Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila*

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Abstract

Energy availability can limit the ability of organisms to survive under stressful conditions. In *Drosophila*, laboratory experiments have revealed that energy storage patterns differ between populations selected for desiccation and starvation. This suggests that flies may use different sources of energy when exposed to these stresses, but the actual substrates used have not been examined. We measured lipid, carbohydrate, and protein content in 16 *Drosophila* species from arid and mesic habitats. In five species, we measured the rate at which each substrate was metabolized under starvation or desiccation stress. Rates of lipid and protein metabolism were similar during starvation and desiccation, but carbohydrate metabolism was several-fold higher during desiccation. Thus, total energy consumption was lower in starved flies than desiccated ones. Cactophilic *Drosophila* did not have greater initial amounts of reserves than mesic species, but may have lower metabolic rates that contribute to stress resistance.
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1. Introduction

Desert animals may be subjected to periods without food and water. Many species avoid long-term stress by estivating, during which time the need to replenish food and water is greatly reduced. Others may be able to acquire sufficient resources to maintain activity, but will still be subjected to episodic starvation or desiccation stress. Survival under these conditions can be maximized by two physiological mechanisms: increasing the storage of resources (energy or water) that are utilized during stress, or conserving resources by reducing the rates at which they are consumed. Flies of the genus *Drosophila* provide an especially good model for stress resistance, because different species inhabit areas with high and low resource availability and predictability, and because selection experiments can be performed to study the evolution of stress resistance under controlled laboratory conditions.

Cactophilic *Drosophila* species in the southwestern deserts of North America reside in necrotic tissues of damaged columnar cacti. Although their abundance decreases dramatically in summer months (Breitmeyer and Markow, 1998), they are not known to estivate and can be collected at all times of the day and year. Even inside necroses, flies can be found where temperature exceed 40°C, and environmental humidities below 10% RH are common (Gibbs et al., 2003b). When a necrosis dries out, *Drosophila* may fly as far as two kilometers to another rotting cactus (Markow and Castrezana, 2000). Thus, desert *Drosophila* are clearly subjected to periods of water stress.

Not surprisingly, desert *Drosophila* are more resistant to high temperatures and desiccation than species from mesic habitats (Stratman and Markow, 1998; Krebs, 1999; Gibbs and Matzkin, 2001; Patton and Krebs, 2001). The mechanisms responsible for these differences include expression of heat-shock proteins (Krebs, 1999)

and reduction of water-loss rates (Gibbs and Matzkin, 2001). Energy metabolism may also play a critical role in stress resistance. Reductions in metabolic rate will increase the amount of time that flies can survive starvation, and will reduce the need to open the spiracles and consequently lose water in desiccating conditions (Lighton, 1994, 1996; Zachariassen, 1996; Addo-Bediako et al., 2001). Experiments with *D. melanogaster* indicate that laboratory selection for stress resistance can lead to a reduction in metabolic rate (Hoffmann and Parsons, 1993; but see Djawdan et al., 1997; Harshman and Schmid, 1998). If desert *Drosophila* are subject to selection for starvation and desiccation resistance in nature, they too should exhibit a reduced metabolic rate for their size.

In addition to rates of energy consumption, both the amount and form of energy storage can affect stress resistance. In inter-specific comparisons, starvation and desiccation resistance are positively correlated with lipid levels (van Herrewege and David, 1997). Selection experiments, on the other hand, provide somewhat contradictory results. Starvation-selected populations of *D. melanogaster* accumulate high lipid and carbohydrate levels (Chippindale et al., 1998), as predicted from comparative studies. Desiccation-selected populations, however, store less lipid but much more glycogen than control populations (Djawdan et al., 1998).

A possible mechanistic relationship between stress resistance and metabolic reserves can be proposed from knowledge of the energy and water contents of different stored compounds. Lipids provide over twice as much energy per gram as carbohydrates (Withers, 1992), so they would be an appropriate fuel to store for starvation resistance. Glycogen is less energy-dense but provides slightly more metabolic water per gram than lipid. Bound water is probably a more important consideration, however, since glycogen also binds three to five times its weight in water (Schmidt-Nielsen, 1990). Lipids do not bind a significant amount of water, so the total amount of water available after metabolism is much lower for lipids than carbohydrates (Gibbs et al., 1997). Thus, carbohydrates may be a more appropriate fuel under water stress.

An important link missing from these studies is knowledge of which energetic substrates are actually consumed. Increased lipid storage will only help flies to survive starvation if they actually metabolize lipids under these conditions. Bound water will be released from stored glycogen only if desiccated flies metabolize carbohydrates; otherwise this water will stay bound. Thus, we can predict that flies will regulate their metabolism to use different energy sources depending on the type of stress imposed. To test this hypothesis, we exposed five species of *Drosophila*, including three mesic and two desert species, to desiccation stress (no food and no water) or starvation stress (water, but no food) and measured the rates of disappearance of energetic substrates (lipids, carbohydrates, and proteins). We also measured initial substrate levels in an additional 11 species of *Drosophila*, to test the hypothesis that flies from arid environments will contain greater levels of metabolic reserves, particularly carbohydrates.

2. Methods and materials

2.1. Fly rearing and maintenance

Table 1 lists the species used and collection information. All flies were reared in milk bottles at 24°C under the laboratory photoperiod regime (~12 h light: 12 h dark). Cactophilic species were reared on banana food containing powdered *Opuntia* cactus, with some species also receiving a piece of their normal host cactus to stimulate egg-laying. Mesic species were reared on standard cornmeal medium, except *D. busckii*, which received Wheeler-Clayton medium. Dry yeast was added to both types of media to provide a protein-rich environment for growth. Larval densities were kept low (~200 per bottle) to prevent overcrowding from affecting adult physiology. For experiments, groups of 20 virgin flies of a given sex were collected and placed in vials containing cornmeal medium and a few grains of live yeast. Five-day old flies were used in all experiments.

Five species were used for starvation and desiccation assays. Two (*D. melanogaster*, *D. pseudoobscura*) were from the subgenus Sophophora, and three (*D. hydei*, *D. mojavensis*, *D. nigrospiracula*) were from the *repleta* group of the subgenus Drosophila. *Drosophila mojavensis* and *D. nigrospiracula* are endemic to the Sonoran Desert of North America, *D. pseudoobscura* is a montane species found in western North America, and the other two species are cosmopolitan. The remaining 11 species were used only for assays of initial lipid, carbohydrate and protein contents.

2.2. Starvation treatment

The starvation treatment involved placement of flies in 35-ml glass vials containing 10 ml of 0.5% agar, thereby allowing flies access to water but not nutrition. Vials were capped with cotton plugs to restrict the flies to the center of the vial, then were capped with Parafilm to ensure that the humidity remained high. Each vial contained five flies of one sex, and ~40 vials of each sex were prepared for each species. The actual experimental sample size was dependent on the number of flies that eclosed on the collection day. The vials were incubated at 24°C, and fifty flies (ten vials) of each sex were removed at pre-determined time points and frozen at −80°C. Removal times for each species were calculated based on independent starvation assays (T.A. Markow

Table 1
Drosophila species used in this study

Species	Collection location and date	Habitat
D. acutilabella	Broward County, Florida USA, 2001	mycophilic, mesic
D. arizonae	Superstition Mountains, Arizona USA, 1997	cactophilic, xeric
D. busckii	Netherlands, 1999	mesic
D. cardini	Broward County, Florida USA, 2001	mycophilic, mesic
D. hydei[a]	Madera Canyon, Arizona USA, 1999	mesic
D. malerkotliana	Panama, 1999	tropical, mesic
D. melanogaster[a]	Madera Canyon, Arizona USA, 1999	mesic
D. mercatorum	Asheville, North Carolina USA, 2001	mesic
D. mettleri	San Carlos, Sonora Mexico, 1997	cactophilic, xeric
D. mojavensi[a]	Guaymas, Sonora Mexico, 2000	cactophilic, xeric
D. nigrospiracula[a]	Tucson, Arizona USA, 2000	cactophilic, xeric
D. pachea	Guaymas, Sonora Mexico, 1999	cactophilic, xeric
D. paulistorum	Panama, 1999	tropical, mesic
D. persimilis	Mather, California USA, 1999	mesic
D. pseudoobscur[a]	Madera Canyon, Arizona USA, 1999	mesic
D. sturtevanti	Panama, 1999	tropical, mesic

[a] Species used for assays of lipid, carbohydrate, and protein metabolism.

and T. Watts, unpublished). The last time point corresponded to the approximate time of 50% mortality in the earlier experiments. Flies survived longer in our experiments, so that almost no mortality was observed (no more than two individuals for any species), indicating that the results were not biased by the deaths of flies which initially had low energy levels.

2.3. Desiccation treatment

Desiccation assays were performed at the same time as the starvation assays, except for the assays using *D. melanogaster*. The same procedure was used as for starvation assays, except that the vials contained five grams of silica gel desiccant instead of agar medium. Five flies were added to an empty vial, followed by a polyethylene sponge, then silica gel desiccant. To ensure that the humidity inside the vials remained below 5%, the vials were then capped with Parafilm and incubated at 24°C. Because flies die much more rapidly under these conditions than when starved, we collected flies for assays at more frequent intervals. Removal times for each species were calculated based on previous desiccation assays. The last time point corresponded to the approximate time of 50% mortality in earlier experiments (Gibbs and Matzkin, 2001). As for the starvation assays, almost no mortality was observed, indicating that the results were not biased by the deaths of desiccation-susceptible flies.

2.4. Lipid assays

Flies were placed in a 50°C oven to dry overnight. To obtain the dry weight, individual flies were weighed to a precision of 0.001 mg using a Cahn microbalance. Flies were then placed in small glass vials containing

approximately 1 ml of ether, which were capped and left overnight to allow time for lipid extraction. The next day, the ether was removed from the vials, and the flies were placed in a 50°C oven for 1 h to evaporate residual ether. Each fly was weighed again, giving the lipid-free weight. Lipid-free mass was subtracted from the dry mass to calculate the amount of lipid per fly.

2.5. Carbohydrate assays

Our carbohydrate assay was based on that of Parrou and Francois (1997). Individual flies were ground in 300 μl water using a hand-held electric mortar and pestle. After centrifuging at ~10 000 g for 2 min, 100 μl supernatant (or 50 μl supernatant + 50 μl water for larger species) was removed for use in assays. Ten μl of *Rhizopus* mold amyloglucosidase (8 mg/ml) was added to each sample to catalyze the conversion of glycogen and trehalose into glucose. The samples were then allowed to sit at room temperature overnight.

The next day, glucose levels were measured by adding 1 ml of Infinity Glucose Reagent (Sigma Chemical Co., St. Louis, Missouri, USA) to each tube. In this assay, glucose oxidation by hexokinase is coupled via glucose-6-phosphate dehydrogenase to the reduction of nicotinamide adenine dinucleotide (NADH). The absorbance by NADH at 340 nm was read within 1 hour to quantify glucose levels. Solutions with known glycogen concentrations were used to make standard curves.

2.6. Protein assays

Protein levels were determined using the bicinchoninic acid (BCA) method. Individual flies were homogenized in 300 μl water and centrifuged at ~10,000 g for 2

min. After centrifugation, 25 μl of supernatant was removed from each sample. One ml of Sigma BCA Protein Assay Reagent was added, then samples were incubated at room temperature overnight. Protein concentrations were determined by comparing the absorbance at 562 nm with standard curves.

2.7. Statistics

Because females were larger than males, and because egg production may cause females to store energy differently than males, the sexes were analyzed separately. All statistical analyses were performed using JMP software (SAS Institute), with Bonferroni corrections for multiple comparisons.

3. Results

Figs. 1 and 2 depict changes in energetic resources during starvation and desiccation stress in five *Drosophila* species. Slopes of regression lines for these data are provided in Table 2. Because of the large number of tests made, we applied a table-wide, sequential Bonferroni correction (Rice, 1989) to assess the statistical significance of the slopes. Male and female flies followed similar patterns in energy consumption. All three fuels were used during starvation stress, as demonstrated by statistically significant decreases ($P < 0.05$ after Bonferroni correction) in 26 out of 30 cases (Table 2). Desiccated flies relied primarily on metabolism of glycogen, with the rate of consumption increasing up to seven-fold relative to starvation ($P < 0.05$ after Bonferroni correction for all 10 glycogen slopes). In some cases, lipid and protein levels actually appeared to increase during desiccation, as indicated by positive regression slopes for data shown in Figs. 1 and 2. None of these positive slopes were significant ($P > 0.05$), whereas five negative slopes were.

In order to determine whether metabolism differed during desiccation and starvation stress, we compared regression slopes for each substrate using the confidence-interval procedure described by Zar (1996). Rates of lipid and protein metabolism did not differ during desiccation and starvation ($P > 0.05$ for both sexes of all five species). Rates of carbohydrate consumption were significantly greater ($P < 0.05$ after Bonferroni correction) under desiccation than starvation stress for every species except *D. pseudoobscura*.

Rates of energy production were calculated using standard conversion factors (Schmidt-Nielsen, 1990) and are presented in Table 3. Across all species, mass-specific metabolic rates of the two cactophilic species were ~60% lower than those of the three mesic species.

Because carbohydrates were the main fuel consumed during water stress, we tested the hypothesis that cacto-
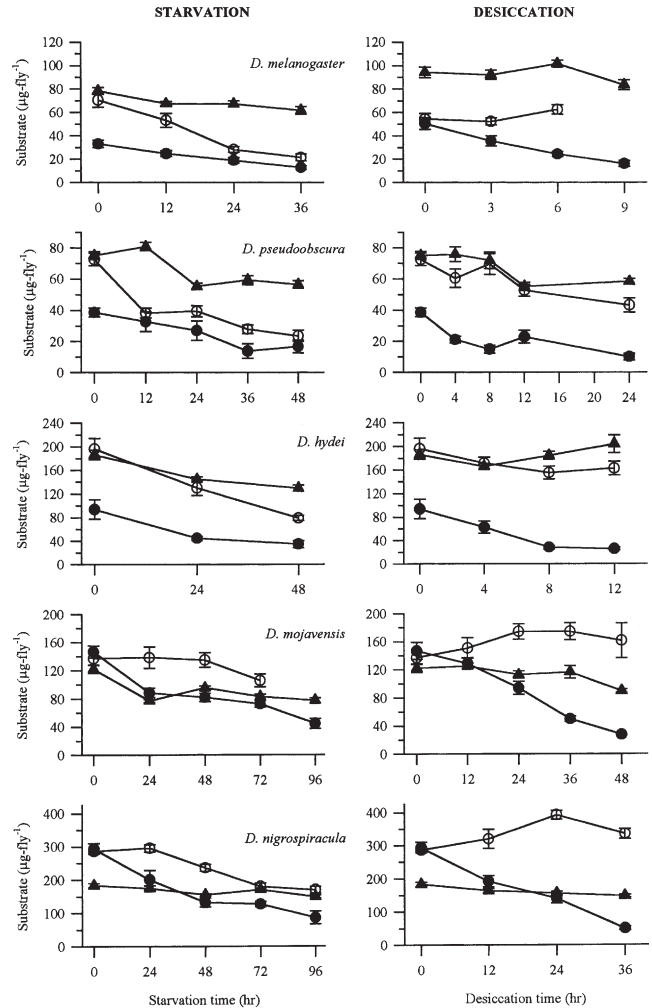

Fig. 1. Effects of starvation and desiccation stress on carbohydrate, lipid and protein levels in females from five species of *Drosophila*. Note the different time scales for the experiments, depending on the treatment and species. Carbohydrates, filled circles; lipids, open circles; proteins, filled triangles. Means (± standard error) of 5–15 measurements are shown. Because starvation and desiccation assays were performed at the same time for four of the species, initial substrate levels are the same for all species except *D. melanogaster*.

philic *Drosophila* initially contain higher carbohydrate levels than mesic species. After correction for body size, carbohydrate contents did not differ between cactophilic and mesic *Drosophila* species (ANCOVA, $P > 0.1$ for males and females; Fig. 3, top panel). Similarly, ANCOVAs revealed that lipid and protein levels were the same for desert and mesic species (Fig. 3; $P > 0.1$ for lipid and protein ANCOVAs). To investigate the possibility that the two major stores, lipid and carbohydrate, might be negatively correlated due to trade-offs between resource synthesis and storage, we calculated residuals of lipid and carbohydrate levels vs. mass. These tended to be positively, though not significantly, correlated ($r = 0.20$, $P > 0.2$ for males; $r = 0.45$, $P > 0.08$ for females). We obtained similar results after controlling for phylogeny using Felsenstein's (Felsenstein,
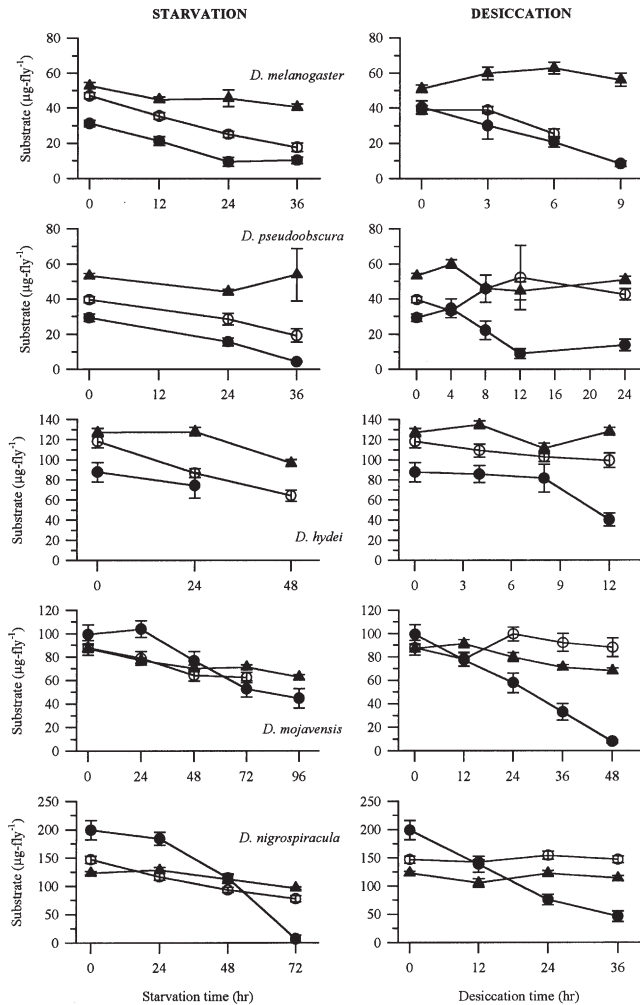
Fig. 2. Effects of starvation and desiccation stress on carbohydrate, lipid and protein levels in males from five species of *Drosophila*. Note the different time scales for the experiments, depending on the treatment and species. Carbohydrates, filled circles; lipids, open circles; proteins, filled triangles. Means (± standard error) of 7–15 measurements are shown. Because starvation and desiccation assays were performed at the same time for four of the species, initial substrate levels are the same for all species except *D. melanogaster*.

1985) method of independent contrasts (data not shown). Thus, there was no apparent storage trade-off.

4. Discussion

Numerous studies have implicated energy storage in desiccation and starvation resistance of *Drosophila* (Clark and Doane, 1983; Service, 1987; Graves et al., 1992; Blows and Hoffmann, 1993; Oudman et al., 1994; van Herrewege and David, 1997; Djawdan et al., 1998). It is therefore surprising that, to our knowledge, no one has determined which substrates these flies actually metabolize under these conditions. Our data reveal that flies from both arid and mesic habitats use a mixture of energy sources when starved, but rely primarily on

carbohydrate metabolism when desiccated. Despite their greater desiccation resistance, however, desert *Drosophila* do not contain higher levels of carbohydrates than mesic species.

4.1. Energy metabolism of Drosophila during starvation and desiccation stress

Energy metabolism during starvation stress has been examined in only a few species of insects. Locusts (*Locusta migratoria*) and fruit beetles (*Pachnoda sinuata*) metabolize glycogen stores during the initial stages of starvation, then switch to lipid and protein metabolism when carbohydrates are gone (Hill and Goldsworthy, 1970; Jutsum et al., 1975; Auerswald and Gade, 2000). Other species, however, may rely more heavily on lipid metabolism (Lim and Lee, 1981; Satake et al., 2000). Diapausing insects also undergo long periods without food, although this condition is not directly comparable to our experiments using active *Drosophila*. Carbohydrates provide most of the energy during diapause, in addition to providing a source of cryoprotectants such as sorbitol and glycerol (Wipking et al., 1995; Alonso Mejia et al., 1997; Kostal et al., 1998). *Drosophila* species, therefore, resemble previously studied species in using a mixture of fuels when starved.

Carbohydrates were clearly the major source of energy during water stress, but our data are somewhat ambiguous regarding lipid and protein metabolism. No significant differences in lipid and protein slopes were detected between starved and desiccated flies, and in some cases desiccated flies actually appeared to synthesize lipids and proteins (as indicated by positive slopes in Table 2). The presumed source for lipogenesis and protein synthesis would be carbohydrates. Lipid synthesis, in particular, would result in the net release of bound water from carbohydrates and might therefore be beneficial for survival. We note, however, that none of the positive slopes were significantly greater than zero, whereas six significant negative values were obtained for lipids and proteins. An important factor may have been that the desiccation treatments were necessarily much shorter than the starvation experiments. Desiccation involves the removal of both water and food, and is clearly a more severe treatment than food deprivation alone. We suggest that lipid and protein metabolism are similar under these stresses, but the desiccation experiments were too short to detect net metabolism consistently.

Net carbohydrate metabolism was observed during both stresses, but was greater during desiccation. The reason for this is unclear, but several possibilities come to mind. Organs involved in water conservation (e.g. the hindgut and Malpighian tubules) may become more metabolically active, and may preferentially metabolize carbohydrates. Alternatively, starved flies may down-

Table 2
Rates of substrate use by *Drosophila* under desiccation and starvation stress

		D. melanogaster	*D. pseudoobscura*	*D. hydei*	*D. mojavensis*	*D. nigrospiracula*
A. Females						
Starvation:	Carbohydrates	−0.562[a]	−0.513[a]	−1.384[a]	−0.919[a]	−2.312[a]
		(0.095)	(0.130)	(0.403)	(0.100)	(0.251)
	Lipids	−1.441[a]	−0.935[a]	−2.433[a]	−0.402	−1.513[a]
		(0.178)	(0.111)	(0.377)	(0.211)	(0.165)
	Proteins	−0.414[a]	−0.437[a]	−1.223[a]	−0.338[a]	−0.277
		(0.100)	(0.100)	(0.206)	(0.064)	(0.091)
Desiccation:	Carbohydrates	−4.000[a]	−0.925[a]	−6.015[a]	−2.633[a]	−6.292[a]
		(0.539)	(0.172)	(1.143)	(0.266)	(0.517)
	Lipids	+1.378	−1.167[a]	−2.898	+0.836	+1.785
		(0.958)	(0.281)	(1.438)	(0.382)	(0.715)
	Proteins	−1.061	−0.914[a]	+1.762	−0.539	−.979[a]
		(0.499)	(0.174)	(1.086)	(0.176)	(0.197)
B. Males						
Starvation:	Carbohydrates	−0.625[a]	−0.632[a]	−0.556	−0.673[a]	−1.584[a]
		(0.091)	(0.100)	(0.668)	(0.103)	(0.222)
	Lipids	−0.818[a]	−0.528[a]	−1.122[a]	−0.376[a]	−0.964[a]
		(0.075)	(0.117)	(0.160)	(0.101)	(0.107)
	Proteins	−0.294[a]	−0.162	−0.629[a]	−0.225[a]	−0.391[a]
		(0.103)	(0.163)	(0.130)	(0.034)	(0.094)
Desiccation:	Carbohydrates	−3.642[a]	−0.868[a]	−3.610[a]	−1.892[a]	−4.132[a]
		(0.608)	(0.234)	(1.104)	(0.171)	(0.460)
	Lipids	−2.244[a]	+0.156	−1.558	+0.134	+0.105
		(0.586)	(0.159)	(0.686)	(0.184)	(0.218)
	Proteins	+0.437	−0.248	−0.554	−0.478[a]	−0.120
		(0.406)	(0.171)	(0.543)	(0.077)	(0.202)

Data are linear regression slopes (S.E.) calculated from 32–80 individuals (Figs. 1 and 2), with negative values indicating that substrate levels decreased over time. We used a table-wide sequential Bonferroni correction (Rice, 1989) to assess statistical significance. Units are µg/h.

[a] Significant slopes ($P < 0.05$ after correction).

Table 3
Rates of energy production (J/h/fly) from different substrates by *Drosophila* under desiccation and starvation stress, calculated from data in Table 2

		D. melanogaster	*D. pseudoobscura*	*D. hydei*	*D. mojavensis*	*D. nigrospiracula*
A. Females		(0.366)	(0.459)	(0.870)	(0.741)	(1.229)
Starvation:	Carbohydrates	0.0099	0.0090	0.0243	0.0161	0.0406
	Lipids	0.0667	0.0368	0.0957	0.0158	0.0595
	Proteins	0.0074	0.0078	0.0217	0.0060	0.0049
	Total	0.0840	0.0536	0.142	0.0379	0.105
Desiccation:	Carbohydrates	0.0703	0.0163	0.106	0.0463	0.111
	Lipids	–	0.0459	0.114	–	–
	Proteins	0.0189	0.0163	–	0.0096	0.0174
	Total	0.0892	0.0785	0.220	0.0559	0.128
B. Males		(0.255)	(0.304)	(0.632)	(0.474)	(0.800)
Starvation:	Carbohydrates	0.0110	0.0111	0.0098	0.0118	0.0278
	Lipids	0.0322	0.0208	0.0441	0.0148	0.0379
	Proteins	0.0052	0.0029	0.0112	0.0040	0.0070
	Total	0.0484	0.0348	0.0651	0.0306	0.0727
Desiccation:	Carbohydrates	0.0640	0.0153	0.0634	0.0332	0.0726
	Lipids	0.0883	–	0.0613	–	–
	Proteins	–	0.0044	0.0098	0.0085	0.0021
	Total	0.152	0.0197	0.134	0.0417	0.0747

Conversion factors used were 17.6 J/mg (carbohydrates), 39.3 J/mg (lipids), and 17.8 J/mg (proteins, assuming uric acid excretion) (Schmidt-Nielsen, 1990). Values below species names are dry masses (mg).
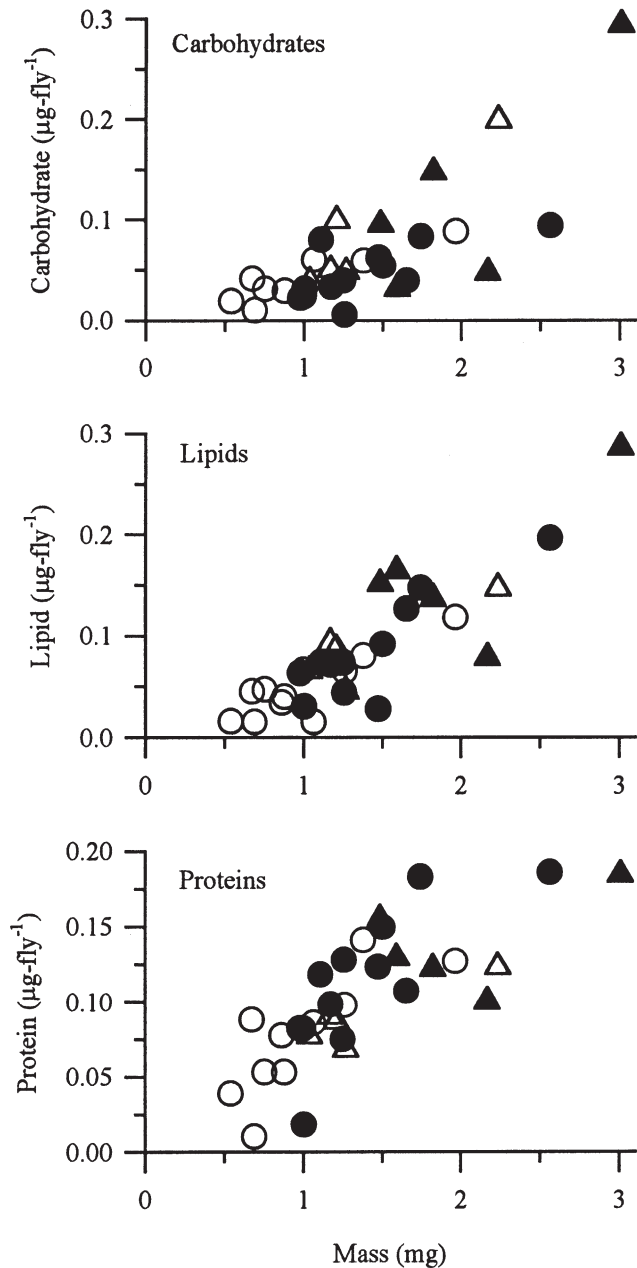
Fig. 3. Carbohydrate, lipid and protein levels in 16 species of *Drosophila*. Females, filled symbols; males, open symbols. Cactophilic species are represented by triangles, mesic species by circles.

would allow us to infer which fuels are being used and whether flies reduce their metabolism in response to starvation, increase metabolism during water stress, or both.

Our understanding of the relationship between energetics and stress resistance in *Drosophila* is based in large part on selection experiments, which have often revealed significant changes in energy storage (Blows and Hoffmann, 1993; Chippindale et al., 1996, 1998; Djawdan et al., 1998; Harshman et al., 1999; Harshman and Hoffmann, 2000). Starvation-selected populations accumulate higher levels than their controls of both lipids and carbohydrates (Chippindale et al., 1998). Our results provide a straightforward physiological explanation: both of these are actually metabolized during starvation (Figs. 1 and 2). Protein metabolism has been ignored in previous work, but we found that it contributed an average of 10% of overall metabolism. It would be very interesting to know whether this represents the general loss of proteins, or whether specific proteins such as storage hexamerins (Telfer and Kunkel, 1991) are metabolized. Unfortunately, protein metabolism has not been examined in stress-selected lines.

Energy storage in desiccation-selected populations is less easy to explain. These accumulate more glycogen and less lipid than their controls (Djawdan et al., 1998). Carbohydrate storage makes sense, since these compounds are metabolized much more rapidly during water stress (Table 2). Reduced lipid storage is more problematic, but it should be noted that the control treatment for desiccation stress (neither food nor water) typically involves mild starvation stress (water but no food). Thus, this 'control' treatment for desiccation entails mild starvation selection, which will tend to favor increased lipid storage. A more appropriate comparison may be to storage patterns in fed controls or in the original founding stocks (Gibbs, 1999). It is interesting to note that desiccation-selected populations accumulate higher levels of *both* lipids and carbohydrates than their ancestral populations (Chippindale et al., 1998; Djawdan et al., 1998).

4.2. Inter-specific variation in energy storage of Drosophila

Little is known regarding the energetic basis for natural variation in stress resistance in *Drosophila*. Starvation and desiccation resistance are often positively correlated across *Drosophila* species (van Herrewege and David, 1997), but these traits generally exhibit opposing directions of clinal variation within species (Karan et al., 1998; Hoffmann and Harshman, 1999; Parkash and Munjal, 2000). Lipid storage is strongly associated with increased starvation resistance, but only weakly correlated with desiccation resistance (van Herrewege and David, 1997). Unfortunately, carbohydrate and protein metabolism have received little attention in comparative studies. Within species, preliminary evi-

regulate metabolic rates. This would be consistent with the results of Djawdan et al. (1997), who found that starved *D. melanogaster* produced less CO_2 than fed or desiccated ones. Our data suggest that this difference results from reduced carbohydrate metabolism, but substrate switching will also affect CO_2 production in the absence of changes in ATP production (Schmidt-Nielsen, 1990). Unfortunately, our biochemical techniques can not be used to examine which energetic sources are metabolized by fed flies. What is needed are simultaneous measurements of CO_2 production and O_2 consumption. Knowledge of respiratory exchange ratios

dence suggests that starvation resistance in high-latitude populations of *D. melanogaster* is associated with glycogen storage (Verrelli and Eanes, 2001), but, as noted above, inter- and intra-specific variation need not follow the same patterns. Because carbohydrates are the preferred metabolic fuel during desiccation stress, and because insects are unable to synthesize carbohydrates *de novo* from lipids (Withers, 1992), one would expect desert *Drosophila* to have higher carbohydrate levels than mesic species. This is not the case, however (Fig. 3), nor do desert flies store and use a different energy source than mesic species (Table 2). A possible explanation is the existence of trade-offs between lipid and carbohydrate storage. These may be as simple as a physical limitation on storage space, so that flies containing the large quantities of carbohydrate required for desiccation resistance can not store as much lipid needed for starvation resistance. Our results, however, suggest a positive relationship between lipid and carbohydrate levels, although a marginally significant correlation was found only in females ($P<0.08$). Thus, we found no evidence for a storage trade-off.

An alternative to increased energy storage in desert *Drosophila* is reduced consumption (i.e. lower metabolic rate). Several authors have proposed that reduced metabolic rates provide an adaptive phenotypic and evolutionary response to stress (Hoffmann and Parsons, 1991), particularly in arid environments (Lovegrove, 2000; Tieleman and Williams, 2000; Addo-Bediako et al., 2001). One implication is that, because desiccation is more stressful than starvation, desiccated flies may have lower metabolic rates than starved flies. In Table 3, we use our data to calculate rates of energy production, based on standard conversion factors for lipid, carbohydrate and protein metabolism. Calculated metabolic rates were generally higher for desiccated flies, in contrast to our prediction and in accordance with previous studies (Djawdan et al., 1997). We also calculated expected rates of CO_2 production using standard conversion factors (not shown). The rates measured for desiccated flies are within 20% of those measured using flow-through respirometry in dry air (A. G. Gibbs, F. Fukuzato and L. M. Matzkin, 2003a), providing independent evidence that our biochemical assays yielded an accurate assessment of metabolic rates.

Mass-specific metabolic rates of the two desert species, *D. mojavensis* and *D. nigrospiracula*, were ~60% lower than those of the three mesic species, under both experimental conditions (Table 3). These were two of the three largest species, so part of this difference may be related to the effects of size on metabolism (Calder, 1984). They also live in warmer habitats (Gibbs et al., 2003b), and insects from warmer environments tend to have lower metabolic rates (Chown and Gaston, 1999; Addo-Bediako et al., 2002). In addition, the desert species are relatively closely related to each other, so their

lower metabolic rates may reflect phylogeny rather than habitat. Thus, our data are consistent with the hypothesis that reduced metabolism contributes to desiccation resistance, but do not provide conclusive evidence.

A potential concern in our experiments is adaptation to laboratory conditions. Life history characters and stress resistance of *D. melanogaster* can change within three-four years of laboratory culture (Sgro and Partridge, 2000; Hoffmann et al., 2001). Reverse evolution experiments also indicate that starvation resistance and lipid content decline rapidly when strong directional selection is relaxed (Teotonio and Rose, 2000; Teotonio et al., 2002), although desiccation resistance does not (Service et al., 1988). Most of the species we used had been in culture less than two years when we performed our experiments (Table 1), and our previous work indicates that cactophilic *Drosophila* species remain desiccation resistant after 15 or more years in culture (Gibbs and Matzkin, 2001). Thus, we feel that adaptation to laboratory conditions probably was not a major factor affecting our results.

4.3. Comparisons to other insects

Our results provide a simple explanation for the evolution of different energy storage patterns in starvation- and desiccation-selected populations of *Drosophila*: flies store the substrates that they will need during the imposition of selection. In natural populations, however, desert *Drosophila* do not store larger quantities of lipids or carbohydrates. Instead, they have depressed metabolic rates relative to mesic congeners, which may allow them to survive food and water stress longer despite having similar storage patterns. These patterns resemble those found in other taxa. Other insects also use multiple substrates, with lipids predominating, during starvation stress (Hill and Goldsworthy, 1970; Edney, 1977; Lim and Lee, 1981; Auerswald and Gade, 2000; Satake et al., 2000), and desert insects tend to have lower metabolic rates than mesic species (Zachariassen et al., 1987; Addo-Bediako et al., 2001; Chown, 2002).

In contrast, *Drosophila* seem to differ from other insects in their metabolic responses to water stress. These responses are poorly understood, as studies of desert insects have generally focused on water budgets or have only measured overall metabolic rates. The limited data available suggest that most insects rely more heavily on lipid metabolism when desiccated, despite the fact that the total amount of water available is lower than for carbohydrates (Buxton and Lewis, 1934; Loveridge and Bursell, 1975; Nicolson, 1980; Nicolson, 1990). It must be noted that these insects (beetles, locusts, tsetse flies) are much larger than *Drosophila*, and therefore may have lower rates of water loss for their size (Edney, 1977). Thus, metabolic water derived from lipids may be sufficient to offset cuticular transpiration and respiratory

losses, whereas *Drosophila* benefit from the bound water released by carbohydrate metabolism.

Acknowledgements

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