The evolution of recovery from desiccation stress in laboratory-selected populations of *Drosophila melanogaster*

Donna G. Folk* and Timothy J. Bradley

Department of Ecology and Evolutionary Biology, University of California, Irvine, Irvine, CA 92697-2525, USA

*Author for correspondence (e-mail: dfolk@adelphia.net)

Summary

We examined the capacity for physiological recovery from the effects of desiccation in five replicate populations of *Drosophila melanogaster* that have been selected for enhanced desiccation resistance (D populations) and in five replicate control populations (C populations). The capacity to recover was signified by the ability to restore three somatic components, namely whole-body water, dry mass and sodium content, all of which are reduced during desiccation. Throughout a period of recovery following a bout of desiccation, the flies were offered one of three fluids: distilled water, saline solution, or saline+sucrose solution. Our findings indicate that, when allowed to recover on saline+sucrose solution, D populations have the capacity to restore water at a greater rate than C populations and are able to fully restore dry mass and sodium content to the levels observed in non-desiccated, hydrated D flies. When provided with this same solution during recovery, C flies are unable to restore dry mass and are faced with an elevated sodium load. Desiccation resistance of the flies subsequent to recovery was also examined. We provide evidence that the greatest desiccation resistance in the D populations is associated with the restoration of all three somatic components, suggesting that not only water content, but also dry mass and sodium, may contribute to the enhanced desiccation resistance that has evolved in these populations.

Key words: *Drosophila melanogaster*, desiccation resistance, evolution, water restoration, sodium content, dry mass.

Introduction

"*The greatest physiological advantage of terrestrial life is the easy access to oxygen; the greatest physiological threat to life on land is the danger of dehydration*" (Schmidt-Nielsen, 1997).

As a consequence of small body size and high mass specific metabolic rate, insects face unique challenges to water conservation, particularly during flight (for reviews, see Edney, 1977; Hadley, 1994). Yet insects are among the most speciose and widely distributed terrestrial animals (Gillott, 1995). The ability of insects to live successfully on land can be attributed to various behavioral, structural and physiological features (e.g. Wigglesworth, 1972; Edney, 1977; Hadley, 1994; Chapman, 1998). We have investigated the evolution of physiological traits related to water conservation in very small insects, specifically fruit flies, by placing populations of *Drosophila melanogaster* under laboratory selection for enhanced desiccation resistance (D populations). Control (C) populations that have not been selected for improved desiccation resistance are maintained concurrently with the D populations. The evolved responses of the D populations to selection have led to an increased ability to survive when desiccated (Rose et al., 1992; Graves et al., 1992; Gibbs et al., 1997; Chippindale et al., 1998; Djawdan et al., 1998; Williams

et al., 1998; Folk et al., 2001; Folk and Bradley, 2003). Phenotypic traits associated with desiccation resistance in the D populations include a large hemolymph pool (>300 nl, ~sixfold increase in hemolymph volume relative to the C populations), which buffers the tissues against water loss for an extended period during desiccation (Folk et al., 2001; Folk and Bradley, 2003); an elevated carbohydrate content, comprising ~30% of total dry mass (Graves et al., 1992; Djawdan et al., 1998; Folk et al., 2001); and a reduced rate of water loss during periods of extreme water stress (Gibbs et al., 1997; Williams and Bradley, 1998; Folk and Bradley, 2003). In contrast, the C populations have a small hemolymph pool (~50 nl), which appears to afford the tissues only minimal protection from water loss during desiccation; a lower carbohydrate content, comprising ~15% of total dry mass; and a relatively high water loss rate.

Drosophilids lose significant quantities of water and dry mass during desiccation (Arlian and Eckstrand, 1975; Graves et al., 1992; Hoffmann and Parsons, 1993; Gibbs et al., 1997; Lehmann et al., 2000; Marron et al., 2003). In addition, inorganic ions, such as Na^+, Cl^- and K^+, are permanently excreted during desiccation as a consequence of osmoregulatory strategies (Folk and Bradley, 2003). In

previous studies, we examined the loss of water and Na^+ from the hemolymph and the tissues in the C and D flies during desiccation. In 24 h, the D flies lost ~60% and ~70% of hemolymph volume and Na^+ content, respectively, while tissue water and Na^+ content were not significantly reduced. Comparable losses from the hemolymph of the C flies during desiccation occurred within only 8 h: ~60% of volume and ~80% of Na^+ content were lost. Furthermore, the C flies lost significant water and sodium content from the tissues within 8 h.

Following desiccation, many adult insects are capable of restoring water content by drinking (Djajakusumah and Miles, 1966; Wall, 1970; Broza et al., 1976; Loveridge, 1975; Hamilton and Seely, 1976; Tucker, 1977; Nicolson, 1980; Naidu and Hattingh, 1988; Naidu, 2001a,b). Some insects will drink saline and/or sugar (e.g. sucrose) solutions to restore water and, presumably, ionic and energy content as well (Evans, 1961; Dethier and Evans, 1961; Browne et al., 1976). Although the ability to restore water content by drinking has been ascertained in various insects, relatively little is known about the ability to restore depleted somatic components, such as inorganic ions and metabolic fuel stores.

We present here the first study of the capacity of populations of *Drosophila melanogaster* selected for enhanced desiccation resistance to recover whole-body water, dry mass and sodium content following a sublethal bout of desiccation. We ascertain and compare the capacities of the D and C flies to restore these somatic components when allowed to recover on one of three fluids: water, saline solution or saline+sucrose solution. We propose that the future physiological health, and thus future stress resistance, of desiccated flies may be contingent upon their ability to restore somatic resources that are expended during periods of desiccation stress.

Materials and methods
Fly populations

Experimental flies (*Drosophila melanogaster*) were from five outbred populations, designated as D_1–D_5, that have undergone laboratory selection for enhanced desiccation resistance since 1988 (Rose et al., 1990, 1992). Each D population is paired with one control population (C_1–C_5), and each Cn/Dn pair 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 the C_1 and the D_1 populations; O_2 is the common ancestor of C_2 and D_2, etc.

Details of the fly 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 all C and D populations for the propagation of the subsequent generation. Following egg collection, the flies were allowed to develop and mature for 14 days (i.e. approximately 4 days into adulthood), at which time the D populations were subjected to selection for enhanced desiccation resistance until 80% mortality was reached. During selection, the D populations were deprived of food and water, while the C populations were deprived only of food. When selection was terminated, all surviving flies were allowed to recover on moist food supplemented with yeast paste for 3 days. Eggs were then collected for rearing of the next generation. Selection for enhanced desiccation has been imposed every generation for more than 250 generations.

Prior to all experiments, subsets of flies from the five D populations were maintained for two generations without undergoing selection. Subsets of flies from the five C populations were supplied a normal diet throughout development and maturation during the same two-generation period. By withholding the flies from the pressures of selection prior to experiments, we eliminated grandparental and parental phenotypic effects that derived from the selection regime. To eliminate the effects of gender, only females were used in the experimental assays. All experiments were performed on mated females that were approximately 4 days old.

Desiccation protocol

Experimental flies from the C and D populations were subjected to an initial, sublethal bout of desiccation. Flies were briefly anesthetized with CO_2 and then placed into 30 ml glass vials. A foam stopper was placed ~3 cm down into the vial and ~4.5 g of Drierite™, a calcium sulfate desiccant (W. A. Hammond Drierite Company, Ltd., Xenia, OH, USA), was placed on top of the foam stopper. The open end of the vial was then sealed with Parafilm™ (Pechiney Plastic Packaging, Chicago, IL, USA). During the initial bout of desiccation, 200 flies (40 vials, 5 flies per vial) from each C and D population were desiccated for 8 h (C flies) or 24 h (D flies). Previous studies have shown that water content, dry mass and sodium levels are significantly reduced during 8 h and 24 h of desiccation in the C and D flies, respectively (Folk et al., 2003).

Following this initial bout of desiccation, flies from each population were divided into three groups, each comprising 65 flies (13 vials, 5 flies per vial). Each group was allowed access for 24 h to only one of three fluids: doubly distilled water, saline solution, or sucrose+saline solution (see below for details of the fluid treatments). Immediately following recovery on the fluids, the flies were desiccated again as described above. During this second desiccation period, flies were desiccated to death. The capacity to resist desiccation was estimated as the time (h) that the flies were able to survive. In summary, the differences between the two desiccation periods were: (1) during the initial bout of desiccation, the C and D flies were desiccated for 8 h and 24 h, respectively; and (2) during the latter bout of desiccation, the flies were desiccated until death in order to estimate recovery of desiccation resistance.

Fluid treatments

Immediately following the initial bout of desiccation, C and D flies were provided one of three fluids: doubly distilled water (ddH$_2$O), isotonic saline solution (20 mmol l^{-1} KCl + 135 mmol l^{-1} NaCl), or saline+sucrose solution [the isotonic saline solution + 5% (146 mmol l^{-1}) sucrose]. (Refer to the previous paragraph for details on the experimental design.)

During the fluid treatment period, flies were held in 30 ml glass vials for 24 h. Prior to placing the flies into the vials, a single Kimwipe™ (laboratory-grade tissue paper, Kimberly-Clark Corporation, Denver, CO, USA) was evenly packed into the bottom of each vial, and either 1.5 ml of ddH₂O, saline solution, or saline+sucrose solution was added. The Kimwipe absorbed the fluid and provided a moist substrate from which it could be extracted and consumed by the flies without danger of drowning.

Gravimetric estimation of wet mass, water content and dry mass

Mature females from each C and D population were anesthetized with CO_2 and immediately weighed using a Cahn 29 automatic electrobalance (Cerritos, CA, USA). The flies were then dried overnight at 60–65°C and reweighed to obtain dry mass. Water content of whole flies was estimated by subtracting the dry mass from the wet mass. Wet mass, dry mass and water content of 10 flies from each C and D population were measured prior to and after the initial desiccation bout, and following the fluid treatments.

Sodium measurements

Ten samples were prepared from each C and D population prior to desiccation and following each of the fluid treatments. Each sample comprised two flies that had been liquefied overnight in 100 μl of concentrated HNO_3 (containing 0.02 p.p.m. sodium) at room temperature (21–23°C). Following solubilization of the flies, 2.9 ml of doubly distilled water was added to each sample. The Na^+ concentration of each sample was determined using atomic absorption spectrophotometry (AA-125 series, Varian Analytical Instruments, Springvale, Australia). The mean whole-body Na^+ content (nmol fly^{-1}) was then calculated from the Na^+ concentration of each sample.

Statistical analyses

We investigate the ability of desiccated C and D flies to restore water, dry mass and Na^+ content to their respective pre-desiccation values when offered one of three different fluids during a period of recovery. We also examine the desiccation resistance of C and D flies following recovery on the different fluids. Previous work has shown that non-desiccated, hydrated D flies have a greater water and Na^+ content than the C flies (Gibbs et al., 1997; Folk and Bradley, 2003). In addition, during desiccation the rates of water loss and Na^+ excretion have diverged significantly between the C and D flies. Within only 8 h of desiccation, the C flies experience significant losses of both water and Na^+ from the hemolymph and the tissues (Folk and Bradley, 2003). The D flies must be desiccated for a longer period of time for comparable losses to occur. For these reasons, we do not compare the recovery of water, Na^+, dry mass and desiccation resistance between the C and D groups in the ANOVA (recovery of water was analyzed using *t*-test, see below); but rather, we examine recovery of these components within the C or D group. Data were analyzed using

Model I ANOVA, in which the C or D populations and the fluid treatments were treated as fixed effects. Bonferroni *post hoc* pairwise comparisons of means within the C or D group were used to determine if: (1) water, Na^+ and dry mass were significantly reduced following the initial bout of desiccation; (2) the somatic components were restored, depending upon the type of fluid provided during recovery; and (3) if desiccation resistant following recovery was affected by the fluid provided during recovery. Student's *t*-test was used to compare restoration of whole-body water in the C and D flies that were provided saline+sucrose. We tested for differences in (1) rate of volume restoration, (2) total volume restored, and (3) proportion of total lost volume that was restored. An arcsine transformation was applied to the proportions prior to statistical analysis.

Results

Restoration of whole-body water content

During 8 h of desiccation, the control flies lost 0.246 μl of whole-body water content, which is a 23% reduction ($P<0.0001$, Fig. 1A). Following 24 h of recovery, during which groups of flies were provided water, saline solution or saline+sucrose solution, the body water content in all three
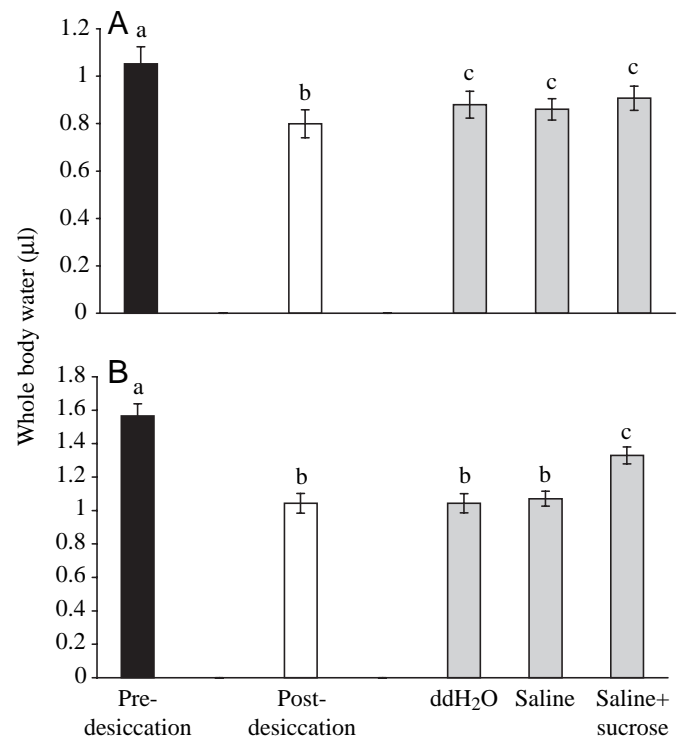
Fig. 1. Whole-body water of (A) control flies prior to and following 8 h of desiccation, and following recovery on distilled H₂O, saline solution or saline+sucrose solution, and (B) desiccation-resistant flies prior to and following 24 h of desiccation, and following recovery on distilled H₂O, saline solution or saline+sucrose solution. Values are means ± S.E.M. of five populations each. Non-matching letters above the columns denote statistically significant differences among treatments.

groups was only partially restored. The level of water restoration in the C flies was the same, regardless of the type of fluid provided: a mean of 0.076 μl, or ~30% of the lost volume, was restored.

During 24 h of desiccation, the desiccation-resistant flies lost 0.523 μl of whole-body water content, which is a 33% reduction ($P<0.0001$, Fig. 1B). Those D flies that had access to water or saline solution during recovery did not increase body water content above the post-desiccation level. A net gain of water was observed only in that group that recovered on saline+sucrose solution. This group did not fully restore body water to the level of the non-desiccated, hydrated flies: ~55% of the total water lost during desiccation (i.e. 0.287 μl) was recovered. The D flies that recovered on saline+sucrose restored water at a significantly higher rate ($0.012 \, \mu l \, h^{-1}$) during recovery than the C flies ($0.003 \, \mu l \, h^{-1}$, $P=0.04$). The proportion of the total lost volume that was restored did not appear to differ between the two groups ($P=0.21$).

Restoration of dry mass

The C flies experienced a reduction (7.5%) in dry mass during 8 h of desiccation ($P<0.0001$, Fig. 2A). The mean rate of dry mass loss was $\sim5 \, \mu g \, h^{-1}$. Dry mass continued to be lost at a mean rate of $\sim3 \, \mu g \, h^{-1}$ during recovery, when flies were

provided either water or the saline solution. When flies were offered the saline+sucrose solution, dry mass was maintained at the post-desiccation level.

The D flies lost 15% of their dry mass at a mean rate of $\sim4 \, \mu g \, h^{-1}$ during 24 h of desiccation ($P<0.0001$, Fig. 2B). Dry mass continued to be lost at the same rate ($\sim4 \, \mu g \, h^{-1}$) during recovery when flies were provided either water or saline solution. In contrast to the C flies, dry mass in the D flies was fully restored to levels observed in non-desiccated, hydrated flies when the saline+sucrose solution was supplied during recovery.

Restoration of whole-body sodium

The mean whole-body Na^+ content in the non-desiccated, hydrated C flies was 43 nmol fly^{-1} (Fig. 3A). A previous study indicated that whole-body Na^+ in the C flies is reduced by ~15% during 8 h of desiccation (Folk and Bradley, 2003); therefore, we estimated that the Na^+ content dropped to ~36 nmol fly^{-1} during the 8 h desiccation period in this study. The Na^+ content following recovery on water was 37 nmol fly^{-1}, which was significantly lower than that in non-desiccated, hydrated flies ($P<0.0001$, Fig. 3A). These data
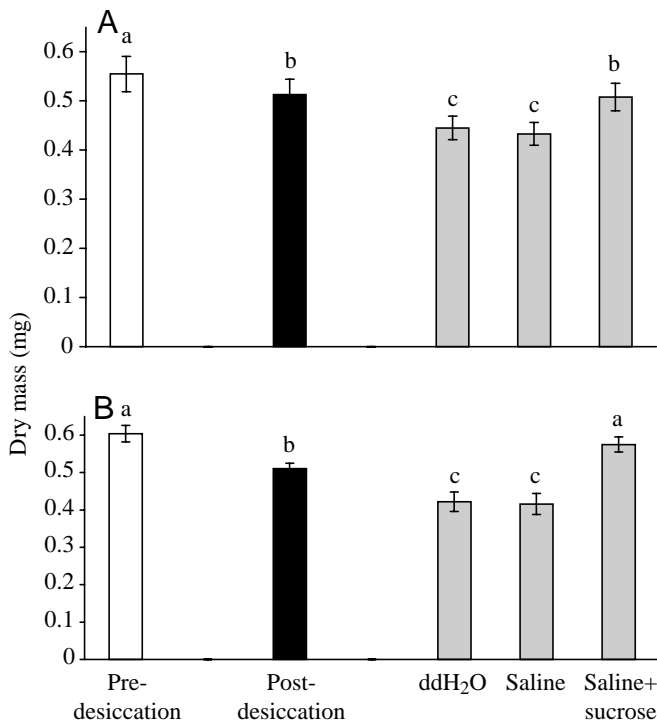

Fig. 2. Dry mass of (A) control flies prior to and following 8 h of desiccation, and following recovery on distilled H_2O, saline solution or saline+sucrose solution, and (B) desiccation-resistant flies prior to and following 24 h of desiccation, and following recovery on distilled H_2O, saline solution or saline+sucrose solution. Values are means ± S.E.M. of five populations each. Non-matching letters above the columns denote statistically significant differences among treatments.
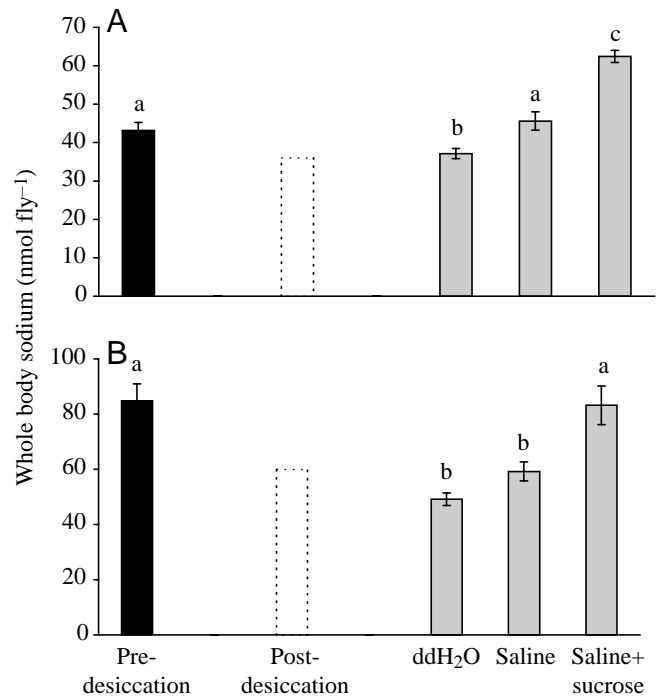

Fig. 3. Whole-body sodium content of (A) control flies prior to and following 8 h of desiccation, and following recovery on distilled H_2O, saline solution or saline+sucrose solution, and (B) desiccation-resistant flies prior to and following 24 h of desiccation, and following recovery on doubly distilled H_2O, saline solution or saline+sucrose solution. [The mean value of the Na^+ content following desiccation was estimated in a previous study (Folk and Bradley, 2003). These data were not included in the statistical analysis of this study.] Values are means ± S.E.M. of five populations each. Non-matching letters above the columns denote statistically significant differences among treatments.
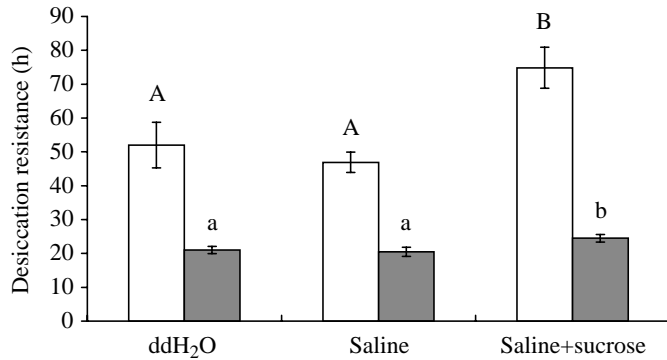
Fig. 4. Desiccation resistance of the D and C populations following recovery from an initial bout of desiccation. Values are means ± S.E.M. of five populations within the C or D group. Open bars, D populations; filled bars, C populations. Non-matching letters above the columns signify statistically significant differences among treatments within the C or D group.

suggest that the Na^+ content in the C flies was maintained at post-desiccation levels when only water was provided. The Na^+ level was fully restored to that observed in non-desiccated, hydrated flies when saline was offered, but it surpassed that of the non-desiccated, hydrated flies by 44% when saline+sucrose was provided ($P<0.0001$).

The mean whole-body Na^+ content in non-desiccated, hydrated D flies was ~85 nmol fly^{-1} (Fig. 3B), approximately twofold higher than that of the C flies. The D flies lose ~30% of Na^+ content during 24 h of desiccation (Folk and Bradley, 2003); thus, we estimated that the Na^+ content fell to ~60 nmol fly^{-1}. The Na^+ content of the D flies following recovery on water or saline (i.e. ~50 and ~60 nmol fly^{-1}, respectively), was significantly lower than that of the non-desiccated, hydrated flies ($P<0.0001$) and was at approximately the same level observed in the desiccated flies. Whole-body Na^+ in the D flies was fully restored to the level observed in the non-desiccated, hydrated flies only when the saline+sucrose solution was provided.

Restoration of desiccation resistance

Desiccation resistance in the C populations following recovery on water or saline solution was the same (21 h), but increased significantly (24.5 h) following recovery on the saline+sucrose solution ($P<0.0001$, Fig. 4). A similar trend was observed in the desiccation resistant populations (Fig. 4). Desiccation resistance did not differ significantly (~50 h) following recovery on water or saline solution in the D flies, but increased to 75 h when the flies were allowed to recover on the saline+sucrose solution ($P<0.0001$).

Discussion
Whole-body water content

Both C and D flies lost significant water volume during desiccation (Fig. 1A,B). The D flies were capable of partial water restoration when provided the saline+sucrose solution

during recovery; the C flies were capable of the same when provided any of the three fluids. The volume regained by the D flies (0.287 µl) was >threefold higher than that regained by the C flies on any solution (0.076 µl on average). These results suggest that the D flies are capable of restoring a much greater volume of body water when provided with the appropriate fluid ($P=0.03$).

A previous study indicated that during 24 h of desiccation, the D flies lose significant water volume, principally from the hemolymph; and after 8 h the C flies lose significant volume from both hemolymph and tissues (Folk and Bradley, 2003). Others (M. A. Albers and T. J. Bradley, unpublished data) examined the restoration of hemolymph volume in the C and D populations using the same experimental design and recovery fluids described in this paper. Their findings indicate that D flies provided saline+sucrose during recovery replenished ~75% of lost hemolymph volume. We estimate that of the total water volume restored, ~50% appears to be allocated to the hemolymph and ~50% to some other compartment. The portion of restored water not allocated to the hemolymph may possibly be water of hydration bound to glycogen, presuming that restoration of dry mass signified replenishment of glycogen stores (see Dry mass below).

The recovered C flies appeared to have restored hemolymph volume to levels above those of the non-desiccated, hydrated flies, regardless of recovery fluid (M. A. Albers and T. J. Bradley, unpublished observations). The average hemolymph volume restored was 0.070 µl, or >90% of the total water restored. Although hemolymph volume was restored, total body water content in the C flies remained significantly lower than that of non-desiccated, hydrated flies, suggesting that tissue-associated water was not replenished.

Restoration of lost water volume following desiccation has been examined in various species of desert tenebrionid beetles (Broza et al., 1976; Nicolson, 1980; Haidu, 2001a,b). During desiccation the beetles lost significant hemolymph volume, which they were able to quickly replenish when allowed to rehydrate on water. Despite complete restoration of hemolymph volume in some of the beetles, the original body mass was not fully restored, suggesting that tissue-associated water was not restored and/or that dry mass had been significantly reduced (Naidu and Hattingh, 1988; Naidu, 2001b). This pattern of rehydration was also observed in cockroaches (Tucker, 1977).

Dry mass

Glycogen appears to be the principal fuel metabolized during desiccation in *Drosophila* (D. G. Folk and T. J. Bradley, unpublished; Marron et al., 2003). Various other insect species preferentially metabolize lipids during desiccation, presumably because metabolic water production is highest (on a 'per gram' basis) when lipids are oxidized (e.g. Tucker, 1977; Nicolson, 1980; Naidu, 2001a). The significance of glycogen to desiccation resistance may be related to the ability of this polymer to bind water, which is released during glycogenolysis (Gibbs et al., 1997; Chippindale et al., 1998). Mammal

glycogen has the ability to bind 3–5 times its mass in water (Schmidt-Nielsen, 1997). Whether insect glycogen is similar to that isolated from mammals remains unclear (Friedman, 1985).

When desiccated for 24 h, the D flies lost dry mass (presumed to be primarily glycogen) at an average rate of ~4 μg h^{-1}. Provided the flies were using glycogen-derived glucose to fuel metabolism, the water of hydration that would be released in 24 h is estimated to range from ~0.290 to ~0.480 μl, while the volume of metabolic water produced is ~0.050 μl. Hence, the volume of water derived from catabolized glycogen would total ~0.340–0.530 μl. If we add the volume of glycogen-associated water produced and potentially lost in 24 h (e.g. 0.340 μl) and the volume of hemolymph lost (~0.200 μl; Folk and Bradley, 2003), we are able to fully account for the 0.523 μl reduction in whole-body water. These data support our previous findings that during 24 h of desiccation, the drought-sensitive tissues of the D flies appear to be protected from loss of water.

When desiccated for 8 h, the C flies lost dry mass (presumed to be primarily glycogen) at an average rate of ~5 μg h^{-1}. If we apply the same calculations used for the D flies, the catabolism of glycogen would contribute an estimated total volume of ~0.140–0.220 μl of water in 8 h. If we add the lower estimated volume of glycogen-associated water produced and potentially lost (i.e. 0.140 μl) and the hemolymph volume lost (~0.011 μl; Folk and Bradley, 2003), only ~60% of the reduction in whole-body water volume is accounted for. These data are consistent with our previous findings that, in contrast to the D flies, tissue water in the C flies is reduced significantly in 8 h of desiccation. (If we used the upper estimated volume of glycogen-associated water in these calculations, we could then account for >90% of the lost water in the C flies; but we chose the lower limit to maintain consistency in calculating water balance within both groups.)

The C and D flies have an average carbohydrate content of 94 μg and 168 μg, respectively, which is presumed to be principally glycogen (Graves et al., 1992; Gibbs et al., 1997; Chippindale et al., 1998; Djawdan et al., 1998; Folk et al., 2001). Our data suggest that glycogen may be a major contributor of water during desiccation until the estimated time of glycogen depletion: ~19 h in the C flies and ~42 h in the D flies, assuming that the rates of glycogen depletion remain constant.

Dry mass continued to decline during the 24 h recovery phase in C and D flies provided only water or saline (Fig. 2A,B). Despite the provision of an energy source, namely sucrose, the C flies only maintained dry mass at the post-desiccation level. The inability of the C flies to recover dry mass fully on the saline+sucrose solution may be related to an excessive accumulation of Na$^+$ (Fig. 3A). Dethier and Evans (1961) demonstrated that the drinking response in blowflies is lowered when the osmotic concentration of the hemolymph increases. If hemolymph osmolality increased in the C flies (even transiently) because of excessive accumulation of Na$^+$, then drinking rates, and thus consumption of sucrose, may have

been negatively affected. This chain of events may have prohibited full recovery of dry mass in the C flies when provided saline+sucrose. The D flies were capable of fully restoring dry mass to the level of the non-desiccated, hydrated flies when provided saline+sucrose (Fig. 2B). In contrast to the C flies, the D flies appear to have the capacity to regulate Na$^+$ levels and replenish dry mass while consuming this solution.

Restoration of sodium content

Sodium is the major inorganic ion in the hemolymph of drosophilids (Sutcliffe, 1963). A consequence of the voluminous hemolymph pool in hydrated D flies is a significant increase in hemolymph Na$^+$ content (Folk and Bradley, 2003). During 24 h of desiccation, the hemolymph Na$^+$ content in the D flies is dramatically reduced, while Na$^+$ level remains unaltered in the tissues. In contrast, during 8 h of desiccation in the C flies, the Na$^+$ content of both the hemolymph and tissues are significantly reduced.

In C flies provided water during recovery, whole-body Na$^+$ content was maintained only at the reduced, post-desiccation level (Fig. 3A). Although whole-body water was only partially restored in these C flies, hemolymph volume was fully replenished. In many insect species, hemolymph Na$^+$ is well-regulated during desiccation (Hadley, 1994); and in those that rehydrate only on water, hemolymph Na$^+$ concentration may be reduced relative to non-desiccated flies (Tucker, 1977; Nicolson, 1980; Naidu and Hattingh, 1988; Naidu, 2001a) or restored to original concentrations (Naidu, 2001b). It remains unclear which strategy is employed by these fruit fly populations.

The C flies provided the saline solution were able to fully restore whole-body Na$^+$ content, even though water volume was only partially restored. These results suggest that during recovery on saline, the osmotic concentration of some compartment in the body may have increased, or that osmotic concentration was regulated and the restored Na$^+$ replaced some other osmolyte. When provided saline+sucrose during recovery, the C flies experienced an increase in whole-body Na$^+$ to a level significantly greater than that of the non-desiccated, hydrated flies, possibly leading to the same consequences discussed above. The apparent Na$^+$ overload may have resulted from the attempt to restore water and/or energy stores, perhaps leading to extensive drinking initially. As a consequence, the Na$^+$ load may have increased to such an extent that the regulatory capacity of the excretory system of the C flies was surpassed, resulting in an elevated Na$^+$ content.

Whole-body Na$^+$ content in the D flies was sustained only at the post-desiccation level when either water or saline were provided (Fig. 3B). The D flies restored Na$^+$ content only when provided saline+sucrose. Although Na$^+$ content was fully restored on this solution, the hemolymph volume was only partially restored. The disparate capacities for restoring Na$^+$ (full restoration) and water content (partial restoration) lead to interesting questions: Was hemolymph Na$^+$ concentration regulated during recovery? Did the Na$^+$ concentration increase in some compartment(s)? Did the restored Na$^+$ replace other

cations within the hemolymph? [Some insects osmoregulate the hemolymph by adjusting the amino acid content (Djajakusumah and Miles, 1966; Broza et al., 1976; Coutchie and Crowe, 1979). The replacement of some cations, such as free amino acids, with Na^+ may be a means by which the recovering D flies maintain hemolymph osmolality, despite full recovery of Na^+ and only partial recovery of hemolymph.] Although these questions cannot be answered within the scope of this study, they would be interesting to address in future studies.

Desiccation resistance

Relative to recovery on water or saline, the C flies had a small, but significant, increase (14%) in desiccation resistance when provided saline+sucrose during recovery (Fig. 4); yet water was only partially restored, dry mass was maintained at post-desiccation levels, and Na^+ content had exceeded that of hydrated flies. Relative to recovery on water or saline, the D flies increased post-recovery desiccation resistance by 50% when allowed to recover on saline+sucrose (Fig. 4). When provided this fluid, the D flies were capable of full replenishment of dry mass (glycogen stores?) and partial replenishment of water content. Previous results suggest that desiccation resistance in the D flies is positively correlated with glycogen content and hemolymph volume (Folk et al., 2001). Our current results suggest that the capacity to replenish these somatic components, leads to the highest recovered desiccation resistance. The D flies were also able to recover whole-body Na^+ content fully when provided the saline+sucrose solution. Sodium may play an important role in water conservation during desiccation. Antidiuretic hormone (ADH) stimulates the uptake of water from the rectal lumen in some insect species, including the cockroach (Wall, 1967; Phillips et al., 1986). The rectal epithelia in the cockroach are rich in Na^+-K^+ ATPase and Na^+ is required during ADH-stimulated water uptake across the rectum, suggesting that the process requires energy (Tolman and Steele, 1976; Steele and Tolman, 1980). Further investigation suggests that glycogen is the principal energy source used to fuel the energy-requiring transport of water across the cockroach rectum (Tolman and Steele, 1980). Although the importance of Na^+ to water conservation during desiccation in our flies remains unclear, these findings suggest that Na^+, as well as glycogen, may play crucial roles in the reabsorption of water from the rectal lumen during desiccation stress.

Conclusion

Selection for enhanced desiccation resistance in *Drosophila melanogaster* leads not only to an improvement in the capacity to resist the stresses of desiccation, but also to a greater ability to recover physiological robustness following desiccation. The desiccation-resistant populations, when compared to the control populations, were able to restore a greater amount of body water and dry mass. Furthermore, the restoration of water, dry mass, Na^+ content and desiccation resistance was greatly improved in D flies that consumed fluid supplemented

with sucrose. We propose that energy consumption appears to be an important aspect of recovery from desiccation in these fruit fly populations.

References

Arlian, L. G. and Eckstrand, I. A. (1975). Water balance in *Drosophila pseudoobscura*, and its ecological implications. *Ann. Entomol. Soc. Am.* **68**, 827-832.

Browne, L. B., Moorhouse, J. E. and van Gerwen, A. C. M. (1976). A relationship between weight loss during food deprivation and subsequent meal size in the locust *Chortoicetes terminifera*. *J. Insect. Physiol.* **22**, 89-94.

Broza, M., Borut, A. and Pener, M. (1976). Osmoregulation in the desert tenebrionid beetle *Trachyderma philistine* Reiche during dehydration and subsequent rehydration. *Isr. Med. J.* **12**, 868-871.

Chapman, R. F. (1998). *The Insects: Structure and Function.* 4th edition. Cambridge, UK: Cambridge University Press.

Chippindale, A. K., Gibbs, A. G., Sheik, M., Yee, K. J., Djawdan, M., Bradley, T. J. and Rose, M. R. (1998). Resource acquisition and the evolution of stress resistance in *Drosophila melanogaster*. *Evol.* **52**, 1342-1352.

Coutchie, P. A. and Crowe, J. H. (1979). Transport of water vapor by tenebrionid beetles. II. Regulation of the osmolarity and composition of the hemolymph. *Physiol. Zool.* **52**, 88-100.

Dethier, V. G. and Evans, D. R. (1961). Physiological control of water ingestion in the blowfly. *Biol. Bull.* **121**, 108-116.

Djajakusumah, T. and Miles, P. W. (1966). Changes in the relative amounts of soluble protein and amino acid in the haemolymph of the locust, *Chortoicetes terminifera* Walker (Orthoptera: Acrididae), in relation to dehydration and subsequent rehydration. *Aust. J. Biol. Sci.* **19**, 1081-1094.

Djawdan, M., Chippindale, A. K., Rose, M. R. and Bradley, T. J. (1998). Metabolic reserves and evolved stress resistance in *Drosophila melanogaster*. *Physiol. Zool.* **71**, 584-594.

Edney, E. B. (1977). *Zoophysiology and Ecology 9: Water Balance in Land Arthropods.* New York: Springer-Verlag.

Evans, D. R. (1961). Control of the responsiveness of the blowfly to water. *Nature* **190**, 1132-1133.

Folk, D. G. and Bradley, T. J. (2003). Evolved patterns and rates of water loss and ion regulation in laboratory-selected populations of *Drosophila melanogaster*. *J. Exp. Biol.* **206**, 2779-2786.

Folk, D. G., Han, C. and Bradley, T. J. (2001). Water acquisition and partitioning in *Drosophila melanogaster*: effects of selection for desiccation-resistance. *J. Exp. Biol.* **204**, 3323-3331.

Friedman, S. (1985). Carbohydrate metabolism. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology.* Vol. 10, *Biochemistry* (ed. G. A. Kerkut and L. I. Gilbert), pp. 43-76. New York: Pergamon Press Inc.

Gibbs, A. G., Chippindale, A. K. and Rose, M. R. (1997). Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *J. Exp. Biol.* **200**, 1821-1832.

Gillott, C. (1995). *Entomology.* 2nd edition. New York: Plenum Press.

Graves, J. L., Toolson, E. C., Jeong, C., Vu, L. N. and Rose, M. R. (1992). Desiccation, flight, glycogen, and postponed senescence in *Drosophila melanogaster*. *Physiol. Zool.* **65**, 268-286.

Hadley, N. F. (1994). *Water Relations of Terrestrial Arthropods.* San Diego, CA: Academic Press.

Hamilton, W. J., III and Seeley, M. K. (1976). Fog basking by the Namib Desert beetle, *Onymacris unguicularis*. *Nature.* **262**, 284-285.

Hoffman, A. A. and Parsons, P. A. (1993). Direct and correlated responses to selection for desiccation resistance: a comparison of *Drosophila melanogaster* and *D. simulans*. *J. Evol. Biol.* **6**, 643-657.

Lehmann, F.-O., Dickinson, M. H. and Staunton, J. (2000). The scaling of carbon dioxide release and respiratory water loss in flying fruit flies (*Drosophila* sp.). *J. Exp. Biol.* **203**, 1613-1624.

Loveridge, J. P. (1975). Studies on the water balance of adult locusts. III. The water balance of non-flying locusts. *Zool. Afr.* **10**, 1-28.

Marron. M. T., Markow, T. A., Kain, K. J. and Gibbs, A. G. (2003). Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila*. *J. Insect Physiol.* **49**, 261-270.

Naidu, S. G. (2001a). Water balance and osmoregulation in *Stenocara gracilipes*, a wax-blooming tenebrionid beetle from the Namib Desert. *J. Insect Physiol.* **47**, 1429-1440.

Naidu, S. G. (2001b). Osmoregulation in *Onymacris rugatipennis*, a free-ranging tenebrionid beetle from the Namib Desert. *Comp. Biochem. Physiol.* **129A**, 873-885.

Naidu, S. G. and Hattingh, J. (1988). Water balance and osmoregulation in *Physadesmia globosa*, a diurnal tenebrionid beetle for the Namib Desert. *J. Insect Physiol.* **34**, 911-917.

Nicolson, S. W. (1980). Water balance and osmoregulation in *Onymacris plana*, a tenebrionid beetle from the Namib Desert. *J. Insect Physiol.* **26**, 315-320.

Phillips, J. E., Hanrahan, J., Chamberlin, M. and Thomson, B. (1986). Mechanisms and control of reabsorption in insect hindgut. *Adv. Insect Physiol.* **19**, 329-422.

Rose, M. R. (1984). Laboratory evolution of postponed senescence in *Drosophila melanogaster. Evol.* **38**, 1004-1010.

Rose, M. R., Graves, J. L. and Hutchison, E. W. (1990). The use of selection to probe patterns of pleiotropy in fitness characters. In *Insect Life Cycles* (ed. F. Gilbert), pp. 29-42. New York: Springer-Verlag.

Rose, M. R., Vu, L., Park, S. U. and Graves, J. L. (1992). Selection on stress resistance increases longevity in *Drosophila melanogaster. Exp Gerontol.* **27**, 241-250.

Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*. 5th edition. Cambridge, UK: Cambridge University Press.

Steele, J. E. and Tolman, J. H. (1980). Regulation of water transport in the cockroach rectum by the corpora cardiaca–corpora allata system: The requirement of Na$^+$. *J. Comp. Physiol.* **13**, 357-366.

Sutcliffe, D. W. (1963). The chemical composition of haemolymph in insects and some other arthropods in relation to their phylogeny. *Comp. Biochem. Physiol.* **9**, 121-135.

Tolman, J. H. and Steele, J. E. (1976). A ouabain sensitive (Na$^+$–K$^+$)-activated ATPase in the rectal epithelium of the American cockroach, *Periplaneta americana. Insect Biochem.* **6**, 513-517.

Tolman, J. H. and Steele, J. E. (1980). The effect of the corpora cardiaca-corpora allata system on oxygen consumption in the cockroach rectum. The role of Na$^+$ and K$^+$. *J. Comp. Physiol.* **66B**, 59-67.

Tucker, L. E. (1977). Effect of dehydration and rehydration on the water content and Na$^+$ and K$^+$ balance in adult male *Periplaneta americana*. *J. Exp. Biol.* **71**, 49-66.

Wall, B. J. (1967). Evidence for antidiuretic control of rectal water absorption in the cockroach, *Periplaneta americana* L. *J. Insect Physiol.* **13**, 565-578.

Wall, B. J. (1970). Effects of dehydration and rehydration on *Periplaneta americana. J. Insect Physiol.* **16**, 1027-1042.

Wigglesworth, V. B. (1972). *The Principles of Insect Physiology.* London: Chapman and Hall.

Williams, A. E. and Bradley, T. J. (1998). The effect of respiratory pattern on water loss in desiccation-resistant *Drosophila. J. Exp. Biol.* **201**, 2953-2959.

Williams, A. E., Rose, M. R. and Bradley, T. J. (1998). Using laboratory selection for desiccation resistance to examine the relationship between respiratory pattern and water loss in insects. *J. Exp. Biol.* **201**, 2945-2952.