

Evolution of stress resistance in *Drosophila*: interspecific variation in tolerance to desiccation and starvation

Luciano M. Matzkin†, Thomas D. Watts and Therese A. Markow*†

Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA

Summary

1. The extent to which variability in desiccation resistance among ecologically diverse *Drosophila* species is related to their ability to resist starvation is unknown. Resistance to desiccation and starvation was measured in females and males of ecologically and phylogenetically diverse *Drosophila* species.
2. We measured resistance to both stressors in ecologically and phylogenetically diverse species. In general females exhibited greater resistance to both stressors than males. Correcting for body size produces a highly significant correlation between resistances to both stressors in both sexes.
3. Phylogenetic relatedness, however, appears to have a large influence not only on resistance to both stressors, but also on the observed correlations between stressors.
4. Species of the *Drosophila* subgenus *Sophophora* examined in this study tend to be fruit breeders inhabiting more temperate and mesic habitats, whereas many of the species in the other major subgenus, *Drosophila*, tend to be cactophilic flies living in more xeric environments.
5. The difference between these two major subgenera, the *Sophophora* and the *Drosophila*, in the nature of the association we observed between desiccation and starvation resistance suggests that selection may have led to different mechanisms underlying resistance to these stressors in the two groups.

Key-words: *Drosophila*, desiccation resistance, starvation resistance, phylogenetic analysis, stress mechanisms, adaptation

Introduction

Among the strongest forces of natural selection are various environmental stressors such as temperature, relative humidity and dietary quantity and quality. It is not surprising, therefore, that evolutionary biologists are interested in understanding the genetic bases of adaptations to stress of many kinds (Hoffmann & Parsons 1993). Flies of the genus *Drosophila* provide a powerful model system for adaptive evolutionary studies of stress responses using both experimental (Telonis-Scott *et al.* 2006) and comparative approaches (Goto & Kimura 1998, Gibert *et al.* 2001, Gibbs & Matzkin 2001).

An unresolved question concerns the relationships among the physiological responses to different types of stress. For example, trade-offs may exist between responses to contrasting

stressors such as heat and cold tolerance. On the other hand, responses to stressors like desiccation and starvation could utilize, at least in part, overlapping physiological mechanisms (Service *et al.* 1985; Rose *et al.* 1992). A review of the literature on *Drosophila* desiccation and starvation resistance (Hoffmann & Harshman 1999), in fact, suggests that response to these two stressors may have at least a partially common basis. As pointed out by Rion & Kawecki (2007), however, understanding the ecological significance and evolution of this apparent relationship has seen little progress in the last decade. Only a few studies have examined both stress responses in the same species (van Herrewege & David 1997).

Desiccation resistance exhibits considerable inter- and intraspecific variability in *Drosophila*: temperate species are more resistant than those from the tropics (van Herrewege & David 1997), and desert species more resistant than mesic ones (Gibbs & Matzkin 2001; Matzkin *et al.* 2007). The higher resistance of desert species may reflect their relatively reduced water loss rates (Gibbs & Matzkin 2001) or lower mass specific metabolic rates (Gibbs *et al.* 2003; Marron *et al.* 2003).

*Corresponding author. E-mail: tmarkow@ucsd.edu

†Present address. Section of Ecology, Behavior and Evolution, Division of Biological Sciences, University of California, San Diego, Muir Biology, Rm. 2208, 9500 Gilman Drive # 0116, La Jolla, CA 92093-0116, USA

Studies of desiccation resistance are confounded, however, by the fact that desiccating conditions simultaneously expose flies to starvation. Because *Drosophila* culture medium contains moisture, it is difficult to expose flies to desiccating conditions without also withholding food. Measures of desiccation resistance thus are likely to contain also a component reflecting starvation resistance. One way to separate these two stressors is to look at starvation alone and determine the degree to which it is correlated with desiccation resistance. Not all species will experience both stressors equally. For example, if a species' feeding sites are far apart but the environment is typically humid, starvation may be a bigger problem than desiccation. In a dry habitat, desiccation would be a larger problem for flies in search of resources. Stress resistance could involve common or different pathways in different species and the degree to which mechanisms overlap will be influenced by phylogenetic and ecological constraints affecting each species.

Far less is known about the evolution of starvation resistance in *Drosophila* compared to desiccation tolerance. A majority of starvation studies have focused on *D. melanogaster* and many of these have been concerned with the relationship between caloric restriction and aging (Service *et al.* 1985; Rose *et al.* 1992; Rion & Kawecki 2007). The most extensive comparative study (van Herrewege & David 1997) tested both desiccation and starvation resistance in 20+ species and sought correlations with weight, water and lipid content. Though data were collected only for males and were not corrected for phylogenetic relatedness, the authors did compare tropical and temperate species, and reported a positive correlation in the latter, but not the former group between resistance to desiccation and starvation. Intraspecific differences that depend on ecology clearly show that the relationship between desiccation and starvation resistance is not simple and is likely to be heterogeneous among different lineages of *Drosophila* (Matzkin *et al.* 2007; van Herrewege & David 1997). Furthermore, species-specific sex differences in desiccation resistance (Matzkin *et al.* 2007) point to the existence of different physiological mechanisms in this trait alone.

Disentangling the question of mechanisms of resistance to desiccation and to starvation and the degree to which they are governed by similar mechanisms must begin with comparative studies of both traits in females and males. By controlling for phylogenetic relationships, underlying genetic correlations can be addressed. Here we report upon stress resistance in species from across the genus *Drosophila* to ask the degree to which desiccation and starvation resistance are phylogenetically constrained as well as the degree to which they are correlated in males and females of the different *Drosophila* subgenera.

Methods

DROSOPHILA SPECIES

We measured desiccation resistance for females and males of 23 *Drosophila* species and starvation resistance on a subset of 16. The species represented three subgenera: *Dorsilopha* (*D. busckii*), *Sophophora*

(*D. melanogaster*, *D. simulans*, *D. malerkotliana*, *D. affinis*, *D. pseudoobscura*, *D. persimilis*, *D. paulistorum* and *D. sturtevantii*), and *Drosophila* (*D. acanthoptera*, *D. pachea*, *D. nanoptera*, *D. hamatofila*, *D. spenceri*, *D. navojoa*, *D. arizonae*, *D. mojaviensis*, *D. hydei*, *D. nigrospiracula*, *D. anceps*, *D. eremophila*, *D. micrometleri* and *D. metleri*). Our interest was to have representation from the two major subgenera of *Drosophila*: *Sophophora* and *Drosophila*, to compare an evolutionarily diverged subgenus, *Dorsilopha*. The collection information for the fly strains used in this study is given in Table 1. With the exception of *D. persimilis*, *D. micrometleri*, *D. acanthoptera* and *D. navojoa* tests were performed on flies that had been in the laboratory a year or less (assays were performed in 2000). More recently collected strains of these species have not been available for testing. All flies were maintained in a 12 : 12 LD cycle at 24 °C and 35% humidity. Flies were reared on standard cornmeal–molasses–agar medium with the exception of *D. busckii*, which required Wheeler–Clayton medium. For *D. pachea*, senita cactus, which contains a required sterol, was added to the surface of the cultures.

DESICCATION RESISTANCE

Virgin females and males were separated under CO₂ and stored separately in banana food vials seeded with yeast until testing. At 3 days of age flies were placed in empty glass shell vials (five flies per vial) with foam plugs and introduced into a Plexiglas desiccation chamber maintained at 1% relative humidity (RH). The chamber was a 30 × 30 × 30-cm clear Plexiglas box with approximately 1.6 kg of Drierite brand desiccant in the bottom. Room air was pumped into the chamber through a column filled with Drierite at a rate of approximately 5 L min⁻¹, allowing the chamber to draw down humidity from ambient to 1% in 2 h or less. Temperature was kept at 24–25 °C. The desiccation chamber had a capacity of 80 vials, permitting males and females of a given species to be tested simultaneously with those of other species. Each species and sex was tested a minimum of three times. Following preliminary determinations of the times at which flies of each species began to die, the number of flies dead was scored at regular hourly intervals, until effectively all flies had died.

STARVATION RESISTANCE

Flies were grown and harvested as in the desiccation experiments. On day 3 post-eclosion, flies were introduced into vials containing 10 mL of 0.5% agar in groups of five flies per vial. The tops of the vials were covered in Parafilm and the vials were changed to fresh medium every 48 h. Deaths were scored three times per day until all flies had died. Data were collated and analysed as per the desiccation experiments.

THORAX LENGTH

For species with previously measured thorax lengths, published data were used (Pitnick *et al.* 1995). For species with no published thorax length (*D. paulistorum*, *D. malerkotliana*, *D. hamatofila*, *D. sturtevantii* and *D. spenceri*) thorax lengths means were determined using 20 males and 20 females per species using an ocular micrometer.

STATISTICAL ANALYSIS

LT_{50s} (lethal tolerance time, in hours, at which 50% of flies had died) were calculated by linear regression analysis of the percent dead over

Table 1. Species used in desiccation and starvation experiments and their collection localities and dates. Flies were collected in nature by members or visitors to the author's laboratory with the exception of *D. busckii*, *D. hamatofila*, *D. acanthoptera*, *D. anceps* and *D. micrometleri* which came from the now closed Bowling Green Stock Centre

Subgenus	Species group	Species	Locality	Date
<i>Dorsilopha</i>		<i>D. busckii</i>	Netherlands	Oct 1999
<i>Sophophora</i>	melanogaster	<i>D. melanogaster</i>	Tempe, AZ	Feb 1999
		<i>D. simulans</i>	Tempe, AZ	Nov 1998
<i>D. malerkotliana</i>		Barro Colorado Isl, Panama	Mar 1999	
	obscura	<i>D. affinis</i>	Baton Rouge, LA	Oct 1999
		<i>D. pseudoobscura</i>	Tempe, AZ	Nov 1998
		<i>D. persimilis</i>	Yosemite Nat'l Park, CA	1996
	willistoni	<i>D. paulistorum</i>	Barro Colorado Isl, Panama	Mar 1999
	saltans	<i>D. sturtevantii</i>	Barro Colorado Isl, Panama	Mar 1999
<i>Drosophila</i>	nannoptera	<i>D. pachea</i>	Ensenada de los muertos, B.C.S., MX	Nov 1998
		<i>D. nannoptera</i>	Tehuacan valley, Puebla, MX	Jul 1998
	repleta	<i>D. acanthoptera</i>	Oaxaca City, Oaxaca, MX	Feb 1976
		<i>D. hamatofila</i>	San Telmo, B.C., MX	1996
		<i>D. spenceri</i>	San Jose del Cabo, B.C.S., MX	Mar 1999
		<i>D. navojoa</i>	El dorado, Sinaloa, MX	Nov 1984
		<i>D. arizonae</i>	Ensenada de los muertos, B.C.S., MX	Nov 1998
		<i>D. mojavenis</i>	San Carlos, Sonora, MX	May 1999
		<i>D. hydei</i>	Madera Canyon, AZ	Jul 1999
		<i>D. nigrospiracula</i>	Organ Pipe Cactus Nat'l Monument, AZ	Oct 1998
		<i>D. anceps</i>	Huauapan de Leon, Oaxaca, MX	May 1992
		<i>D. eremophila</i>	Ensenada de los muertos, B.C.S., MX	Nov 1998
		<i>D. mettleri</i>	Organ Pipe Cactus Nat'l Monument, AZ	Oct 98
<i>D. micrometleri</i>	Port Henderson, Jamaica	Unknown		

Table 2. Mean, standard error and sample size (in parentheses) for desiccation and starvation LT₅₀

Species	Desiccation resistance		Starvation resistance	
	Females	Males	Females	Males
<i>D. busckii</i>	15.23 ± 0.58 (20)	10.51 ± 0.29 (20)	104.90 ± 1.99 (20)	58.10 ± 1.52 (20)
<i>D. melanogaster</i>	15.56 ± 0.15 (32)	9.49 ± 0.16 (31)	45.09 ± 1.79 (32)	59.37 ± 3.72 (31)
<i>D. simulans</i>	14.11 ± 0.45 (33)	8.06 ± 0.15 (32)	58.91 ± 0.80 (33)	48.69 ± 1.05 (32)
<i>D. malerkotliana</i>	15.18 ± 0.33 (27)	8.28 ± 0.30 (29)	71.23 ± 3.12 (27)	75.62 ± 3.64 (29)
<i>D. sturtevantii</i>	7.7 ± 0.9 (28)	7.1 ± 0.8 (32)		
<i>D. affinis</i>	13.18 ± 0.43 (15)	9.27 ± 0.28 (18)		
<i>D. pseudoobscura</i>	30.77 ± 0.60 (36)	30.28 ± 0.60 (33)	48.04 ± 2.85 (36)	53.80 ± 2.12 (33)
<i>D. persimilis</i>	22.81 ± 0.35 (17)	21.78 ± 0.43 (17)		
<i>D. paulistorum</i>	10.66 ± 0.96 (15)	8.91 ± 0.24 (15)	93.13 ± 3.16 (15)	79.38 ± 1.69 (15)
<i>D. acanthoptera</i>	18.63 ± 0.46 (18)	15.90 ± 0.47 (20)	106.18 ± 1.03 (18)	104.94 ± 1.01 (20)
<i>D. pachea</i>	33.28 ± 0.76 (51)	35.41 ± 0.78 (58)		
<i>D. nannoptera</i>	34.57 ± 0.99 (22)	31.14 ± 1.07 (24)	103.87 ± 3.91 (22)	106.41 ± 4.07 (24)
<i>D. hamatofila</i>	39.90 ± 1.49 (14)	37.04 ± 1.02 (14)	85.67 ± 1.50 (14)	84.10 ± 3.53 (14)
<i>D. spenceri</i>	30.58 ± 0.95 (20)	30.68 ± 1.15 (18)		
<i>D. navojoa</i>	29.11 ± 0.71 (20)	25.02 ± 0.61 (19)		
<i>D. arizonae</i>	42.70 ± 1.62 (38)	31.94 ± 1.19 (38)	141.12 ± 2.72 (38)	129.96 ± 3.00 (38)
<i>D. mojavenis</i>	48.14 ± 1.32 (51)	46.29 ± 1.54 (51)	170.73 ± 7.24 (51)	141.01 ± 4.65 (51)
<i>D. hydei</i>	18.12 ± 0.50 (38)	21.44 ± 0.54 (45)	71.44 ± 3.04 (38)	73.24 ± 2.19 (45)
<i>D. nigrospiracula</i>	44.62 ± 1.21 (35)	38.95 ± 0.88 (35)	138.03 ± 4.10 (35)	123.23 ± 4.01 (35)
<i>D. anceps</i>	31.03 ± 0.96 (24)	23.20 ± 1.33 (25)	129.46 ± 2.74 (24)	98.98 ± 2.53 (25)
<i>D. eremophila</i>	22.10 ± 0.38 (23)	19.44 ± 0.38 (22)		54.74 ± 5.07 (22)
<i>D. micrometleri</i>	14.4 ± 1.2 (17)	13.5 ± 1.0 (20)		
<i>D. mettleri</i>	47.37 ± 1.88 (21)	36.65 ± 1.04 (21)	123.30 ± 4.31 (21)	112.22 ± 4.41 (21)

time in each vial and two-way ANOVA (for species and sex) was performed for each stress. To examine species-specific differences for desiccation and starvation resistance between sexes we performed *t*-test, correcting for multiple comparisons using a Bonferroni correction.

To remove the possible correlation associated with phylogenetic relatedness (Felsenstein 1985) we calculated phylogenetically independent contrasts of size-independent measurements of desiccation and starvation resistance. Using size-independent measurements is paramount as body size in *Drosophila* is known to correlate with

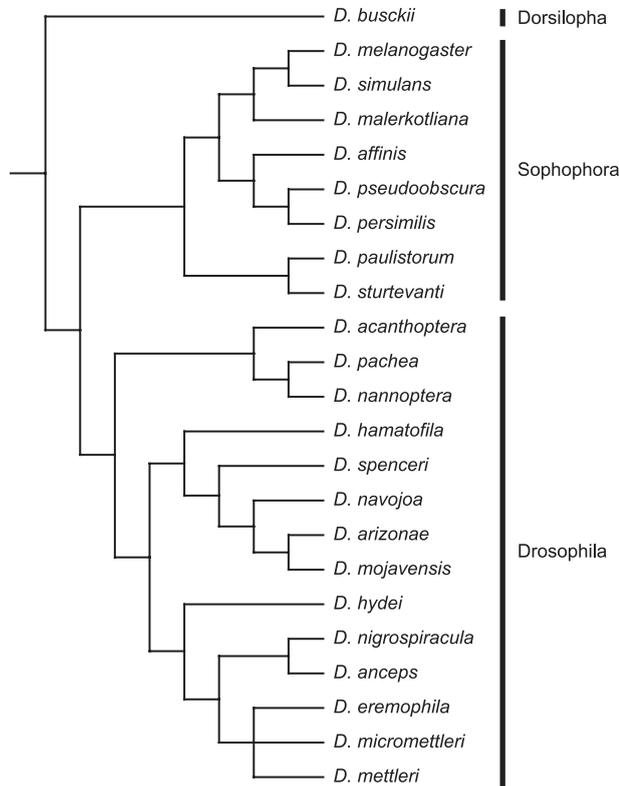


Fig. 1. Phylogenetic relationships of *Drosophila* species used in this study. Vertical bars indicated subgenus membership.

stress resistance (Gibbs & Matzkin 2001). Body size effects were removed by utilizing the residuals of the regression between thorax length and stress resistance. Phylogenetically independent contrasts were calculated using the relationship of *Drosophila* species shown in Fig. 1, assuming equal branch lengths, and the CAIC v. 2.6.9 software (Purvis & Rambaut 1995). Given the uncertainty in divergence times between certain lineages in this study, we opted to utilize a cladogram of the independent contrast analysis. Additionally, previous studies on the evolution of desiccation resistance in *Drosophila* have suggested that phylogenetic independent contrast analysis using divergence times or a cladogram yielded similar results (Gibbs & Matzkin 2001). The phylogenetic relationships shown in Fig. 1 is a cladogram generated from previous studies (Markow & O'Grady 2005a; Pitnick *et al.* 1995). The relationships between the phylogenetic independent contrast of desiccation and starvation resistance were examined by calculating the product-moment coefficients of 'positivized' contrasts through the origin as suggested by Garland *et al.* (1992). All statistical analyses (ANOVA, regression and correlations) were performed using the JMP ver. 5 software.

Results

DESICCATION RESISTANCE

Species ($F = 332.6$, $P < 0.001$) and sexes ($F = 122.1$, $P < 0.001$) varied widely in their resistance to low relative humidity (Fig. 2, Table 2 and Table S1 in Supporting Information). The

desert endemic *D. mojavensis* survived the longest compared to the rapid desiccation of *D. sturtevantii*, a tropical species. For 12 species (*D. acanthoptera*, *D. affinis*, *D. anceps*, *D. arizonae*, *D. busckii*, *D. eremophila*, *D. malerkotliana*, *D. melanogaster*, *D. metleri*, *D. navojoa*, *D. nigrospiracula* and *D. simulans*) females were more desiccation resistant (significant at $P < 0.0021$ with Bonferroni correction see Table S2). The opposite pattern, greater resistance in males than females, was observed only for *D. hydei*.

STARVATION RESISTANCE

Starvation resistance was measured for fewer of the species than desiccation but considerable variability across species ($F = 163.2$, $P < 0.001$) and sex ($F = 33.2$, $P < 0.001$) nonetheless was observed (Fig. 2 Table 2 and Table S3). Most resistant to starvation were the cactophilic *D. mojavensis*, *D. nigrospiracula*, *D. arizonae* and *D. nannopectera* while *D. simulans*, *D. malerkotliana*, and *D. paulistorum* (fruit breeders) were the least resistant. In species with significant sex effect, as with desiccation, females were in general more resistant (Table S4). The most extreme sex difference was observed in *D. busckii*, at 104 h for females compared to 58 h for males. Additionally we observed females having a significantly greater starvation resistance for four other species (*D. anceps*, *D. mojavensis*, *D. paulistorum* and *D. simulans*) (Table S4). Males significantly resisted starvation better than females only in *D. melanogaster*. Sex differences in starvation resistance were not observed in nine species (see Table S4).

SIZE AND RESISTANCE

Drosophila exhibit substantial interspecific size variation as well as species differences in the degree of sexual size dimorphism (Table S5). As expected a positive association exists between body size and stress resistance (see Fig. S1 in Supporting Information). The strongest, but not always significant, relationship was found for desiccation resistance in both females ($b_{y,x} = 29.7$, $F_{1,19} = 3.20$, $P = 0.08$) and males ($b_{y,x} = 37.9$, $F_{1,19} = 5.29$, $P = 0.03$). For starvation resistance, although a positive relationship was observed (Fig. S1) it was not significant for either females or males ($b_{y,x} = 36.9$, $F_{1,13} = 0.46$, $P = 0.51$ and $b_{y,x} = 62.6$, $F_{1,14} = 1.98$, $P = 0.18$, respectively).

RELATIONSHIP BETWEEN RESISTANCES TO STRESSORS

After removing the effect of body size (using the residuals of a stress resistance vs. body size regression) a strong positive correlation between desiccation and starvation resistance is detected for both sexes ($r = 0.67$, $P = 0.006$ and $r = 0.66$, $P = 0.005$, for females and males respectively, see Fig. 3). While these correlations suggest some common mechanism underlying resistance to both types of stress, the species studied do not represent phylogenetically independent points. Thus a correction for evolutionary relatedness is necessary to infer any correlations. When the size corrected data are analysed

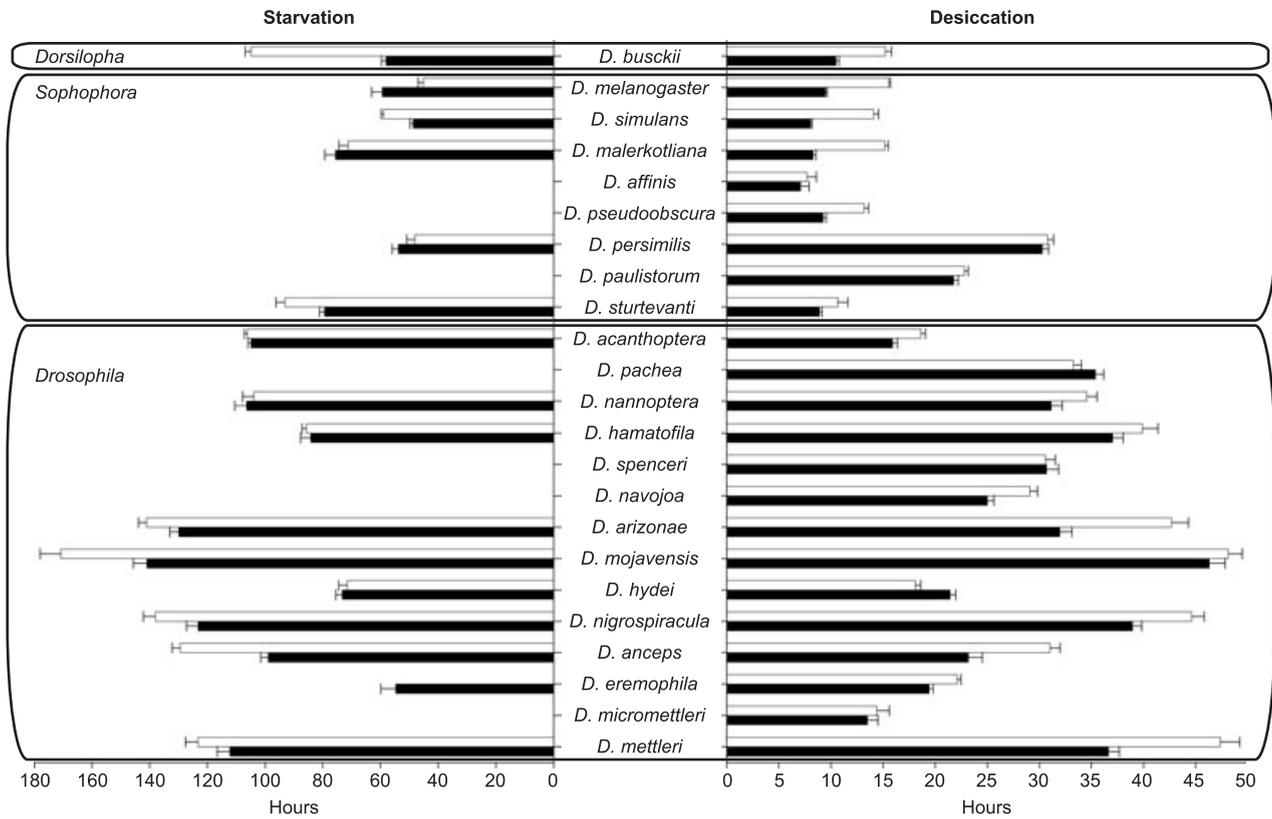


Fig. 2. Mean and standard error of LT_{50} for starvation (left panel) and desiccation (right panel). White and black bars are females and males, respectively. Boxes represent subgenera membership.

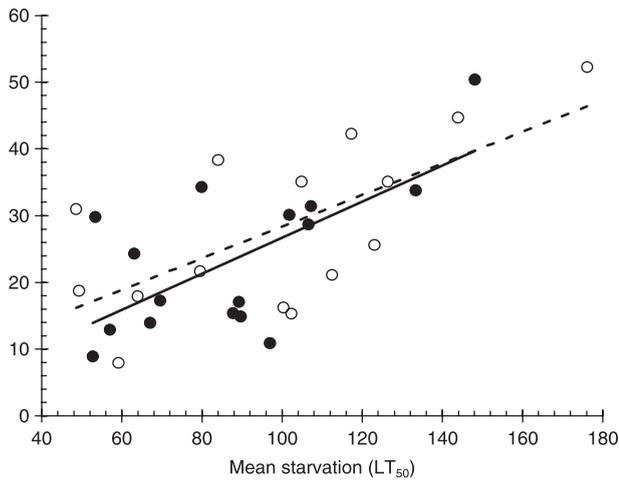


Fig. 3. Relationship between starvation and desiccation after removing the effects of size (using residuals). Open circles are females and closed circles males. Correlation for females, dashed line, ($r = 0.67$, $P = 0.006$) and males, solid line, ($r = 0.66$, $P = 0.005$) are shown.

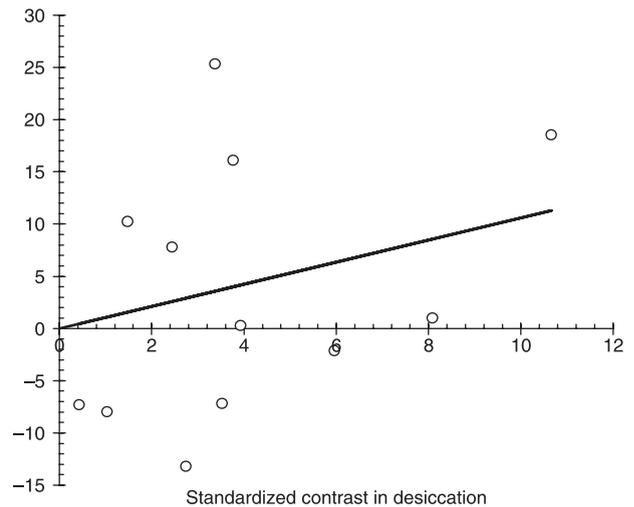


Fig. 4. Size corrected and phylogenetically standardized contrasts for desiccation and starvation resistance in females. Correlation ($r = 0.33$, $P = 0.30$) is shown.

using phylogenetically independent contrasts, the positive relationship is still observed but no longer is statistically significant either for females ($r = 0.33$, $P = 0.30$, see Fig. 4) or males ($r = 0.46$, $P = 0.11$, see Fig. 5). A distinct pattern was

observed when the species are grouped according to their subgenera, either *Sophophora* or *Drosophila* (*D. busckii* was omitted from these analyses as it is the only member of the subgenus *Dorsilopha* used in this study). Although not

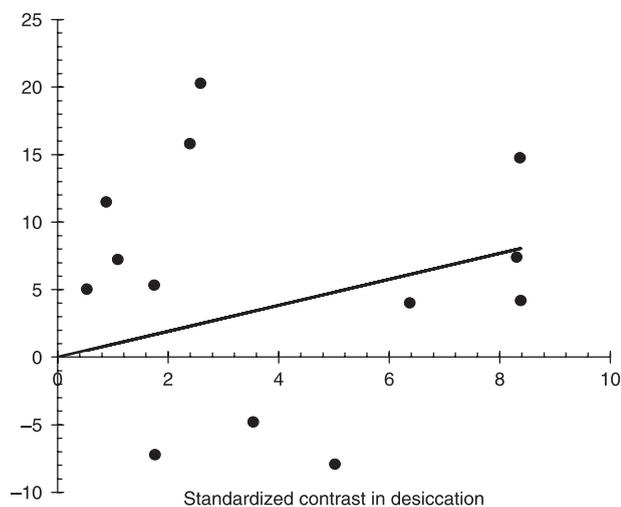


Fig. 5. Size corrected and phylogenetically standardized contrasts for desiccation and starvation resistance in males. Correlation ($r = 0.46$, $P = 0.11$) is shown.

significant the correlation between desiccation and starvation in *Sophophora* appears to be negative ($r = -0.33$, $P = 0.67$ and $r = -0.44$, $P = 0.56$ for females and males respectively), whereas it is positive in *Drosophila* ($r = 0.44$, $P = 0.32$ and $r = 0.44$, $P = 0.38$ for females and males respectively). This association appears stronger when pooling both sexes ($r = -0.44$ and $r = 0.41$, for *Sophophora* and *Drosophila*, respectively) and is marginally significantly different ($P = 0.10$) from each other (Test of Homogeneity, Sokal & Rohlf 1995).

Discussion

Desiccation and starvation resistance both exhibit significant sex and species differences. Interspecific variation in desiccation resistance is sixfold, whereas starvation resistance differences among species are only threefold. For both stressors, on the whole, females outperform males.

Comparative studies conducted in the laboratory will never fully reproduce conditions faced by organisms in nature. Each species used in the current study experiences different relative humidities in the wild. While clearly it was impractical to rear each species under different abiotic conditions or use more than one type of test of resistance for these experiments, other rearing or testing protocols may yield different outcomes. Adaptation to laboratory conditions is another factor that can confound comparative studies of this type. Whereas *Drosophila* are no exception, Rego *et al.* (2007) showed that for two species, *D. subobscura* and *D. madeirensis*, several years in the laboratory produced no change in starvation resistance.

After correcting for body size, a highly significant positive correlation between desiccation and starvation resistance is observed for both sexes. Once the effect of phylogenetic

history is removed, however, the relationship is no longer statistically significant. The influence of phylogenetic relatedness is very obvious from the graphs presented in Fig. 2. It was not our original intent to examine each subgenus separately. We were able, however, to use the limited number of species for each major subgenus (*Sophophora* and *Drosophila*) for which we had both desiccation and starvation data to perform separate independent contrast analyses. The correlations between independent contrasts for desiccation and starvation resistance appear to be in opposite directions albeit not significant, between *Sophophora* and *Drosophila* both in females and males. Pooling across both sexes the correlation coefficient of *Sophophora* and *Drosophila* are marginally significantly different from each other. At the subgeneric level, mechanisms underlying resistance to desiccation and starvation appear to be correlated. It is likely, however, that the subgeneric differences reflect the existence of different mechanisms underlying stress resistance in the two groups, but testing additional species is needed to verify this pattern.

For the most part, the species examined from the *Sophophoran* subgenus are cosmopolitan (human commensals) and/or tropical in their distributions and they primarily feed and breed in decaying fruits (Markow & O'Grady 2005a,b, 2008). The species from the subgenus *Drosophila*, on the other hand, are associated with necrotic cacti and because of the distribution of the cactus hosts, are found in more xeric habitats. Thus the abiotic environments in which members of the two subgenera live tend to be different. Cacti and fruit also differ tremendously in nutritional profiles from the elemental to the biochemical levels and these differences are reflected in the body compositions of the flies that consume them (Markow *et al.* 1999, Jaenike & Markow 2003). Nutritional differences easily can be envisioned as driving differences in energy metabolism and storage and therefore starvation resistance. Cactophilic species are characterized by a lower metabolic rate and water loss rate than non-cactophilic congeners (Gibbs & Matzkin 2001, Marron *et al.* 2003).

Given the low frequency of viable cactus hosts in the field (Breitmeyer & Markow 1998), cactophilic *Drosophila* not only have to survive periods of low humidity but also extended periods of starvation. Therefore, it is expected that a correlation would exist between starvation and desiccation resistance of cactophilic flies. This is potentially what is driving the positive correlation observed between desiccation and starvation phylogenetically independent contrasts in the subgenus *Drosophila*, as the majority of species sampled from that subgenus are cactophiles. Furthermore, it is expected that selection for increased desiccation resistance would not be as severe in *Drosophila* inhabiting more mesic environments, such as the ones sampled in this study from the subgenus *Sophophora*. Independence of the evolution of desiccation and starvation resistance has been shown to occur under certain artificial selection regimes in *D. melanogaster* (Graves *et al.* 1992, Passananti *et al.* 2004a,b). Our study suggests that under certain ecological conditions decoupling of the mechanisms involved in desiccation and starvation resistance also can occur in nature.

References

- Breitmeyer, C.M. & Markow, T.A. (1998) Resource availability and population size in cactophilic *Drosophila*. *Functional Ecology*, **12**, 14–21.
- Felsenstein, J. (1985) Phylogenies and the comparative method. *American Naturalist*, **125**, 1–15.
- Garland, T., Jr., Harvey, P.H. & Ives, A.R. (1992) Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Systematic Biology*, **41**, 18–32.
- Gibbs, A., Fukuzato, F. & Matzkin, L. (2003) Evolution of water conservation mechanisms in *Drosophila*. *Journal of Experimental Biology*, **206**, 1183–1192.
- Gibbs, A.G. & Matzkin, L.M. (2001) Evolution of water balance in the genus *Drosophila*. *Journal of Experimental Biology*, **204**, 2331–2338.
- Gibert, P., Moreteau, B., Petavy, G., Karan, D. & David, J.R. (2001) Chill-coma tolerance, a major climatic adaptation among *Drosophila* species. *Evolution*, **55**, 1063–1068.
- Goto, S.G. & Kimura, M.T. (1998) Heat- and cold-shock responses and temperature adaptations in subtropical and temperate species of *Drosophila*. *Journal of Insect Physiology*, **44**, 1233–1239.
- Graves, J.L., Toolson, E.C., Jeong, C., Vu, L.N. & Rose, M.R. (1992) Desiccation, flight, glycogen, and postponed senescence in *Drosophila melanogaster*. *Physiological Zoology*, **65**, 268–286.
- Hoffmann, A.A. & Harshman, L.G. (1999) Desiccation and starvation resistance in *Drosophila*: patterns of variation at the species, population and intrapopulation levels. *Heredity*, **83**, 637–643.
- Hoffmann, A.A. & Parsons, P.A. (1993) *Evolutionary Genetics and Environmental Stress*. Oxford University Press, Oxford.
- Jaenike, J. & Markow, T.A. (2003) Comparative elemental stoichiometry of ecologically diverse *Drosophila*. *Functional Ecology*, **17**, 115–120.
- Markow, T.A. & O'grady, P.M. (2005a) *Drosophila: A guide to Species Identification and Use*. Academic Press, London.
- Markow, T.A. & O'grady, P.M. (2005b) Evolutionary genetics of reproductive behavior in *Drosophila*: Connecting the dots. *Annual Review of Genetics*, **39**, 263–291.
- Markow, T.A. & O'grady, P.M. (2008) Reproductive ecology of *Drosophila*. *Functional Ecology*, **22**, 747–759.
- Markow, T.A., Raphael, B., Dobberfuhl, D., Breitmeyer, C.M., Elser, J.J. & Pfeiler, E. (1999) Elemental stoichiometry of *Drosophila* and their hosts. *Functional Ecology*, **13**, 78–84.
- Marron, M.T., Markow, T.A., Kain, K.J. & Gibbs, A.G. (2003) Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila*. *Journal of Insect Physiology*, **49**, 261–270.
- Matzkin, L.M., Watts, T.D. & Markow, T.A. (2007) Desiccation resistance in four *Drosophila* species: sex and population effects. *Fly*, **1**, 268–273.
- Passananti, H.B., Beckman, K.A. & Rose, M.R. (2004a) Relaxed stress selection in *Drosophila melanogaster*. *Methuselah Flies: A Case Study in the Evolution of Aging* (eds M.R. Rose, H.B. Passananti & M. Matos), pp. 323–352. World Scientific, Singapore.
- Passananti, H.B., Deckert-Cruz, D.J., Chippindale, A.K., Le, B.H. & Rose, M.R. (2004b) Reverse evolution of aging in *Drosophila melanogaster*. *Methuselah Flies: A Case Study in the Evolution of Aging* (eds M.R. Rose, H.B. Passananti & M. Matos), pp. 297–322. World Scientific, Singapore.
- Pitnick, S., Markow, T.A. & Spicer, G.S. (1995) Delayed male maturity is a cost of producing large sperm in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 10614–10618.
- Purvis, A. & Rambaut, A. (1995) Comparative analysis by independent contrasts (caic): an apple macintosh application for analysing comparative data. *Computer Applications in the Biosciences*, **11**, 247–251.
- Rego, C., Rose, M.R. & Matos, M. (2007) Do species converge during adaptation? A case study in *Drosophila*. *Physiological and Biochemical Zoology*, **80**, 347–357.
- Rion, S. & Kawecki, T.J. (2007) Evolutionary biology of starvation resistance: what we have learned from *Drosophila*. *Journal of Evolutionary Biology*, **20**, 1655–1664.
- Rose, M.R., Vu, L.N., Park, S.U. & Graves, J.L. (1992) Selection on stress resistance increases longevity in *Drosophila melanogaster*. *Experimental Gerontology*, **27**, 241–250.
- Service, P.M., Hutchinson, E.W., MacKinley, M.D. & Rose, M.R. (1985) Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. *Physiological Zoology*, **58**, 380–389.
- Sokal, R.R. & Rohlf, F.J. (1995) *Biometry*. W.H. Freeman and Co., New York.
- Telonis-Scott, M., Guthridge, K.M. & Hoffmann, A.A. (2006) A new set of laboratory-selected *Drosophila melanogaster* lines for the analysis of desiccation resistance: Response to selection, physiology and correlated responses. *Journal of Experimental Biology*, **209**, 1837–1847.
- Van Herreweghe, J. & David, J.R. (1997) Starvation and desiccation tolerances in *Drosophila*: Comparison of species from different climatic origins. *Ecoscience*, **4**, 151–157.

Received 15 August 2008; accepted 28 November 2008

Handling Editor: Frank Messina

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Relationship between thorax length and desiccation and starvation resistance.

Table S1 Two-way ANOVA for desiccation resistance

Table S2 *t*-tests for desiccation resistance between sexes for each species

Table S3 Two-way ANOVA for starvation resistance

Table S4 *t*-tests for starvation resistance between sexes for each species

Table S5 Mean thorax length for all *Drosophila* species in this study

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.