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).
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 not find correlations with weight, water and lipid content.

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. sturtevanti), and Drosophila (D. acanthoptera, D. pachea, D. nan Алексоиа, D. hamatoфília, D. spenceri, D. navоjоа, D. artизона, D. mojaуенсис, D. hydei, D. nigrospiracula, D. anepа, D. emеуопhília, D. micromettleri and D. mettleri). 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.

**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 (Ptinick et al. 1995). For species with no published thorax length (D. paulistorum, D. malerkotliana, D. hamatoфília, D. sturtevanti and D. spenceri) thorax lengths means were determined using 20 males and 20 females per species using an ocular micrometer.

**STATISTICAL ANALYSIS**

LT₅₀ (lethal tolerance time, in hours, at which 50% of flies had died) were calculated by linear regression analysis of the percent dead over
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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. micromettleri* which came from the now closed Bowling Green Stock Centre

<table>
<thead>
<tr>
<th>Subgenus</th>
<th>Species group</th>
<th>Species</th>
<th>Locality</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsilopha</td>
<td>melanogaster</td>
<td><em>D. busckii</em></td>
<td>Netherlands</td>
<td>Oct 1999</td>
</tr>
<tr>
<td>Sophophora</td>
<td>melanogaster</td>
<td><em>D. melanogaster</em></td>
<td>Tempe, AZ</td>
<td>Feb 1999</td>
</tr>
<tr>
<td></td>
<td>simulans</td>
<td><em>D. simulans</em></td>
<td>Tempe, AZ</td>
<td>Nov 1998</td>
</tr>
<tr>
<td></td>
<td>malerkotiiana</td>
<td><em>D. malerkotiiana</em></td>
<td>Barro Colorado Isl, Panama</td>
<td>Mar 1999</td>
</tr>
<tr>
<td></td>
<td>affinis</td>
<td><em>D. affinis</em></td>
<td>Baton Rouge, LA</td>
<td>Oct 1999</td>
</tr>
<tr>
<td></td>
<td>pseudoobscura</td>
<td><em>D. pseudoobscura</em></td>
<td>Tempe, AZ</td>
<td>Nov 1998</td>
</tr>
<tr>
<td></td>
<td>persimilis</td>
<td><em>D. persimilis</em></td>
<td>Yosemite Nat’l Park, CA</td>
<td>1996</td>
</tr>
<tr>
<td>willistoni</td>
<td><em>D. willistoni</em></td>
<td>Barro Colorado Isl, Panama</td>
<td>Mar 1999</td>
<td></td>
</tr>
<tr>
<td>G. saltans</td>
<td><em>D. saltans</em></td>
<td>Barro Colorado Isl, Panama</td>
<td>Mar 1999</td>
<td></td>
</tr>
</tbody>
</table>

| | melanogaster | *D. melanogaster* | Tempe, AZ | Jul 1998 |
| | simulans | *D. simulans* | Tempe, AZ | Nov 1998 |
| | malerkotiiana | *D. malerkotiiana* | Barro Colorado Isl, Panama | Mar 1999 |
| | sturtevanti | *D. sturtevanti* | Barro Colorado Isl, Panama | Mar 1999 |

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Desiccation resistance</th>
<th>Starvation resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td><em>D. busckii</em></td>
<td>15.23 ± 0.58 (20)</td>
<td>10.51 ± 0.29 (20)</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>15.56 ± 0.15 (32)</td>
<td>9.49 ± 0.16 (31)</td>
</tr>
<tr>
<td><em>D. simulans</em></td>
<td>14.11 ± 0.45 (33)</td>
<td>8.06 ± 0.15 (32)</td>
</tr>
<tr>
<td><em>D. malerkotiiana</em></td>
<td>15.18 ± 0.33 (27)</td>
<td>8.28 ± 0.30 (29)</td>
</tr>
<tr>
<td><em>D. sturtevanti</em></td>
<td>7.7 ± 0.9 (28)</td>
<td>7.1 ± 0.8 (28)</td>
</tr>
<tr>
<td><em>D. affinis</em></td>
<td>13.18 ± 0.43 (15)</td>
<td>9.27 ± 0.28 (18)</td>
</tr>
<tr>
<td><em>D. pseudoobscura</em></td>
<td>30.77 ± 0.60 (36)</td>
<td>30.28 ± 0.60 (33)</td>
</tr>
<tr>
<td><em>D. persimilis</em></td>
<td>22.81 ± 0.35 (17)</td>
<td>21.78 ± 0.35 (17)</td>
</tr>
<tr>
<td><em>D. paulistorum</em></td>
<td>10.66 ± 0.96 (15)</td>
<td>8.91 ± 0.24 (15)</td>
</tr>
<tr>
<td><em>D. acanthoptera</em></td>
<td>18.63 ± 0.46 (18)</td>
<td>15.90 ± 0.47 (20)</td>
</tr>
<tr>
<td><em>D. pachea</em></td>
<td>33.28 ± 0.76 (51)</td>
<td>35.41 ± 0.78 (58)</td>
</tr>
<tr>
<td><em>D. arizonae</em></td>
<td>34.57 ± 0.99 (22)</td>
<td>31.14 ± 1.07 (24)</td>
</tr>
<tr>
<td><em>D. mojavensis</em></td>
<td>39.90 ± 1.49 (14)</td>
<td>37.04 ± 1.02 (14)</td>
</tr>
<tr>
<td><em>D. spenceri</em></td>
<td>30.58 ± 0.95 (20)</td>
<td>30.68 ± 1.15 (18)</td>
</tr>
<tr>
<td><em>D. navojoa</em></td>
<td>29.11 ± 0.71 (20)</td>
<td>25.02 ± 0.61 (19)</td>
</tr>
<tr>
<td><em>D. arizonae</em></td>
<td>42.70 ± 1.62 (38)</td>
<td>31.94 ± 1.19 (38)</td>
</tr>
<tr>
<td><em>D. mojavensis</em></td>
<td>48.14 ± 1.32 (51)</td>
<td>46.29 ± 1.54 (51)</td>
</tr>
<tr>
<td><em>D. hydei</em></td>
<td>18.12 ± 0.50 (38)</td>
<td>21.44 ± 0.54 (45)</td>
</tr>
<tr>
<td><em>D. nigrospiracula</em></td>
<td>44.62 ± 1.21 (35)</td>
<td>38.95 ± 0.88 (35)</td>
</tr>
<tr>
<td><em>D. anceps</em></td>
<td>31.03 ± 0.96 (24)</td>
<td>23.20 ± 1.33 (25)</td>
</tr>
<tr>
<td><em>D. eremophila</em></td>
<td>22.10 ± 0.38 (23)</td>
<td>19.44 ± 0.38 (22)</td>
</tr>
<tr>
<td><em>D. micromettleri</em></td>
<td>14.4 ± 1.2 (17)</td>
<td>13.5 ± 1.0 (20)</td>
</tr>
<tr>
<td><em>D. mettleri</em></td>
<td>47.37 ± 1.88 (21)</td>
<td>36.65 ± 1.04 (21)</td>
</tr>
</tbody>
</table>

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 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.
were performed using the ‘positivized’ contrasts through the origin as suggested by Gar
were examined by calculating the product-moment coefficients of independent contrast of desiccation and starvation resistance
in 2005a; a cladogram generated from previous studies (Markow & O’Grady & Matzkin 2001). The phylogenetic relationships shown in Fig. 1 is using divergence times or a cladogram yielded similar results (Gibbs
have suggested that phylogenetic independent contrast analysis of
times between certain lineages in this study, we opted to utilize a relationship of
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. acanchoptera, D. affinis, D. aneps, D. arizone, D. busckii, D. eremophilia, D. malerkotliana, D. mela
nogaster, D. mettleri, D. navojoa, D. nigrosipricula 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. nigrosipricula, D. arizone and D. nannoptera 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. aneps, 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 ($b_{x} = 29·7, F_{1,15} = 3·20, P = 0·08$) and males ($b_{x} = 37·9, F_{1,19} = 5·29, P = 0·03$). For starvation resistance, although a positive relationship was observed (Fig. S1) it was not significant for either females or males ($b_{x} = 36·9, F_{1,11} = 0·46, P = 0·51$ and $b_{x} = 62·6, F_{1,11} = 1·98, P = 0·18$, 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. 1. Phylogenetic relationships of Drosophila species used in this study. Vertical bars indicated subgenus membership.
Evolution of stress resistance in *Drosophila*


Using phylogenetically independent contrasts, the positive relationship is still observed but no longer is statistically significant either for females \( (r = 0.33, P = 0.30) \) 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. 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.

significant the correlation between desiccation and starvation resistance 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 congeneres (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


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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

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