8 h of desiccation. We propose that the increased hemolymph volume of the D flies plays a role in buffering water loss from the tissues. One consequence of this large hemolymph pool is that the hydrated D flies contained approximately seven times more sodium within the hemolymph than did the hydrated C flies. Despite a continual loss of hemolymph volume in the D flies during lengthy periods of desiccation, the sodium content of the hemolymph was significantly reduced only during a single event. We provide evidence that the regulation of extracellular sodium, as well as chloride, occurred *via* excretory processes during desiccation. In addition, whole-body potassium was not significantly decreased in the D flies during desiccation but was reduced (i.e. excreted) in the C flies; hence, we suggest that the potassium content paralleled tissue water level.

Key words: water loss, ion regulation, hemolymph, *Drosophila melanogaster*, desiccation, Na^+, K^+, Cl^-, excretion, osmoregulation.

Introduction

Organisms, such as *Drosophila melanogaster*, that are amenable to laboratory selection studies have been employed to investigate physiological characters that can evolve in response to a desiccating environment (Graves et al., 1992; Hoffman and Parsons, 1993; Gibbs et al., 1997; Djawdan et al., 1998; Chippindale et al., 1998; Williams and Bradley, 1998; Williams et al., 1998; Folk et al., 2001). Evolutionary responses to selection for enhanced desiccation resistance in laboratory populations of *Drosophila melanogaster* are multifold and include increased water stores and lowered water loss rates (Gibbs et al., 1997; Bradley et al., 1999). In the present study, we examined the patterns and rates of water loss, as well as ion regulation and osmoregulatory strategies, during desiccation in 10 populations of *Drosophila melanogaster*: five replicate populations that have undergone laboratory selection for enhanced desiccation resistance (D populations) and five control (C) populations.

Mellanby (1939) proposed that a vital function of insect hemolymph is to store water, which is then available for distribution to the tissues during periods of water stress. One of the evolutionary responses in our laboratory-selected, desiccation-resistant populations is a striking increase in hemolymph volume (~330 nl; a >6-fold increase in hemolymph volume relative to the control populations). We propose that water from this large hemolymph pool can buffer the tissues against water loss during periods of water stress, thus supporting tissue homeostasis. The control populations, which have not been subjected to selection for enhanced desiccation resistance, have a relatively small hemolymph volume [~50 nl]. We expect that these populations may have a reduced ability to prevent water loss from other body compartments during desiccation.

During lengthy bouts of desiccation, rates of water loss in insects tend to decline as water stress proceeds (Loveridge, 1968; Edney, 1971, 1977; Hadley et al., 1986; Noble-Nesbitt and Al-Shukur, 1987; Hadley, 1994). Previous studies have shown that our populations of desiccation-resistant flies have a significantly lower mean rate of water loss than the control populations (Gibbs et al., 1997; Williams et al., 1998). We wished to determine if the desiccation-resistant populations had diverged from the control flies in the ability to modulate water loss rates during extended periods of desiccation.

Sodium is the principle ion in the hemolymph of dipterans and generally makes up approximately two-thirds of the cation

pool (Sutcliffe, 1963). The loss of water during desiccation without a concomitant loss of Na^+ would lead to an increase in the Na^+ concentration of the hemolymph. As hemolymph osmotic concentration rises, water would tend to move out of the cells. As a consequence, ion regulation of the hemolymph during desiccation may be necessary to prevent intracellular water loss.

Most insect species regulate hemolymph osmotic concentration during desiccation (Edney, 1977; Hadley, 1994). One component of hemolymph osmoregulation in terrestrial insects during water loss is the removal of inorganic ions, principally Na^+, Cl^- and, to a lesser extent, K^+, which are either permanently excreted or transported to and sequestered within the tissues. Two general disadvantages related to the strategy of ion excretion combined with hemolymph osmoregulation are: (1) by necessity, water is lost during excretion and (2) upon rehydration the insects are committed to replacing the excreted ions *via* consumption. Although some insects have been shown to use excretory osmoregulation, use of this strategy has been shown for only a small number of species (Laird and Winston, 1975; Hyatt and Marshall, 1978; Albaghdadi, 1987; Naidu and Hattingh, 1986).

One strategy of hemolymph osmoregulation in terrestrial insects during desiccation is the transport of ions out of the hemolymph and into some tissues, where they may be stored in an osmotically inactive form. This strategy has been demonstrated in the cockroach *Periplaneta* (Wall, 1970; Tucker, 1977a,b; Hyatt and Marshall, 1977, 1985a,b). Hemolymph osmoregulation in *Periplaneta* is achieved primarily by the removal of Na^+ and K^+. Only a small fraction of the ions are excreted; the greater proportion is sequestered within the fat body, probably as urate salts. The advantages of this strategy over ion excretion are: (1) water is not lost during ion storage and (2) upon rehydration the ions are available for transport back into the hemolymph.

We present here the first study of water and ion distribution in *Drosophila melanogaster* during desiccation. We examined water levels and Na^+ content of both the hemolymph and tissues. If the flies osmoregulate the hemolymph during desiccation using Na^+ excretion, we predict that the Na^+ content of the hemolymph will decline, while tissue Na^+ will be unchanged (or perhaps reduced). Alternatively, if the flies use the 'removal and sequestration' strategy, the hemolymph Na^+ content should drop, while tissue Na^+ increases.

We also measured whole-body potassium and chloride content during desiccation. Potassium is the principal intracellular cation and is found only in very low concentrations within the hemolymph of dipterans (Sutcliffe, 1963). We anticipated that because of the increased capacity of the desiccation-resistant populations to prevent cell shrinkage during desiccation, the maintenance of cellular ion composition would also be enhanced. If true, the K^+ content of the tissues should not change, considering net tissue water loss is minimized. Chloride, the principle inorganic anion in dipteran hemolymph, generally comprises ~20% of the total anion pool (Sutcliffe, 1963). We propose that in order to maintain electroneutrality, as well as osmolarity, loss of Na^+ and/or K^+ content may be accompanied by loss of Cl^-.

Materials and methods

Fly populations

The flies were from five large, outbred populations (designated D_1–D_5) of *Drosophila melanogaster* L., which have undergone laboratory selection since 1988 for enhanced desiccation resistance (Rose et al., 1990, 1992). Each D population is paired and concurrently maintained with one of five control populations (C_1–C_5). Each of the five C_n/D_n pairs derives from one of five ancestral populations (O_1–O_5; Rose, 1984). For example, the O_1 population is the shared, common ancestor of both the C_1 population and the D_1 population; O_2 is the common ancestor of C_2 and D_2, etc.

Details of the maintenance protocol and selection regime are provided in Folk et al. (2001). Briefly, multiple batches of eggs (60–80 eggs per batch) were collected from each population, and each was placed into a food vial. The insects were allowed to metamorphose and mature for 14 days, at which time the D populations were subjected to the selection protocol until 80% mortality was reached. During selection, the D populations were deprived of food and water, while the C populations were provided water. Following selection, all surviving flies were allowed to recover on moist food for 3 days. Eggs were then collected for rearing of the next generation. (Approximately the same number of females from all C and D populations was available for establishment of each new generation.) Selection for enhanced desiccation has been imposed for more than 250 generations.

Prior to all experiments, flies from the C and D populations were removed from the pressure of selection for two generations. By rearing C and D flies for two generations under identical conditions, and without the stress of selection, we removed grandparental and parental effects that derived from the selection regime. We were then able to attribute divergences between the C and D populations to genetic differences. Additionally, to eliminate the effects of gender, only females were studied. All experiments were performed on females that were (on average) 4-day-old, mated adults.

Desiccation protocol

Five or six flies were briefly anesthetized with CO_2 and placed in a 30 g vial. A foam stopper was placed ~3 cm into the vial, and ~4.5 g of Drierite (calcium sulfate desiccant) was poured on top of the stopper. The open end of the vial was then tightly covered with Parafilm. Flies were allowed to desiccate undisturbed within the vials for 8 h, 16 h, 24 h (D flies only) or 48 h (D flies only).

Estimation of extractable hemolymph volume and gravimetric measurement of water content in exsanguinated flies

Mature females were anesthetized with CO_2 and then weighed using a Cahn 29 automatic electrobalance (Cerritos, CA, USA). Flies were kept under CO_2 anesthesia while the

abdomen was gently torn with fine-tipped, surgical forceps. A Kimwipe (i.e. low-lint, laboratory-grade tissue paper) moistened with isotonic saline (250 mosmol l^{-1}; Singleton and Woodruff, 1994) was used to absorb the extractable hemolymph. By slightly moistening the Kimwipe, tissue from the fat body was less likely to stick to it. To obtain the total wet mass of the exsanguinated flies (i.e. of the tissue–gut–cuticle), each fly was re-weighed immediately following hemolymph extraction. The hemolymph volume was then estimated by subtracting the wet mass of the exsanguinated flies from the whole-body wet mass (for a brief review of estimating hemolymph volume by use of the blotting technique, see Hadley, 1994). Hemolymph volume and total wet mass of the exsanguinated flies were measured in 20 individuals from each C and D population at the following time intervals: prior to desiccation (designated as 0 h) and after 8 h, 16 h, 24 h (D flies only) and 48 h (D flies only) of desiccation. Because control flies survive for an average of 23 h when desiccated, measurements of these flies were not made at 24 h and 48 h. The exsanguinated flies were dried overnight at 60°C and reweighed to obtain dry mass. Water content of the exsanguinated flies was estimated by subtracting dry mass from total wet mass.

Ion measurements

Sodium and potassium

Eight samples of whole flies (Na^+ and K^+ measurements) and eight samples of exsanguinated flies (Na^+ measurements only) were prepared from each population prior to desiccation (designated as 0 h) and after 8 h, 16 h, 24 h (D flies only) and 48 h (D flies only) of desiccation. Each sample consisted of three flies that had been solubilized overnight in 100 μl of concentrated HNO_3 (containing 0.02 p.p.m. Na^+, 0.05 p.p.m. Cl^- and <0.002 p.p.m. K^+) at room temperature (21–23°C). Following solubilization of the flies, 2.9 ml of doubly distilled water (ddH_2O) was added. Sodium and potassium concentrations of the samples were determined using atomic absorption spectrophotometry (AA-125 series; Varian Analytical Instruments, Springvale, Australia). The mean Na^+ content of either the intact flies or the exsanguinated flies was calculated from the Na^+ concentration of the sample. The Na^+ content of the hemolymph was estimated by subtracting Na^+ content of the exsanguinated flies from whole-body Na^+ content.

Chloride

Whole-body chloride was determined using a colorimetric assay (Gonzalez et al., 1998). Each sample was made up of three flies that had been solubilized overnight in 50 μl of concentrated HNO_3 at 21–23°C. Following liquefaction of the flies, 0.95 ml ddH_2O was added to each sample. Eight samples were prepared from each population at the time intervals described in the previous section. Chloride was quantified through a two-step assay: (1) thiocyanate ions were released from mercuric thiocyanate through the formation of mercuric chloride and (2) in the presence of ferric ions, a colored compound (ferric thiocyanate) was produced in proportion to the Cl^- content in each sample. The absorbance of each sample was measured spectrophotometrically at 480 nm. Chloride concentration of the fly samples was then calculated from standard curves that were constructed from samples of known Cl^- concentrations.

Statistical analyses

The changes in hemolymph volume, water and Na^+ content of the tissue–gut–cuticle and whole-body K^+ were compared between the C and D groups prior to and throughout desiccation using a linear mixed-effects statistical model, in which the pairing of each C_n with D_n was treated as a block effect. The model included C and D treatments and the paired-population blocks as fixed effects, and time and within-population variance as random effects. The analyses were performed using the statistical program, R. Within the C and D treatment groups, we performed one-way analyses of variance with Bonferroni *post-hoc* comparison of means to determine the time period(s) at which significant changes in each dependent variable occurred. (To obtain Na^+ content of the hemolymph, we had subtracted the mean Na^+ content in exsanguinated flies from the mean Na^+ content in whole flies for each C_n or D_n population at each time interval. Hence, we could include in the statistical analyses only the five population means from both treatments at each time interval. As a result, we used only treatment and time when analyzing changes in hemolymph Na^+ content.) Least-squares linear regression was used to examine the relationship between chloride content and hemolymph volume during desiccation. Each point in the regression analyses represented the estimated mean from all five of the C or D populations. The significance level for all analyses was 0.05.

Results

Patterns and rates of water loss

The C and D flies differed in net rates of water loss during desiccation from both the hemolymph (P=0.01; Fig. 1) and the tissue–gut–cuticle (P=0.02; Fig. 2). Due to a continual loss of water from the large hemolymph pool, the D flies had an overall rate of hemolymph reduction that was ~3-fold higher than that of the C flies. Conversely, the C flies had a rate of water loss from the tissue–gut–cuticle that was ~4-fold higher than that of the D flies.

The D flies experienced loss of hemolymph throughout the entire desiccation period, and the volume was ultimately reduced by >80%. The mean rate of hemolymph loss during the initial 8 h of desiccation was 14 nl h^{-1}. Between 8 h and 24 h, this rate was reduced by ~50% to 6 nl h^{-1} and dropped to ~2.5 nl h^{-1} during the 24–48 h period. While the volume of hemolymph in the D flies declined continually throughout desiccation, the water content of the tissue–gut–cuticle was maintained during the initial 24 h (Fig. 2). At 24–48 h, the D flies had a significant reduction in water from the tissue–gut–cuticle (P=0.0001). The overall rate of water loss

in the D flies during 24–48 h totaled ~10 nl h^{-1} (~2.5 nl h^{-1} and 7.5 nl h^{-1} from the hemolymph and tissue–gut–cuticle, respectively).

The patterns and rates of water loss in the control flies were distinct from those observed in the desiccation-resistant flies. Approximately two-thirds of the hemolymph volume of the C flies was lost during the initial 8 h (P=0.001; Fig. 1), after which the volume was not significantly reduced. In contrast to the D flies, the C flies lost significant volume from the tissue–gut–cuticle by 8 h (P<0.0001; Fig. 2). The mean net rate of water loss in the C flies during the initial 8 h was 39 nl h^{-1} (4 nl h^{-1} and 35 nl h^{-1} from the hemolymph and tissue–gut–cuticle, respectively), which was ~3 times that observed in the D flies during the same time period. Net water loss rate dropped by ~90% to a mean rate of ~4 nl h^{-1} during 8–16 h.

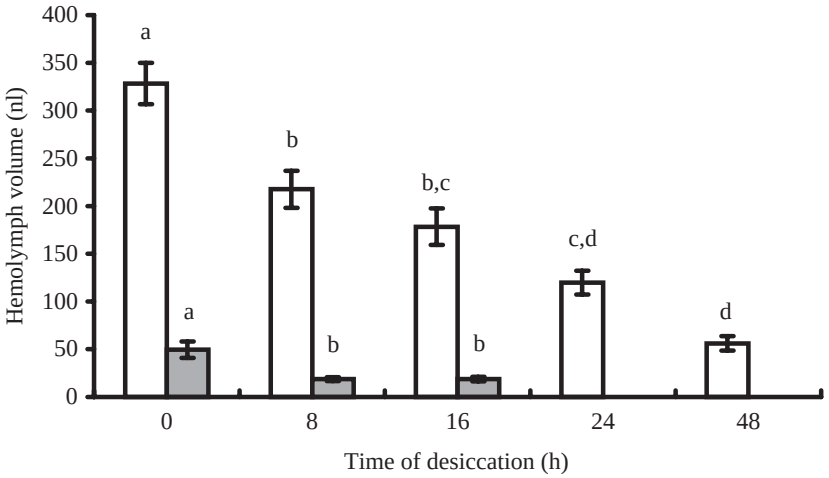

Fig. 1. Hemolymph volume of desiccation-resistant flies (D flies) and their controls (C flies) prior to desiccation (0 h) and at time intervals during desiccation. Values are means ± S.E.M. of five populations from either the C or D treatments. Open bars represent values from the D populations; filled bars represent values from the C populations. Significant differences within each treatment (C or D) are denoted by non-matching letters above the bars.

Hemolymph sodium

The desiccation-resistant flies had a higher rate of Na^+ removal from the hemolymph, which was a consequence of the greatly increased Na^+ content (P=0.002). The hemolymph Na^+ content of the D populations was significantly reduced due to the excretion of Na^+ between 8 h and 16 h (P=0.004; Table 1). Despite this excretory event, the hemolymph Na^+ content of desiccated D flies remained more than twice as high as that of fully hydrated C flies. Hemolymph Na^+ content of the D flies appeared to be regulated; yet Na^+ concentration was not strongly controlled (Table 1). Hemolymph Na^+ concentration increased by ~40% during the first 8 h of desiccation and was reduced by >50% following the Na^+ excretion event (8–16 h). Between 16 h and 48 h, the Na^+ content was stable and, as a result, Na^+ concentration increased as hemolymph volume continued to decline. By 48 h, Na^+ concentration exceeded pre-desiccation values by >60%.

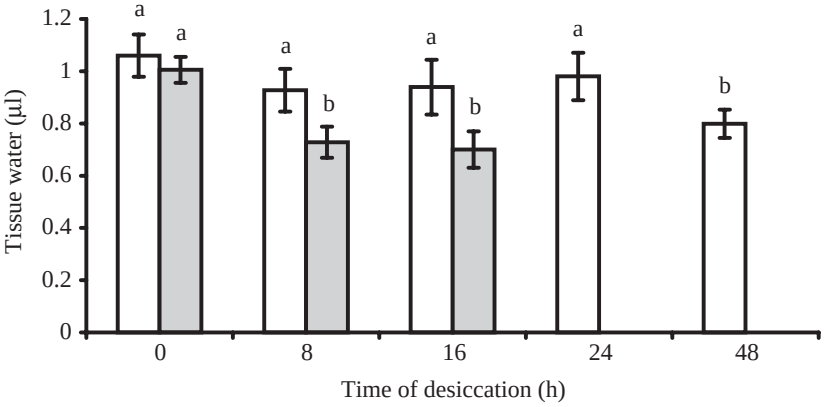

Fig. 2. Water content of exsanguinated desiccation-resistant flies (D flies) and their controls (C flies) prior to desiccation (0 h) and at time intervals during desiccation. Values are means ± S.E.M. of five populations from either the C or D treatments. Open bars represent values from the D populations; filled bars represent values from the C populations. Significant differences within each treatment (C or D) are denoted by non-matching letters above the bars.

Hemolymph Na^+ content of the C flies dropped significantly (84% reduction) during the initial 8 h (P=0.04; Table 1) and was maintained at a very low level during 8–16 h. The hemolymph Na^+ concentration in the C flies declined >50% during the initial 8 h, and by 16 h it had been reduced by ~75%, following the removal of much of the Na^+ from the hemolymph.

Sodium content in exsanguinated flies

The rate of loss of Na^+ from the tissue–gut–cuticle of the C flies was significantly higher (~2-fold increase) than that observed in the D flies (P=0.007). The Na^+ content of the tissue–gut–cuticle of the D flies was maintained during the initial 24 h of desiccation (Fig. 3); then, between 24 h and 48 h, a significant amount (14%) of Na^+ was removed (P=0.01) and subsequently excreted. The exsanguinated control flies had a significant reduction (16%) in Na^+ following 8 h (P=0.01; Fig. 3); the Na^+ content was unchanged following this reduction (P=0.52).

Whole-body potassium

Potassium content did not change significantly in the D flies throughout the entire desiccation period (P=0.13; Fig. 4). By contrast, the control flies had an excretory loss of 17% K^+ following 8 h of desiccation (P=0.01; Fig. 4), following which no significant loss of K^+ was detected (P=0.31).

Table 1. *Hemolymph volume, sodium content and sodium concentration in the C and D flies prior to desiccation (0 h) and after desiccation for 8 h, 16 h, 24 h (D flies only) and 48 h (D flies only)*

	Control populations			Desiccation-resistant populations		
Time (h)	Hemolymph volume (nl)	Hemolymph sodium content (nmol fly^{-1})	Hemolymph sodium concentration (mmol l^{-1})	Hemolymph volume (nl)	Hemolymph sodium content (nmol fly^{-1})	Hemolymph sodium concentration (mmol l^{-1})
0	50±19^{a}	3.1±1.3^{a}	62±19	328±22^{a}	22.9±3.3^{a}	70±4
8	19±5^{b}	0.5±0.2^{b}	28±12	218±19^{b}	21.2±3.2^{a}	97±8
16	19±5^{b}	0.3±0.3^{b}	16±16	178±19^{b,c}	7.5±2.7^{b}	42±7
24				120±12^{c,d}	7.4±1.5^{b}	62±14
48				56±8^{d}	6.4±2.1^{b}	114±46

Each value is the mean ± S.E.M. derived either from the five C or five D populations. The cells in a column that contain different letters are significantly different at the P=0.05 level.

Whole-body chloride

Both control and desiccation-resistant flies had significant losses of whole-body Cl^- during desiccation. The mean rate of Cl^- reduction in the C flies was 0.22 nmol h^{-1} (P=0.001, r^2=0.99, y=–0.223x+8.323; Fig. 5), which was >100% higher than the rate of Cl^- reduction in the D flies (P=0.023, r^2=0.94, y=–0.094x+9.875; Fig. 5). At the end of the desiccation periods (16 h and 48 h for the C and D flies, respectively), pre-desiccation levels of Cl^- had been reduced by 43% in both groups.

A significant positive relationship between Cl^- content and hemolymph volume was found in both the C populations (regression not shown; P=0.01, r^2=0.65, y=0.081x+4.205) and the D populations (P=0.001, r^2=0.88, y=0.0163x+5.123; Fig. 6), suggesting that the decline in Cl^- was associated with the loss of hemolymph.

Discussion

In this paper, we report for the first time that sodium is regulated through excretion during desiccation in *Drosophila melanogaster*. Additionally, in populations selected for enhanced desiccation resistance, hemolymph appears to serve as a buffer against water loss from the intracellular space.

Sodium regulation during desiccation

The Na^+ content of the hemolymph of a hydrated D fly was >7-fold higher than that of a hydrated C fly (Table 1). Hemolymph water loss coupled with unregulated Na^+ content could result in exceedingly high Na^+ concentrations within the hemolymph of the D flies. The osmotic concentration of Na^+ within the hemolymph could increase to >400 mosmol l^{-1}

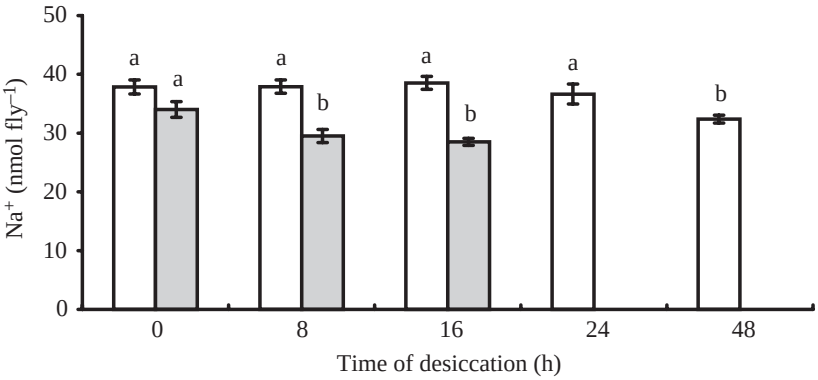

Fig. 3. Sodium content of tissue–gut–cuticle of desiccation-resistant flies (D flies) and the controls (C flies) prior to desiccation (0 h) and at time intervals during desiccation. Values are means ± S.E.M. of five populations from either the C or D treatments. Open bars represent values from the D populations; filled bars represent values from the C populations. Significant differences within each treatment (C or D) are denoted by non-matching letters above the bars.

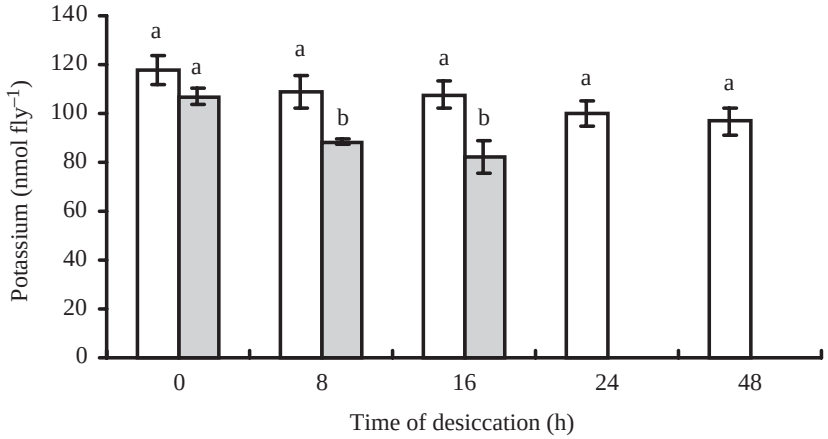

Fig. 4. Whole-body potassium of desiccation-resistant flies (D flies) and their controls (C flies) prior to desiccation (0 h) and at time intervals during desiccation. Values are means ± S.E.M. of five populations from either the C or D treatments. Open bars represent values from the D populations; filled bars represent values from the C populations. Significant differences within each treatment (C or D) are denoted by non-matching letters above the bars.

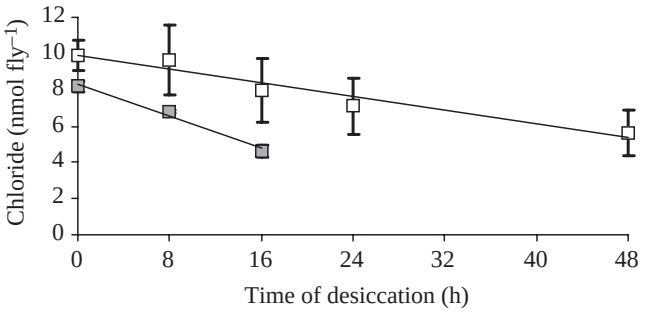

Fig. 5. Chloride content in flies prior to (0 h) and during desiccation. Both the control (r^2=0.99, P=0.001, y=–0.223x+8.323) and selected (r^2=0.94, P=0.023, y=–0.094x+9.875) groups showed a significant decline in chloride during desiccation. Each filled symbol represents the mean ± S.E.M. of the five C populations; each open symbol represents the mean ± S.E.M. of the five D populations.

following 48 h desiccation if Na^+ content remained unadjusted. Yet, the highest hemolymph Na^+ concentration that we observed in the D flies was 114 mosmol l^{-1}, indicating that Na^+ content was regulated during desiccation.

In dehydrated *Periplaneta*, Na^+ is removed from the hemolymph and subsequently stored in the fat body (Wall, 1970; Tucker, 1977a,b; Hyatt and Marshall, 1977, 1985a,b). We examined Na^+ content of the hemolymph and of exsanguinated flies to determine if Na^+ was removed from the hemolymph and stored in another compartment. Sodium was removed from the hemolymph in both C and D flies, yet the Na^+ content of the other compartments did not increase; hence we found no evidence of Na^+ sequestration (Table 1; Fig. 3). Instead, our data suggest that after Na^+ was removed from the hemolymph, it was permanently excreted.

Sodium content of the tissue–gut–cuticle in the D flies did not change significantly until 24–48 h. To determine if the drop in Na^+ was due merely to gut clearance, we estimated the Na^+ content of a full gut. Total gut volume was estimated to be ~0.1 μl (based on morphometric data from Miller, 1994). The Na^+ concentration of the food was ~10 mmol l^{-1}. Given these values, we estimate that only ~1 nmol of Na^+ is contained within a full gut. Clearing of the gut, therefore, could not account for the loss of Na^+. We conclude that a reduction in Na^+ content must have occurred within some tissues.

Relative to the D flies, the C flies had a much lower hemolymph volume and Na^+ content when fully hydrated (Table 1; Fig. 1); yet, during the initial 8 h of desiccation, the C flies lost a high proportion of both. The loss of Na^+ from the hemolymph was not followed by an increase in Na^+ content of the tissue–gut–cuticle (Fig. 3), indicating that the control flies also excreted Na^+ during desiccation.

Potassium regulation

Potassium is the predominant intracellular cation and is found at relatively low concentrations (25 mmol l^{-1}) in the hemolymph of *Drosophila melanogaster* (Van der Meer and Jaffe, 1983). The control flies lost substantial whole-body K^+ within the first 8 h of desiccation. Overall, our data suggest that the initial 8 h period was physiologically stressful in the C flies: they lost 28% of the water and 16% of the Na^+ content from the tissue–gut–cuticle, as well as 17% of whole-body K^+ (Figs 2–4). We propose that the reduction in K^+ occurred in parallel with the acute loss of water and Na^+ from the tissues and as a means of regulating osmotic concentration of the tissues.

In response to selection for enhanced resistance to desiccation, the D populations have evolved osmoregulatory capacities divergent from those observed in the C flies. Despite the loss of volume from the tissue–gut–cuticle in the latter 24 h, the whole-body potassium content of the D flies did not significantly decline during the entire 48 h of desiccation (Fig. 4).

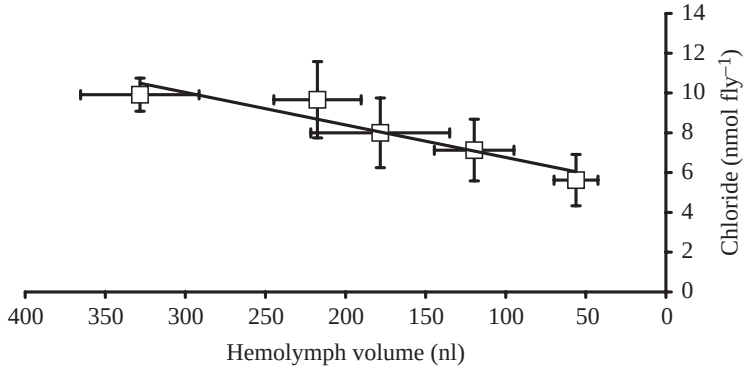

Fig. 6. A strong positive relationship was observed between chloride content and hemolymph volume in the desiccation-resistant populations prior to and during desiccation (r^2=0.88, P=0.001, y=0.0163x+5.123). As desiccation proceeds, hemolymph volumes move from left to right on the graph as it is drawn. Each point represents the mean ± S.E.M. of the five D populations.

Regulation of chloride

Both the C and D groups of flies lost substantial whole-body Cl^- during desiccation (Fig. 5). Several lines of evidence suggest that Cl^- was lost principally from the hemolymph, at least in the D flies. (1) Loss of Cl^- was strongly associated with a reduction in hemolymph volume (P=0.001; Fig. 6). (2) The water, K^+ and Na^+ content of the tissue–gut–cuticle were not reduced during 24 h of desiccation (Figs 2–4). Therefore, loss of Cl^- from any body compartment other than the hemolymph during 0–24 h is doubtful. (Between 24 h and 48 h, the D flies had a small, but significant, loss of Na^+ from the tissue–gut–cuticle. Chloride ions may have been lost concurrently with Na^+ from the tissue–gut–cuticle between 24–48 h.) (3) Following the single Na^+ excretion event, hemolymph Na^+ and Cl^- content were maintained at almost equimolar levels (Fig. 7). (4) Finally, intracellular Cl^- concentration is usually quite low in relation to

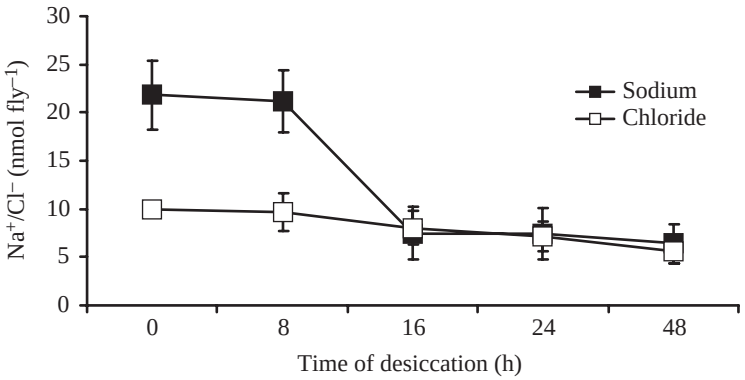

Fig. 7. Changes in hemolymph Na^+ and whole-body Cl^- in the desiccation-resistant flies (D flies) prior to and during desiccation. Values are means ± S.E.M. of the five D populations.

extracellular Cl^- (Alberts et al., 1994). For these reasons, we propose that most of the Cl^- measured in the whole flies was located in the hemolymph and that regulation of both Na^+ and Cl^- plays a role in hemolymph osmoregulation in the D flies.

During the initial 8 h period, the C flies had significant losses of K^+ and Na^+ from the tissue–gut–cuticle, and some Cl^- may have been lost concomitantly from these sites. For this reason, we are unable to conclude that the reduction in Cl^- was a major component of hemolymph osmoregulation in the control flies.

Patterns and rates of water loss

The control flies experienced a net loss of ~30% of water from the tissue–gut–cuticle during the initial 8 h of desiccation. Although we were unable to separate the volume of water lost from the tissues from that removed from cuticle or gut, the following evidence supports the idea that water was, indeed, lost from the tissues early in desiccation: (1) The C flies had a relatively small hemolymph volume even when hydrated, thus the ability to replace tissue water with that stored in the hemolymph was presumably minimal. (2) The substantial loss of K^+ suggests that intracellular osmoregulatory adjustments were made in response to acute cellular water loss. It is feasible that the flies also lost some water from the cuticle and gut. The importance to water balance of the water absorbed by the cuticle remains controversial (Loveridge, 1968; Winston and Beament, 1969; Machin et al., 1985; Machin and Lambert, 1987). The significance of water storage within the gut of drosophilids is unclear. We estimated gut volume of the flies to be ~100 nl. Assuming that the gut was filled with food containing 80% water, clearing of the gut would only account for ~30% of the water lost from the entire tissue–gut–cuticle. Therefore, we propose that substantial water was lost from the tissues.

After the initial 8 h period, hemolymph volume remained unchanged in the C flies (Table 1). At this time, the volume was low (19 nl) and may have reached a critical level, which if further reduced could be physiologically detrimental, if not fatal. The hemolymph has multiple circulatory and osmoregulatory functions (Chapman, 1998). If hemolymph levels are reduced below some critical level, distributive and regulatory functions of the hemolymph may be compromised. We suggest that the adverse effects deriving from a severe reduction in hemolymph volume may play a role in defining the lower limits of hemolymph volume, necessitating that additional water loss comes from the intracellular compartment.

The patterns and rates of water loss during desiccation in the D flies is distinct from that observed in the C flies. During 24 h of desiccation, water was only lost from the hemolymph, and the tissue water content was conserved. Interestingly, whole-body K^+ content did not significantly decrease during the 48 h period in the D flies; therefore, it remains unclear if the water lost from the tissue–gut–cuticle was principally lost from tissues.

During desiccation, the rate of absolute water loss declined in both C and D groups. The C and D flies had net water loss rates in the range of 4–39 nl h^{-1} and 6–14 nl h^{-1}, respectively. The phenomenon of diminishing water loss rates as time of exposure to drying conditions increases has been observed in various insects and has been hypothetically attributed to: (1) a decline in activity as the insect familiarizes itself with its new conditions, resulting in a lower metabolic rate and a reduction in spiracular water loss; (2) a reduction in hemolymph volume leading to an increase in ion concentration, which may cause structural changes in cuticular proteins and a reduction in cuticular permeability; and (3) hormonal control of water movement across the integument (Treherne and Willmer, 1975; Edney, 1977; Noble-Nesbitt and Al-Shukur, 1988a,b). The mechanistic explanation for diminishing rates of water loss in our populations remains unclear.

Conclusions

We have shown that flies selected for enhanced desiccation resistance have evolved a large hemolymph pool, which acts to protect intracellular volume during periods of water stress. Gibbs and Matzkin (2001) have shown that desiccation resistance in cactophilic species of *Drosophila* is not correlated with total body water content. The lack of increasing total body water in the desert species may reflect a trade-off between locomotor capacity and the storage of water for desiccation resistance (Lehman and Dickinson, 2001). The precise role of water compartmentalization in desert flies remains unclear. The findings reported herein suggest that this would be a valuable area to explore.

The capacity to regulate the sodium, chloride and potassium levels of the hemolymph in conjunction with volume loss has been observed to varying degrees in insects, including desert beetles (Coutchie and Crowe, 1979; Naidu and Hattingh, 1986). We demonstrate for the first time that drosophilids have the capacity to adjust the inorganic solute content of the hemolymph when volume declines during desiccation and the degree to which those patterns have been affected by selection.

Special thanks to R. Josephson and an anonymous reviewer for valuable comments on the manuscript. In addition, we thank L. Mueller and D. Campbell for their help with the statistical analyses. This work was supported by NSF grant IBN-0079501 to T.J.B.

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