The Journal of Experimental Biology 207, 2313-2321
Published by The Company of Biologists 2004
doi:10.1242/jeb.01024

Osmotic regulation in adult *Drosophila melanogaster* during dehydration and rehydration

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Accepted 13 April 2004

Summary

We have examined the osmoregulatory capacities of laboratory populations of the insect *Drosophila melanogaster* by measuring hemolymph osmotic concentration during desiccation and upon recovery from a bout of desiccation. Recovery treatments entailed allowing the flies access to distilled water, a saline solution or a saline+sucrose solution after a desiccation bout shown to reduce hemolymph volume by ~60%. Prior to desiccation, the hemolymph osmotic concentration was 353±11 mOsm. We found that *Drosophila* display strict osmotic regulation under prolonged conditions of dehydration. Osmotic regulation continued during recovery from desiccation, regardless of the fluid provided. This result is evidence that this insect does not require an external source of osmolytes or energy to regulate its hemolymph osmotic concentration or to restore hemolymph volume, which is reduced during desiccation. We also examined populations that have been selected for over 250 generations for enhanced desiccation resistance to identify physiological characters that have evolved in response to the selection regime. The selected lines displayed a reduced pre-desiccation hemolymph osmotic concentration (315±7 mOsm) and a marginally improved capacity for osmoregulation.

Key words: osmoregulation, hemolymph, desiccation, rehydration, *Drosophila melanogaster*.

Introduction

Terrestrial insects are particularly susceptible to dehydration due to their relatively small size and large surface-to-volume ratio in relation to other classes of terrestrial animals. Insect populations exposed to dehydrating conditions on a regular basis will either perish or become adapted to the given environment (Bradley et al., 1999; Watanabe et al., 2002). The danger of desiccation is amplified in insects that rely on flight for transportation to food sources (Markow and Castrezana, 2000), for mating (Eiko et al., 2002; Norio, 2002) or for migration (Coyne et al., 1987; Drake and Gatehouse, 1995). Water loss through the respiratory system is enhanced during flight (Lehmann, 2001). In addition to respiratory water loss, insects also lose water through the cuticle (Ramsay, 1935) and *via* excretion (Bradley, 1985). On the other hand, insects can gain water through food and drink, metabolic water (Showler and Moran, 2003) and (rarely) water vapor absorption (Ramsay, 1964; Grimstone et al., 1968; Machin 1980, 1983). Given the potential for desiccation in insects, they are remarkable at withstanding even the most arid environments.

Several osmotic strategies have been observed in insects to deal with the stress of desiccation; these include tolerance to osmotic variability (Naidu and Hattingh, 1986; Garrett and Bradley, 1994; Patrick and Bradley, 2000), osmotic regulation by sequestration (Hyatt and Marshall, 1977, 1985) and osmotic regulation by excretion (Bradley, 1985; Hadley, 1994). Although numerous studies have been conducted examining osmoregulation in insects, studies on small terrestrial insects are notably absent. An exception to this statement is found in investigations regarding water vapor absorption in small insects (Holmstrup et al., 2001).

We chose to study osmoregulation in a small insect that is of great importance in the fields of genetics, evolution and molecular biology, namely *Drosophila melanogaster*. The paucity of information regarding the patterns and mechanisms of osmotic regulation in this species is regrettable, given the numerous studies investigating the evolution and population genetics of enhanced desiccation resistance in both wild and laboratory-selected *Drosophila* populations (Dobzhansky, 1952; Gibbs et al., 1997, 2003; Bradley et al., 1999; Hoffmann and Harshman, 1999; Nghiem et al., 2000; Pfeiler and Markow, 2001; Marron et al., 2003).

In the current study, we have undertaken an analysis of patterns of osmoregulation in adult *Drosophila* during desiccation. Since some degree of desiccation is inevitable in these small insects as they fly about seeking mates, food sources and oviposition sites, the pattern of osmoregulation during water loss and subsequent rehydration are of considerable interest. We chose to study these phenomena in

five replicate populations of *Drosophila* that have been maintained in the laboratory for over 250 generations (C populations). We also examined five replicate populations that have undergone selection for enhanced desiccation resistance (D populations) to determine if osmotic regulation or patterns of rehydration have evolved during this selection process.

Previous studies involving the populations of flies used in this experiment have established that, relative to the C populations, the D populations have a reduced rate of water loss both prior to and during a bout of desiccation (Gibbs et al., 1997; Williams et al., 1998) and a greater body water content (Gibbs et al., 1997). The majority of this additional water is found extracellularly as hemolymph (Folk et al., 2001). It has also been demonstrated that hemolymph volume decreases substantially in the C and D populations during desiccation and that some ions are removed from this fluid compartment and excreted (Folk and Bradley, 2003). What is not known is whether the flies allow their hemolymph osmolality (number of solutes per kg of water) to increase substantially or whether they are strictly regulating their internal fluids. The absence of regulation could result in intolerable concentrations of osmolytes that lead to cellular and metabolic malfunctioning. In this study, we measure the hemolymph osmolality of flies of five D populations and five C populations before desiccation, during desiccation and upon recovery from desiccation to determine the osmoregulatory capabilities of the C and D populations under these circumstances. In addition, we examine the ability of the C and D populations to replenish hemolymph volume during recovery from desiccation.

Materials and methods

Fly stock

The populations of *Drosophila melanogaster* Meigen used in this experiment have been involved in a long-term, on-going selection study. They were derived from five large, outbred populations selected for postponed reproduction (O populations; Rose, 1984). From each of the five O populations, two additional lines were created, a D population and a C population. There exist, therefore, five paired D and C populations. The D populations undergo selection for enhanced desiccation resistance every generation, and the C populations serve as paired controls. Population sizes at each generation were >1000 flies to prevent inbreeding. All flies were maintained at 25°C with 24 h light. Standard Rose lab banana food was used for the rearing of all populations. This food consisted of 1.1 liter water, 16.7 g agar, 150 g peeled bananas, 18.3 ml light corn syrup, 18.3 ml dark corn syrup, 27.5 ml barley malt and 40 g active dry yeast dissolved in 95% ethanol (an anti-fungal solution). The yeast was inactivated during cooking by vigorous boiling. Flies were provided with a diet rich in yeast for a source of protein prior to egg collection. Eggs were collected from flies ~6–9 days post-eclosion, and larvae were grown at moderate densities of 60–80 per 40 ml vial containing approximately 10 ml of fly food. Adult flies were transferred to Plexiglas cages (24 cm×19 cm×13 cm) 14 days after egg collection. All flies on which physiological measurements were to be made were reared separately from the ongoing colony without selection for two generations to eliminate parental and grandparental phenotypic effects. Only females were used for experimental purposes.

Selection regime

The D populations were selected for enhanced desiccation resistance at every generation. Fourteen days after egg collection (at approximately 4 days post-eclosion), each C and D population was transferred from food vials to separate large Plexiglas cages, one for each population. At this point, selection was initiated. The D populations were placed in cages along with a cheesecloth bag of desiccant (Drierite; W. A. Hammond, Drierite Company, Ltd, Xenia, OH, USA) and no food or water. Cage entrances were sealed with plastic wrap to retard the entrance of water vapor from the ambient environment. The C populations were placed in identical cages but with a water source (a non-nutritive agar plate), no food and no desiccant. When each D population reached 80% mortality, selection was removed and food was presented to both the D population and its paired control population. Therefore, the difference in the treatment of the C and the D populations took place in the adult stage and consisted only of the presence or absence of water.

Measuring hemolymph osmolality

Hemolymph osmolality was measured in individual flies of all 10 populations (N=10). Hemolymph samples were collected by piercing the lateral thoracic segment of individual flies, under oil, with a pulled micropipette (micropipette puller; Narishig Scientific Instruments Lab, Setagaya-Ku, Tokyo, Japan). Through capillary action, hemolymph was drawn into the micropipette. Oil was collected in the micropipette before and after hemolymph collection to avoid evaporative water loss from the hemolymph sample. The samples were immediately expelled *via* mouth pipetting into oil wells of a calibrated nanoliter osmometer (Clifton Technical Physics, Hartford, NY, USA) under a dissecting microscope (500×), and osmolality (mOsm) was determined by melting point depression (Bradley and Phillips, 1975). Measured hemolymph samples ranged from volumes of ~0.05 to 1.4 nl. No melanization of the hemolymph was observed subsequent to collection.

Hemolymph osmolality during desiccation

Hemolymph osmolality was determined in 10 individual female flies in each population at various time intervals during a bout of desiccation stress. The D flies were desiccated for 8, 16, 24 and 48 h and the C flies for 8 and 16 h. Five flies were placed in a 40 ml glass vial containing approximately 5 g of indicator Drierite. Flies were allowed to occupy the lower three-quarters of the chamber and were isolated from the Drierite by a thin foam plug. Entrances to the desiccating chambers were sealed with Parafilm (American Can Company, Greenwich, CT, USA). After the allotted desiccation period, live flies were removed from desiccating chambers and directly

submerged in oil. Hemolymph samples were drawn and osmolality was measured as described above.

Recovery

We also examined hemolymph osmolality and hemolymph volumes following recovery from a bout of desiccation. After 8 h of desiccation in the C populations and 24 h desiccation in the D populations, live flies were removed from desiccating chambers and placed in recovery chambers. Recovery chambers were 40 ml vials containing a Kimwipe (Kimberly-Clark, Roswell, GA, USA) saturated with 1.5 ml of one of three recovery fluids: (1) distilled water, (2) a saline solution (25 mmol l^{-1} KCl, 135 mmol l^{-1} NaCl) or (3) a saline+sucrose solution (5% sucrose, 35 mmol l^{-1} KCl, 135 mmol l^{-1} NaCl). The flies were allowed to recover in these chambers for 24 h (five flies per vial). Hemolymph osmolalities were then obtained as described above and the hemolymph volumes were estimated gravimetrically using the blotting technique described by Folk et al. (2001).

Data analysis

Initial hemolymph osmotic concentrations and those of dehydrated flies were plotted against proportional dehydration of the hemolymph. Proportional dehydration of the hemolymph is defined by the expression V_i/V_e (Hadley, 1994), where V_i is the initial volume of the hemolymph (i.e. the hemolymph volume prior to desiccation) and V_e is the volume of hemolymph remaining after a given amount of time in the desiccating chamber. Volumes were obtained in a previous study examining rates of water loss and ion regulation in the C and D populations (Folk and Bradley, 2003). Using a gravimetric blotting technique, these authors determined the average extractable hemolymph volume in individual flies of each population before desiccation and after 8, 16, 24 and 48 h of desiccation (8 and 16 h in the C flies).

To determine the degree to which flies of each population osmoregulate, a theoretical regression line was constructed. This theoretical line was the osmolality for a given hemolymph volume if no ion regulation were to occur. The theoretical osmolality (osmolality_e) was defined by:

$$\text{Osmolality}_e = (\text{osmolality}_i \times V_i)/V_e ,$$

where V is the volume of hemolymph and subscripts i and e are initial (prior to desiccation) and experimental (after given increment of desiccation period), respectively. If the observed hemolymph osmolality and corresponding volume fall on the theoretical line, then the flies failed to osmoregulate. If they fall below the theoretical line, the flies osmoregulated to some extent.

To quantify the extent of osmoregulation, a modification of a method proposed by Riddle (1985) was used. The ratio of observed osmolality slope/theoretical osmolality slope served as an index of the extent of osmoregulation. If this ratio is equal to 1, there is no evidence of osmoregulation. The closer this value is to 0, the greater the extent of osmoregulation. This index allows comparison between the C and D populations because it ignores differences in initial hemolymph osmolality or volume (Hadley, 1994).

Statistics

We tested the difference in the means of hemolymph osmolality for each population (N=5) prior to desiccation between the C populations and the D populations using a paired Student's *t*-test to determine whether the populations were at the same hemolymph osmolality at the onset of desiccation. We determined whether the hemolymph osmotic concentration of the C and D populations increased as a result of desiccation by performing a linear regression test, which established if the observed slopes differed from zero. To evaluate osmoregulatory abilities of flies within a given selection treatment, a paired Student's *t*-test was performed comparing the five C_n (or D_n) slopes of the observed regression lines to the five slopes of the theoretical regression lines. To determine whether the C populations were osmoregulating differently from the D populations, we compared the ratios of the slopes of the observed regression line with the slopes of the theoretical regression lines. This ratio, calculated for each population, was used to evaluate the extent of osmoregulation. The ratio did not satisfy the assumptions of a parametric test; therefore, a one-tailed Wilcoxon signed rank test was used to determine whether the D populations exhibit a greater extent of osmoregulation than their paired control populations. We performed two analysis of variance (ANOVA) tests with Bonferroni/Dunn *post-hoc* tests on hemolymph volume and another two for hemolymph osmolality to determine differences between initial values, values after the prescribed desiccation and values after recovery on water, saline or saline+sucrose. One ANOVA detected differences within the C populations and the other within the D populations. Hemolymph volume data obtained from Folk and Bradley (2003) were used for the pre-desiccation volume and volume after desiccation.

Results

Hemolymph osmolality prior to desiccation

The hemolymph osmotic concentration varies considerably between individuals, even within a single population (Table 1).

Table 1. *Mean hemolymph osmolalities of* Drosophila melanogaster

Population	Osmolality (mOsm)	Population	Osmolality (mOsm)
C_1	338±11	D_1	317±8
C_2	352±15	D_2	314±6
C_3	387±9	D_3	324±12
C_4	325±3	D_4	289±6
C_5	365±15	D_5	331±7
C mean	353±11	D mean	315±7

C populations ($C_{1–5}$) are control flies and D populations ($D_{1–5}$) are those selected for enhanced desiccation resistance. Values are given in mOsm ± S.E.M.

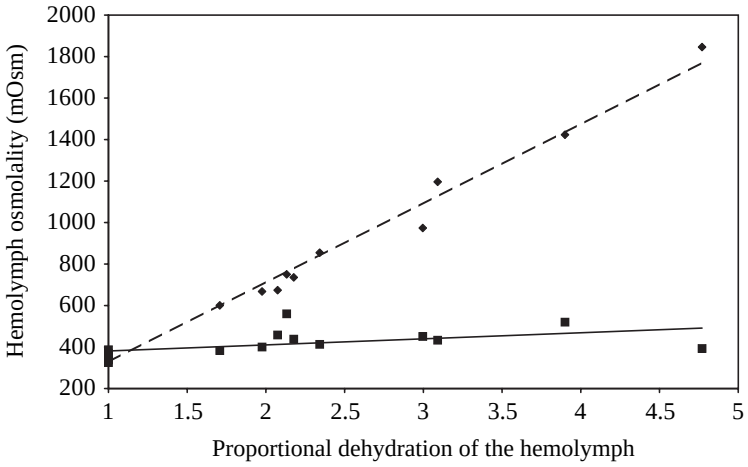

Fig. 1. Observed hemolymph osmolality (■) and theoretical hemolymph osmolality (◆) in C populations as a function of proportional dehydration of the hemolymph. Proportional dehydration of the hemolymph represents a decline of hemolymph as numbers increase. For example, at a value of 2, the flies lost 50% of their hemolymph, at 4 they lost 75%, etc. The solid and broken lines are the means of the slopes for the five observed and theoretical slopes, respectively.

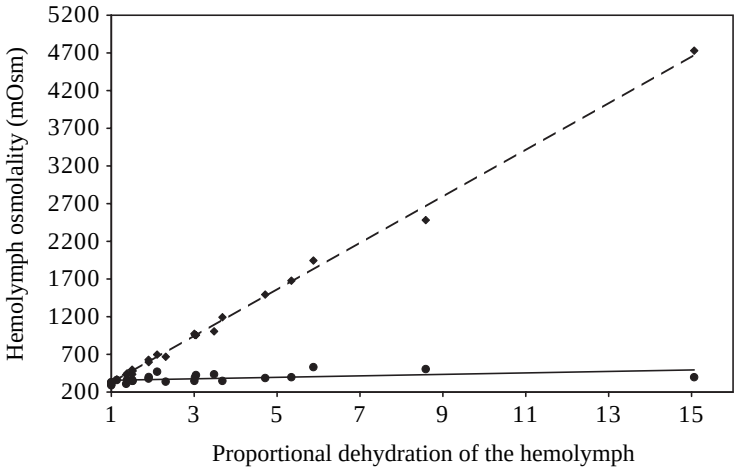

Fig. 2. Observed hemolymph osmolality (●) and theoretical hemolymph osmolality (◆) in D populations as a function of proportional dehydration of the hemolymph. The solid and broken lines are the means of the slopes for the five observed and five theoretical slopes, respectively.

Table 2. *The ratio of observed to theoretical slopes for five C populations ($C_{1–5}$) and five D populations ($D_{1–5}$)*

Population	Obs./theor.	Population	Obs./theor.
C_1	0.231	D_1	0.066
C_2	0.483	D_2	0.006
C_3	0.005	D_3	0.003
C_4	0.200	D_4	0.073
C_5	0.148	D_5	0.115

The ratio of observed to theoretical slopes serves as an index of the extent of osmoregulation. The closer this value is to 0, the greater the osmoregulatory ability in a given population.

Prior to desiccation, when all flies were presumably in a fully hydrated state, the D populations had a lower mean hemolymph osmolality (315±7 mOsm) than the C populations (353±11 mOsm) (P<0.05).

Hemolymph osmolality during desiccation

Throughout desiccation, the hemolymph osmolality in flies from both selection treatments increased gradually as hemolymph volume decreased, that is to say as proportional dehydration of the hemolymph increased (Figs 1, 2). The linear relationship for each selection treatment is the regression line of mean values of each population ($C_{1–5}$ or $D_{1–5}$). The positive slopes of these mean lines were found to be statistically significantly different from zero (P<0.05). The point that represents the largest value on the *x*-axis is a measured value during a non-lethal prescribed bout of desiccation and does not represent the hemolymph osmotic concentration at death.

The observed hemolymph osmotic concentration of the C and D populations during dehydration (Figs 1, 2) is plotted adjacent to slopes representing the theoretical osmolalities that would arise if no osmotic regulation occurred. The observed increase in hemolymph during dehydration is substantially lower (P<0.001) than the theoretical increase for both C and D populations. This discrepancy between observed and theoretical slopes is a clear indication that all populations were osmoregulating.

The ratio generated by dividing the observed change in hemolymph osmotic concentration by the theoretical concentrations can be used as a measure of the extent of osmoregulation that occurred during dehydration (Table 2). There is some variation in this ratio among populations within selection treatments; however, when we compare the paired populations (i.e. compare C_n with D_n) each D population has a lower ratio than its paired control population. Therefore, at least with regard to this parameter, selection for enhanced desiccation resistance has led to a greater capacity for osmoregulation (P<0.05).

Recovery (hemolymph volume)

Simple visual observations of the flies under a dissecting scope following 24 h of recovery suggested that the responses during recovery were not entirely uniform. A few flies appeared to have drunk nothing at all and were still in a rather dehydrated state while other individuals had very little hemolymph despite a largely distended gut. Nonetheless, most flies were able to increase their hemolymph volume during recovery. Pre- and post-desiccation values are included in Figs 3–6 to show what is happening to hemolymph volume and hemolymph osmolality during desiccation.

Following a drop in hemolymph volume during an 8 h desiccation bout, the C populations increased hemolymph

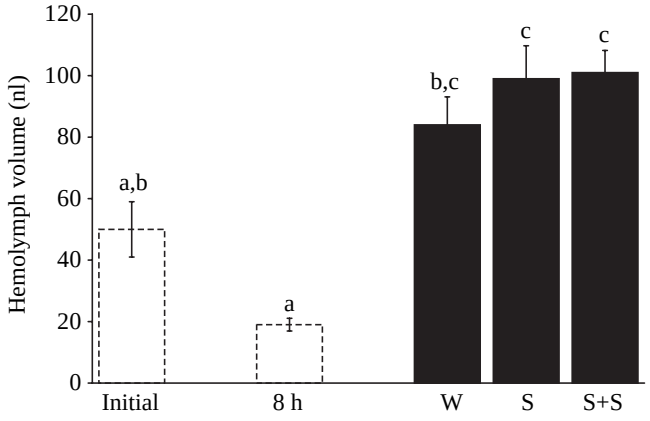

Fig. 3. Mean hemolymph volume of the C populations prior to desiccation, after 8 h of desiccation and after recovery from desiccation on distilled water (W), saline (S) or saline+sucrose (S+S). Broken-lined bars represent data published in a previous study (Folk and Bradley, 2003). Error bars represent S.E.M.

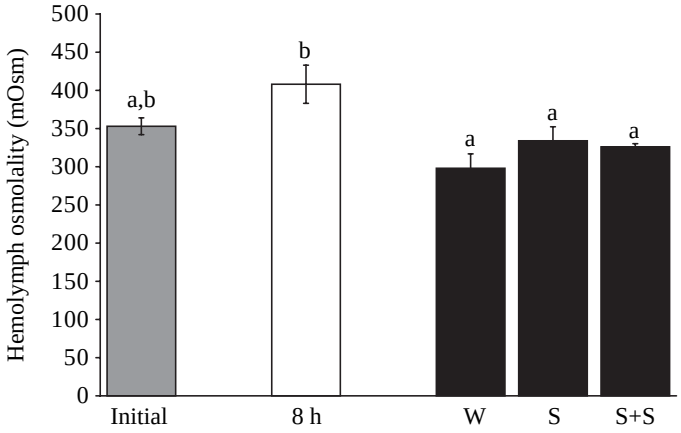

Fig. 5. Mean hemolymph osmolality of the C populations prior to desiccation, after 8 h desiccation and after recovery from desiccation on distilled water (W), saline (S) or saline+sucrose (S+S). Error bars represent S.E.M.

volume after rehydration on the three recovery solutions (Fig. 3). Hemolymph volumes averaged 84±9 nl on water, 99±11 nl on saline and 101±7 nl on saline+sucrose. The type of recovery fluid had no significant effect on the mean hemolymph volume following the 24 h rehydration period (P<0.05). Flies that recovered on saline or saline+sucrose had a significantly higher hemolymph volume than when they were in their pre-desiccated state (P<0.05).

The D populations showed a different response following the recovery treatment. Hemolymph volumes were 185±44 nl, 177±41 nl and 273±47 nl for water, saline and saline+sucrose, respectively (Fig. 4). There were no statistically significant differences in hemolymph volume between the flies hydrated on water, saline or saline+sucrose (P>0.05). These volumes also did not differ from either pre- or post-desiccation hemolymph volumes (P>0.05), although when comparing hemolymph volumes of pre- and post-desiccation, there was a statistically significant difference (P<0.05).

Recovery (hemolymph osmolality)

Hemolymph osmolality following recovery was not dependent on the recovery treatment. The hemolymph osmolalities in flies of the C populations were 298±19 mOsm, 334±18 mOsm and 329±4 mOsm after recovery on water, saline and saline+sucrose, respectively (Fig. 5). These values were not statistically different from pre-desiccated hemolymph osmolalities (P>0.05).

Following recovery, the osmolality of the hemolymph in the D populations was measured as 296±22 mOsm, 315±14 mOsm and 305±24 mOsm after rehydration on water, saline or saline+sucrose, respectively (Fig. 6). These values are not

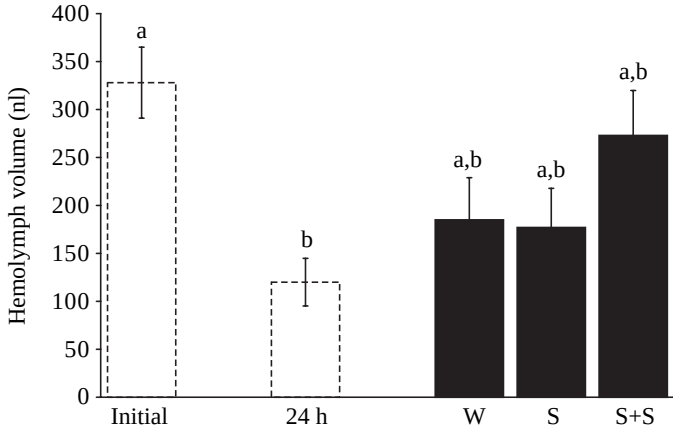

Fig. 4. Mean hemolymph volume of the D populations prior to desiccation, after 24 h of desiccation and after recovery from desiccation on distilled water (W), saline (S) or saline+sucrose (S+S). Broken-lined bars represent data published in a previous study (Folk and Bradley, 2003). Error bars represent S.E.M.

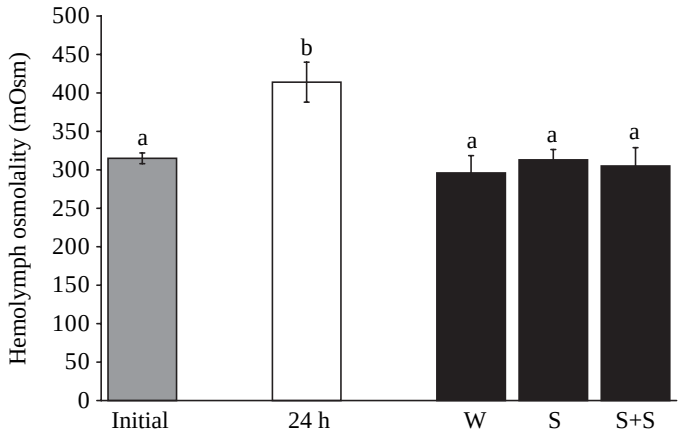

Fig. 6. Mean hemolymph osmolality of the D populations prior to desiccation, after 24 h desiccation and after recovery from desiccation on distilled water (W), saline (S) or saline+sucrose (S+S). Error bars represent S.E.M.

statistically distinguishable from the pre-desiccation values (P>0.05).

Discussion

In the present study, we demonstrate that *Drosophila melanogaster* are very strong osmoregulators. They can maintain their hemolymph osmotic concentration in a narrow range even when subjected to a bout of desiccation that reduces their hemolymph volume to less than 25% of its initial value.

Hemolymph osmolality prior to desiccation

Insects exhibit a much larger variation in the concentration of the extracellular fluid, both individually and in response to environmental variation, than do vertebrates (Buck, 1953; Jeuniaux, 1971; Bosquet, 1977). It has been argued that the selectively permeable sheath that protects the insect central nervous system permits a greater tolerance of both osmotic and ionic variability in the hemolymph (Treherne and Pichon, 1973; Jones, 1977; Ashhurst, 1985).

In the present study, the control populations have a mean osmotic concentration prior to desiccation of 353 mOsm. This value is considerably higher than the single other measurement of 251±9 mosmol l^{-1} from individual *D. melanogaster* (Singleton and Woodruff, 1994). Other values from adult dipterans include 400 mOsm for blowflies (Phillips, 1969) and 354 mOsm for the mosquito *Aedes aegypti* (Williams et al., 1983).

Interestingly, the osmotic concentration of the hemolymph of the control populations is higher than that of the D flies under the same conditions. The hemolymph osmolality in the O populations, the ancestors of both the C and D populations, is unknown. It is therefore unclear whether the C populations have experienced an increase in hemolymph concentration relative to their ancestor following reproductive isolation from the D populations or whether the D populations have evolved a lower hemolymph osmolality in response to selection for enhanced desiccation resistance.

Hemolymph osmolality during desiccation

As water is lost during desiccation, the hemolymph osmolality increases in all populations tested. This increase in hemolymph osmolality is still well below the theoretical osmolality values, which are expected in the absence of osmoregulation, demonstrating a strong capacity for osmoregulation in *D. melanogaster*. Other insects have a similar pattern of osmotic regulation, including, for example, the orthopteran *Carausius morosus* (Nicolson et al., 1974) and the coleopterans *Stips stali* (Naidu and Hattingh, 1986) and *Onymacris plana* (Nicolson, 1980). Phillips (1969) found that the dipteran *Calliphora erythrocephala* increased its hemolymph osmotic concentration during dehydration by 25% after two days of water deprivation, concomitantly increasing its urine concentration 15-fold. Wall (1970) reported a similar trend in the cockroach *Periplaneta americana* during dehydration.

In examining populations that have undergone 250 generations of selection for enhanced desiccation resistance, we found that their capacities for osmotic regulation have been marginally improved in response to this selection regime. This difference in osmotic regulatory capacity is not the physiological trait that is considered the most important for enhanced desiccation resistance, however. More important evolved physiological differences between the C and D populations are a reduced rate of water loss (Gibbs et al., 1997; Williams et al., 1998) and an increase in water content (Gibbs et al., 1997; Folk and Bradley, 2003).

The mechanistic details of osmoregulation in *Drosophila* are yet to be worked out. Folk and Bradley (2003) found that as the flies lose water, both C and D populations excrete sodium, potassium and chloride. The quality of these excreted solutes, as reported in their study, does not account for all of the osmolytes removed from the hemolymph to maintain hemolymph osmolalities as were measured during desiccation. Further studies are required to determine what additional solutes are removed and to where they are transferred. Of the organs engaged in osmoregulation in *Drosophila*, only the Malpighian tubules have been examined in mechanistic detail (Maddrell and O'Donnell, 1992; Dow et al., 1994; O'Donnell and Maddrell, 1995; O'Donnell et al., 1996; Linton and O'Donnell, 1999; O'Donnell and Spring, 2000; Rheault and O'Donnell, 2001). It would be valuable to determine the relative roles of the osmoregulatory organs in *Drosophila*, particularly the rectum, which Phillips (1969) demonstrated is the site of urine concentration in adult dipterans. The specific osmolytes that are important in the various fluid compartments (intracellular fluid, hemolymph, urine) are also unknown.

Recovery

The full selection regime of these fly populations involves not only resistance to desiccation but also the capacity for recovery. We were therefore interested in the capacity of the flies to resist desiccation and their capacity for osmotic recovery. We therefore examined recovery on various fluids.

Recovery in the control populations

Recovery on distilled water. Following an 8 h bout of desiccation, the C populations lost almost 60% of their hemolymph volume yet did not increase their hemolymph osmotic concentration significantly. During a 24 h recovery period on distilled water, the C populations were able to increase hemolymph volume to pre-desiccation values. Hemolymph osmolality after rehydration on this fluid was also returned to pre-desiccation values. It follows that the flies must have obtained osmolytes from the body compartment in order to replace hemolymph volume at the appropriate osmotic concentration. Folk and Bradley (2003) examined the changes in ion content of the C populations under identical conditions of desiccation. They found that the flies excrete some sodium during desiccation but retain approximately 85% of whole body sodium content, 83% of potassium and 60% of chloride. A detailed study of the location of these ions following desiccation and the degree to which they are mobilized upon

rehydration has yet to be carried out. Diptera normally have a fairly sodium-rich hemolymph compared with other insects (Sutcliffe, 1963). It might be expected that ion mobilization, particularly that of sodium, would be a major aspect of hemolymph reconstitution during recovery in *Drosophila*. The fat body in *Periplaneta americana* acts as a sink for sodium and potassium ions from the hemolymph during dehydration (Hyatt and Marshall, 1977). Upon rehydration on deionized water, these ions are removed from the fat body and replaced in the hemolymph (Hyatt and Marshall, 1985). In *Drosophila*, however, a role for other osmolytes, including amino acids, organic acids and peptides, in the rehydration process cannot at this time be ruled out. Dipterans and other species of insects have been shown to break down proteins into osmotically active amino acids in response to perturbations in hemolymph osmotic concentration (Collett, 1976a,b; Woodring and Blakeney, 1980). Further studies will be required to determine hemolymph composition before and after recovery in *D. melanogaster* as well as the source of hemolymph osmolytes.

Recovery on a saline or a saline+sucrose solution. In the C flies, rehydration on a saline solution isosmotic to the hemolymph resulted in an increase in hemolymph volume following a decline during the 8 h desiccation period. The restored hemolymph volume actually surpassed pre-desiccation volume and was statistically indistinguishable from that of flies rehydrated on distilled water. As in the flies that recovered on distilled water, the hemolymph osmolality was returned to the original pre-desiccated values after rehydration on the saline solution. It is clear that *Drosophila* can fully rehydrate and maintain osmotic concentration using only saline without a supplemental energy source. Hemolymph volume subsequent to a bout of dehydration and recovery has been shown to increase beyond levels of pre-stressed values in the orthopteran *Chortoicetes terminifera* (Djajakusumah and Miles, 1966).

When the control populations were rehydrated on a saline+sucrose solution, hemolymph volume was higher than pre-desiccation values. Clearly, *Drosophila* can restore water lost from the hemolymph by the consumption of fluids of variable composition. Hemolymph osmolality was also restored after recovery on the saline+sucrose solution.

Recovery in the populations selected for enhanced desiccation resistance

Following a 24 h bout of desiccation, the D populations had lost on average 66% of their hemolymph volume and increased their hemolymph osmolality by ~100 mOsm. When provided with any of the three recovery fluids, the D flies were able to return to a hemolymph volume statistically indistinguishable from the initial hemolymph volume. The final hemolymph volumes achieved were intermediate to the pre-desiccation and post-desiccation levels.

Although the flies did not fully recover lost hemolymph, they did manage to regain their original hemolymph osmolality after recovery on distilled water. Like the C populations, the D populations can replace substantial volumes of hemolymph at the appropriate osmolality while imbibing only distilled water, as well as by drinking isosmotic saline or saline+sucrose solutions. Clearly, their capacities for osmotic regulation of the hemolymph are substantial and the flies are capable of dealing with a variety of environmental conditions.

Osmoregulation in Drosophila

Drosophila face intermittent desiccation in their normal environment. Competition for mates and searching for food and oviposition sites inevitably lead the insects away from dietary sources of water. Their exceptionally small surface area to volume ratio exacerbates water loss, with rates of water loss of 20–30 $\mu l\ g^{-1}\ h^{-1}$ being reported for control populations in the laboratory (Gibbs et al., 1997; Williams et al., 1998) under non-flying conditions. Lehmann (2001) measured the rate of water loss in *D. melanogaster* during flight and found that, as metabolic demand increased, spiracles must be open more frequently and the rate of water loss increases accordingly (60–140 $\mu l\ g^{-1}\ h^{-1}$).

The present study was designed to determine the degree of osmotic regulation that occurs in *Drosophila* during the periods of water loss, as well as during the rehydration events that must occur when the insects again encounter a source of water. We found that *Drosophila* display surprisingly strict osmotic regulation under conditions of dehydration, being able to regulate osmotic concentration when over two-thirds of the hemolymph volume has been lost. Similarly, recovery of hemolymph volume can be achieved with a variety of recovery fluids, including distilled water. Neither external sources of sodium nor energy in the form of sugar are required. This implies that *Drosophila* could rehydrate in the wild using a variety of sources of water such as rainwater or dew, nectar and the fruit juices associated with their oviposition sites.

Hoffmann (1990) reported an acclimation response in *D. melanogaster* when subjected to a non-lethal dry environment. Subsequent to this temporary bout of desiccation, the flies became more resistant to a further desiccation stress. We now know that this species osmoregulates; therefore, it would be interesting to determine the pattern of osmoregulation during the second bout of desiccation. Potentially, this increase in desiccation resistance could result from a higher tolerance to the elevation of hemolymph osmotic concentration (due perhaps to the presence of heat shock proteins; Lindquist, 1986), a decrease in cuticular water loss, an increase in body water during the recovery phase or another physiological mechanism. When Hoffmann (1991) carried out these experiments on various field-collected *Drosophila* species of differing habitats, he found that the acclimation response was well established in *Drosophila* with the exception of the species *D. birchii*, which is found exclusively in tropical rainforests. It would be interesting to measure physiological mechanisms behind this result and to determine other osmoregulatory differences in this tropical species.

In the course of examining osmotic regulation in *Drosophila*, very interesting new questions have arisen. Of the populations of *Drosophila* examined in this study (both control

and selected), all are able to replace hemolymph lost during desiccation using only distilled water while osmoregulating, suggesting that the flies can restore this hemolymph using internally stored or produced osmolytes. Clearly, this result deserves further study. The processes by which these two osmotic strategies, osmotic regulation and volume homeostasis, are maintained in *Drosophila* are of considerable interest, given the extensive physiological, molecular, genetic and now genomic techniques available for their investigation.

This work was supported by National Science Foundation grant IBN 0079501 awarded to T.J.B.

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