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Research Paper

Desiccation Resistance in Four Drosophila Species

Sex and Population Effects

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population variation, adaptation, stress resistance, mesic adaptation, xeric adaptation, Drosophila, desiccation resistance

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ABSTRACT

Desiccation resistance and body mass were measured in multiple populations of each of four species of Drosophila: two desert endemic species (*D. nigrospiracula* and *D. mojavensis),* and two with more widespread distributions (*D. melanogaster* and *D. pseudoobscura*). While flies from the desert species were more desiccation tolerant, there was, in certain cases, significant variation in desiccation resistance among populations of the same species. A significant difference in desiccation resistance was observed between the sexes, females were more resistant than males, but this relationship was reversed when taking into account body mass differences between the sexes. The degree of observed within-species variability demonstrates that studies focusing upon differences between species can produce different conclusions if they rely on observations for only single populations of a given species. Our data also suggest the existence of multiple mechanisms for desiccation resistance.

INTRODUCTION

Desiccation is a serious problem for small insects, thus natural selection is expected to have favored increased resistance to low humidity in species residing in arid environments.[1] Flies of the genus Drosophila are attractive for studies of the genetic, molecular, and ecological bases of desiccation resistance because of the wide variety of habitats in which they are found and because of the ease with which most species are reared and manipulated in the laboratory. For Drosophila, variation in desiccation resistance has been observed both at the inter- and intraspecific levels.[1-5] Although clear and predictable associations with obvious environmental variables have not always been found at either level, an overall pattern suggests that among Drosophila, species from more arid places are more resistant to desiccation.[6] These species differences are expected to originate at the intraspecific level, during the process of differentiation between geographically distant populations. It is not always the case, however, that such clear antecedents are observed. Coyne et al.[7] found that while geographic populations of *D. pseudoobscura* differed in their resistance to environmental stress, the resistance was not always as predicted by local climatic conditions. Additionally, evidence suggests that the level of genetic variation for desiccation resistance can vary greatly between species and could have drastic effects on a species' ability to survive climatic change.[5,8,9]

Mechanisms underlying the evolution of desiccation resistance are likely to be multiple, complex, and possibly even different in between species. Associations with certain environmental variables may be obscured by a lack of knowledge of the ecologies of the species examined, suggesting that Drosophila species for which more detailed information about microhabitats and basic biology in the wild are better candidates for future studies.

Four Drosophila species are endemic to the Sonoran Desert of North America, utilizing various species of necrotic cacti as feeding and breeding sites.[10,11] The macro- and microhabitats and biology of these desert species have been well characterized,[12-16] and their ecology differs considerably from other Drosophila such as *D. melanogaster* and *D. pseudoobscura*, which also have been popular model systems for evolutionary studies. Endemism to the harsh desert predicts that flies of these species should be more desiccation resistant than any other Drosophila. Although previous studies have detected increased desiccation resistance in xeric-adapted Drosophila associated with decreased metabolic rate and cuticular water loss[6,17] they did not examine within-species variability, using only one laboratory strain for each species tested.

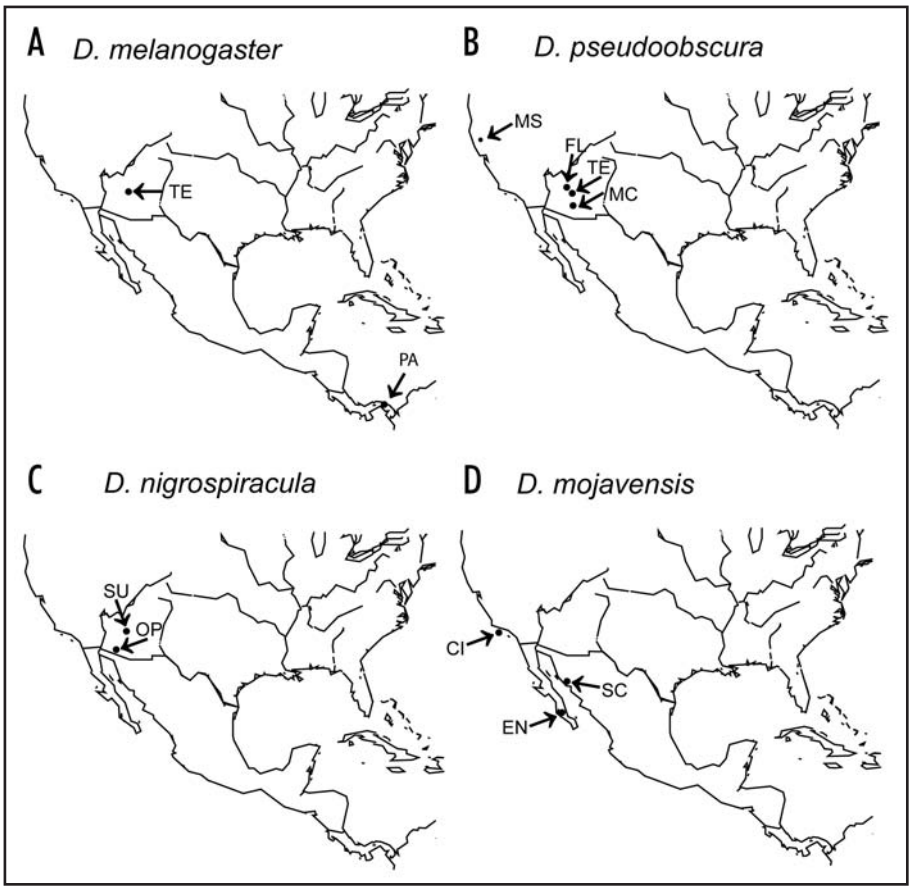

Figure 1. Collecting sites for all *D. melanogaster* (A), *D. pseudoobscura* (B), *D. nigrospiracula* (C) and *D. mojavensis* (D) samples. PA = Canal Zone, Panama; TE = Tempe, AZ; MC = Madera Canyon, AZ; FL = Flagstaff, AZ; MS = Mount Saint Helena, CA; SC = San Carlos, Sonora, MX; EN = Ensenada de los Muertos, Baja California Sur, MX; CI = Catalina Island, CA; OP = Organ Pipe Cactus National Monument, AZ; SU = Superstition Mts, AZ.

In this study, we ask the following questions: (i) Do species of Drosophila endemic to the Sonoran Desert of North America exhibit greater resistance to desiccation than species not endemic to this arid region? (ii) Is there a consistent sex difference in desiccation resistance across species? (iii) To what extent do populations of the same species from different geographic areas exhibit variation in desiccation resistance? We address these questions by measuring desiccation resistance in two species endemic to the Sonoran Desert, *D. mojavensis* and *D. nigrospiracula* and in two other species, *D. melanogaster* and *D. pseudoobscura*, that have much more widespread distributions.

MATERIALS AND METHODS

Drosophila strains. Four species, *D. melanogaster, D. pseudoobscura, D. mojavensis* and *D. nigrospiracula* were collected at the localities shown for each in Figure 1. Collections of *D. melanogaster* from Tempe, Arizona were made in February 1999 (designated TE), and from Gamboa, Panama (PA), in March 1998, by Therese A. Markow. *Drosophila pseudoobscura* were collected from Madera Canyon (MC), Arizona by Therese A. Markow in July 1999, from Tempe (TE), Arizona in November 1998 by Therese A. Markow, from Flagstaff (FL), Arizona and from Mount Saint Helena (MS), California by M. Noor in July 1997. Collections of *D. mojavensis* were made by Therese A. Markow from San Carlos (SC), Sonora Mexico in May 1999 and Ensenada de los Muertos (EN) in November 1998 by Therese A. Markow. and from Santa Catalina Island (CI) in April 1997 by G. Hocutt. The collections of *D. nigrospiracula* from Organ Pipe Cactus National Monument (OP) were made by G. Hocutt in October 1998 and from the Superstition Mountains (SU) of Arizona by Therese A. Markow in October 1998. The *D. melanogaster*, *D. pseudoobscura* and *D. nigrospiracula* stocks were generated by pooling two isofemale lines each. An initial analysis showed no line-to-line variation. The *D. mojavensis* stock consisted of one isofemale line from San Carlos and mass populations for the other two locales. All flies were kept in low-density cultures in bottles of banana/opuntia food. Flies were tested within several generations of their capture, with experiments completed in early 2000.

Analysis of desiccation resistance and body size. Virgin females and males were stored separately in banana food vials seeded with yeast until testing. At three days of age flies were placed in empty glass shell vials (five flies per vial) with foam plugs and placed in a Plexiglas desiccation chamber maintained at 1% relative humidity (RH). The desiccation chamber consisted of a 15x15x15-inch clear Plexiglas box floored with approximately 1600 grams of Drierite brand desiccant. Room air was pumped into the chamber through a column filled with Drierite at a rate of 300 cubic inches per minute. This air exchange allowed the chamber to draw down humidity from ambient to 1% in two hours or less. Temperature was kept at 24–25°C. For a given population and sex, a minimum of four vials were tested per run (17–43 vials per population and sex, see Table 1). The desiccation chamber had a capacity of 80 vials, permitting males and females of a given species to be tested simultaneously with males and females of other species and populations. Each population and sex was tested a minimum of three times. After preliminary tests to determine the times at which flies of each species began to die, scoring of the number of flies dead was performed at regular intervals, usually hourly, until effectively all flies had died. LT_{50}s (lethal tolerance time, hours, at which 50% of flies had died) were calculated by linear regression analysis on the percent dead over time in each vial. Dry weight was used as a measure of body size. Virgins were collected on the day of eclosion, separated by sex, stored in cornmeal vials with yeast until three days post eclosion and dried at 50°C for 72 hours. A total of ten dried flies (per species, population and sex) were then weighed on a Cahn Model C-31 microbalance.

LT_{50}s and dry mass for various populations were analyzed by a nested ANOVA using JMP Version 4.04 software (SAS Institute Inc., Cary, NC) for each sex separately. Since mass could influence desiccation resistance, the data was also analyzed in a similar nested ANOVA as the ratio of LT_{50} over dry mass. As suggested by Sokal and Rohlf[18] these values were square-root transformed. To determine the extent

Table 1 **Mean ± SE for LT_{50} and Dry mass (in micrograms)**

	LT_{50}		Dry mass	
	Female	**Male**	**Female**	**Male**
D. melanogaster				
PA	16.2 ± 0.2 (39)	10.0 ± 0.2 (24)	318.3 ± 9.4 (10)	200.2 ± 4.4 (10)
TE	15.6 ± 0.1 (32)	9.5 ± 0.2 (30)	373.6 ± 12 (10)	254.0 ± 7.4 (10)
D. pseudoobscura				
TE	30.5 ± 0.6 (33)	29.7 ± 0.5 (31)	589.4 ± 18.7 (10)	373.7 ± 9.3 (10)
MC	29.0 ± 0.5 (42)	28.1 ± 0.4 (43)	582.3 ± 16.3 (10)	356.9 ± 12.9 (10)
FL	29.0 ± 0.7 (28)	25.4 ± 0.6 (29)	545.5 ± 12.3 (10)	315.3 ± 7.0 (10)
MS	28.7 ± 0.5 (25)	24.6 ± 0.4 (28)	566.4 ± 10.9 (10)	369.0 ± 15.5 (10)
D. mojavensis				
CI	50.4 ± 2.5 (17)	51.4 ± 2.7 (19)	653.8 ± 16.3 (10)	517.8 ± 17.8 (10)
SC	47.8 ± 2.0 (23)	45.3 ± 2.0 (23)	742.3 ± 28.9 (10)	497.3 ± 18.0 (10)
EN	46.5 ± 1.2 (19)	38.0 ± 0.9 (24)	513.6 ± 33.1 (10)	286.9 ± 21.8 (10)
D. nigrospiracula				
SU	40.5 ± 1.2 (37)	40.9 ± 1.4 (37)	986.8 ± 40.6 (10)	707.6 ± 19.3 (10)
OP	44.6 ± 1.2 (35)	39.2 ± 0.9 (34)	956.0 ± 52.4 (10)	665.4 ± 21.6 (10)

Sample size for LT_{50} (number of vials) and for dry mass (number of flies) is given in parentheses after the mean and standard error. PA = Panama, TE = Tempe, MC = Madera Canyon, FL = Flagstaff, MS = Mt. Saint Helena, CI = Catalina Island, SC = San Carlos, EN = Ensenada de los Muertos, SU = Superstition Mountains, OP = Organ Pipe National Monument.

Table 2 **Nested analysis of variance for each sex for dry mass, LT_{50} and LT_{50}/mass**

	Female				Male			
	df	**SS**	**F**	**% variance**	**df**	**SS**	**F**	**% variance**
Dry mass								
Species	3	4076315.1	31.98***	82.4%	3	2344715.2	14.76**	78.7%
Pop(Species)	7	297383.1	6.06***	5.9%	7	370709.7	22.66***	14.6%
Error	99	694139.6		11.7%	99	231356.1		6.7%
LT_{50}								
Species	3	352.06	234.74***	88.4%	3	448.6011	70.23***	88.1%
Pop(Species)	7	3.50	2.75**	0.7%	7	14.9051	11.01***	3.1%
Error	320	58.20		10.0%	311	60.1351		8.9%
LT_{50}/mass								
Species	3	166.4923	10.51**	59.4%	3	489.2258	14.62**	72.3%
Pop(Species)	7	36.9533	20.58***	16.3%	7	78.0553	28.80***	13.6%
Error	320	82.0956		24.4%	311	120.4050	1	4.2%

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

of sex-specific desiccation difference and its possible interaction among populations of a given species a two-way ANOVA (using population as a random effect) was performed on the transformed ratio of LT_{50} over dry mass.

RESULTS

Sex-specific and species-specific differences in desiccation resistance. The means of desiccation resistance, as LT_{50}, for females and males of the populations of all four species are provided in Table 1. Even prior to any statistical analyses, several patterns are obvious. First, despite the presence of intraspecific variability in desiccation resistance, the two desert species, *D. mojavensis* and *D. nigrospiracula*, survive on average at least 10 hours longer than do *D. pseudoobscura*. *Drosophila pseudoobscura* in turn has a mean resistance that is 15 hours longer than *D. melanogaster*. Testing for the influence of body size on interspecific differences is not one of the goals of this study, as it requires data for a larger number of species analyzed phylogenetically. It is clear, however, that desert endemic species are larger than flies of the other two species (Fig. 2 and Table 1). *Drosophila nigrospiracula* is approximately three times the dry weight of *D. melanogaster*; the dry weight of *D. mojavensis* is nearly twice that of *D. melanogaster*. *Drosophila pseudoobscura* are, on average, smaller than *D. mojavensis*, but intraspecific differences in *D. mojavensis* size created some degree of overlap. Significant differences in desiccation resistance (LT_{50}) and dry mass were observed between species as well as between populations within species (Table 2). Furthermore, significant differences between and within species were seen for size-corrected desiccation

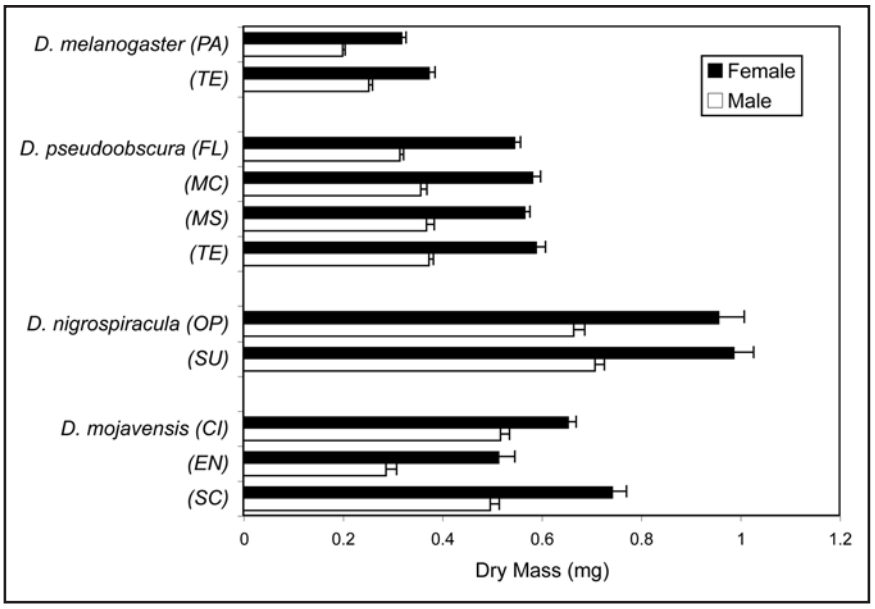

Figure 2. Mean and std. error of dry mass (in mg) for each species, population and sex. PA = Panama, TE = Tempe, MC = Madera Canyon, FL = Flagstaff, MS = Mt. Saint Helena, CI = Catalina Island, SC = San Carlos, EN = Ensenada de los Muertos, SU = Superstition Mountains, OP = Organ Pipe National Monument.

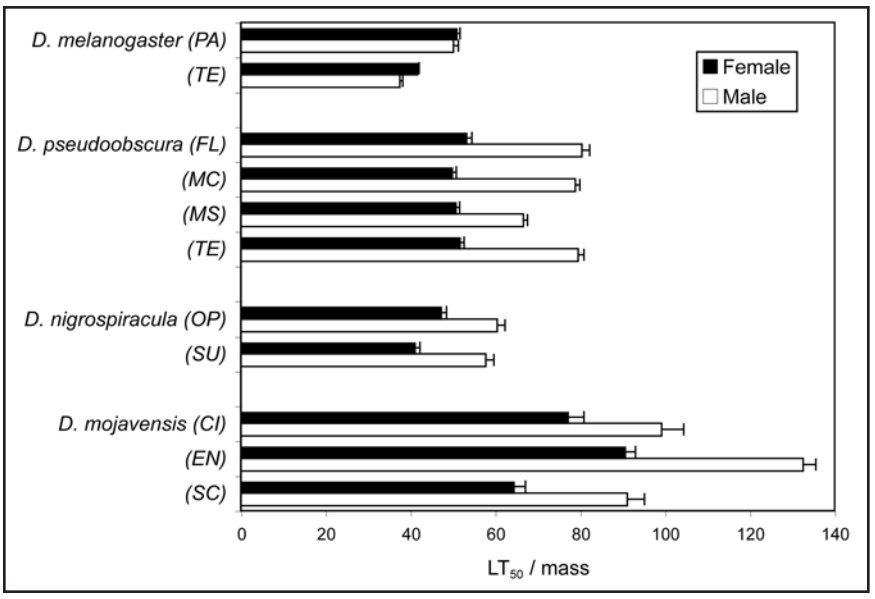

Figure 3. Mean and std. error of LT_{50}/dry mass for each species, population and sex.

resistance (LT_{50}/mass) in both sexes (Table 2). The relationship between body mass and desiccation resistance does not appear to be linear. A significant regression was observed for both females and males between log (LT_{50}) and log (dry mass) (slope = 0.94, $r^2 = 0.65$, $p < 0.001$ and slope = 1.07, $r^2 = 0.58$, $p < 0.001$, for females and males, respectively). Thus while body size may be associated with increased desiccation resistance, desiccation resistance also exists that is independent of body size.

Figure 3 illustrates the differences in LT_{50}/mass across our study. *Drosophila mojavensis* females and males had significantly greater LT_{50}/mass than all other species (Tables 1 and 3). For size-corrected desiccation resistance the general order was *D mojavensis* > *D. nigrospiracula* ≈ *D. pseudoobscura* ≈ *D. melanogaster* for females and *D. mojavensis* > *D. pseudoobscura* > *D. nigrospiracula* > *D. melanogaster* for males.

Intraspecific variation in desiccation resistance. Our second and third question was concerned with detecting intraspecific differences in desiccation resistance, as well as differences between the sexes. Although between and within species differences were observed for LT_{50}, dry mass and LT_{50}/mass, differences between the species explained the largest percent of the variation (Table 2). When examined individually, significant population effects in LT_{50}/mass were observed for each of the four species (Table 4). In addition to population differences, there were large significant differences in LT_{50}/mass between the sexes (Table 4). A significant interaction between population and sex was found only for *D. melanogaster* and *D. pseudoobscura* (Table 4).

DISCUSSION

A large amount of variation in desiccation resistance was observed both within and between the four species examined in this study, although the greatest amount of variation was across species. A similar pattern was observed for male Drosophila in the multi-species desiccation resistance analysis of van Herrewege and David[2] (a study that did not focus on females). The variation found in our study, whether between collection localities and/or between sexes, permitted us to examine the relationship in each population between desiccation resistance and other variables such as body size.

Resistance to desiccation is a function both of the water content of the organism and its rate of water loss.[19] Water content can be further subdivided into that which comes via ingestion and that from metabolic sources such as glycogen and lipids.[20] In many terrestrial arthropods loss of water can occur via the cuticle, respiration and excretion. In Drosophila, the pattern of respiration can be drastically different between mesic and xeric adapted species[17] and this could be one of the causes for the inter- and intraspecific variation in desiccation resistance we observed in this study.

Overall, the two xeric adapted species (*D. mojavensis* and *D. nigrospiracula*) exhibited a greater desiccation resistance (t-test, d.f. = 306, t = -23.6, $p < 0.001$, and d.f. = 319, t = -20.2, $p < 0.001$ for females and males, respectively) than the two mesic adapted species (*D. melanogaster* and *D. pseudoobscura*). Interspecific differences in desiccation resistance between habitats (temperate vs. tropical) were also observed by van Herrewege and David.[2] The only species in common (*D. melanogaster*) with that study had about twice the desiccation resistance observed in this study. That study used a different methodology to examine desiccation resistance than the one used here. The difference between xeric- and mesic-adapted species is not as strong (*t*-test, d.f. = 151, t = -4.3, $p < 0.001$, and d.f. = 224, t = -5.0, $p < 0.001$ for females

Table 3 **Pairwise difference in LT_{50}/mass in females and males between populations and species**

	D. melanogaster (PA)	*D. melanogaster* (TE)	*D. pseudoobscura* (FL)	*D. pseudoobscura* (MC)	*D. pseudoobscura* (MS)	*D. pseudoobscura* (TE)	*D. nigrospiracula* (OP)	*D. nigrospiracula* (SU)	*D. mojavensis* (CI)	*D. mojavensis* (EN)	*D. mojavensis* (SC)
D. melanogaster (PA)	-	**0.147**	-0.3915	-0.4162	-0.5554	-0.4657	-0.2325	**0.237**	**0.9668**	**1.7631**	**0.2806**
D. melanogaster (TE)	**0.2214**	-	**0.2574**	**0.0811**	**0.0744**	**0.182**	-0.1403	-0.4626	**1.6191**	**2.4146**	**0.9308**
D. pseudoobscura (FL)	**1.136**	**2.1421**	-	-0.3088	-0.4422	-0.4681	-0.1235	**0.3457**	**0.7745**	**1.5695**	**0.0846**
D. pseudoobscura (MC)	**1.1091**	**2.1189**	-0.5673	-	-0.4929	-0.3826	-0.2981	**0.1714**	**1.0482**	**1.8448**	**0.3627**
D. pseudoobscura (MS)	**0.3313**	**1.3371**	**0.0802**	**0.0559**	-	-0.5189	-0.3069	**0.1622**	**0.9243**	**1.7188**	**0.2331**
D. pseudoobscura (TE)	**1.1025**	**2.1093**	-0.656	-0.5992	**0.0471**	-	-0.1981	**0.2712**	**0.8931**	**1.6888**	**0.2051**
D. nigrospiracula (OP)	-0.0418	**0.9659**	**0.5177**	**0.4965**	-0.2875	**0.4852**	-	-0.0507	**1.2362**	**2.0322**	**0.5491**
D. nigrospiracula (SU)	-0.2169	**0.7916**	**0.7179**	**0.6981**	-0.0875	**0.6856**	-0.457	-	**1.7049**	**2.501**	**1.0181**
D. mojavensis (CI)	**1.9864**	**2.9881**	**0.1345**	**0.2721**	**0.9275**	**0.1893**	**1.3613**	**1.5599**	-	**0.0357**	**0.0416**
D. mojavensis (EN)	**3.6444**	**4.6485**	**1.7946**	**1.9362**	**2.5872**	**1.8501**	**3.0231**	**3.2226**	**0.7753**	-	**0.8357**
D. mojavensis (SC)	**1.6308**	**2.6345**	-0.2194	-0.0784	**0.5733**	-0.1639	**1.0088**	**1.2082**	-0.4436	**1.2138**	-

Pairwise Tukey-Kramer test. Abs(Difference) - LSD is show for each comparison. Females differences are on top of the diagonal and male differences are below the diagonal. Values in bold are significant ($p < 0.0009$). PA = Panama, TE = Tempe, MC = Madera Canyon, FL = Flagstaff, MS = Mt. Saint Helena, CI = Catalina Island, SC = San Carlos, EN = Ensenada de los Muertos, SU = Superstition Mountains, OP = Organ Pipe National Monument.

and males, respectively) when corrected for body size differences between the species (Fig. 3). This is due to the fact that although *D. nigrospiracula* is one of the most desiccation resistant flies (Table 1), it is also the largest out of the four species studied (Fig. 2). For *D. nigrospiracula* size thus may be an important component of desiccation resistance, although it appears less important for *D. mojavensis*. Females are heavier than males, potentially explaining the observed differences in LT_{50}/mass between the sexes. In three of four species, males exhibit higher LT_{50}/mass values, suggesting that they may be under selection to survive as long as females and maintain a 1:1 sex ratio in nature.

Making predictions about interspecific resistance based upon climatic conditions is complicated by the fact that, for widespread species like *D. melanogaster* or *D. pseudoobscura*, the range of habitats is much broader than for endemic species. This is especially true for *D. melanogaster* a species which is believed to have originated in tropical Africa and has recently (< 500 years) colonized North America.[21] Despite its recent arrival to North America, evidence suggests that *D. melanogaster* populations have rapidly adapted to their newly colonized habitats.[22,23] In the present study, this is exemplified by the several-fold difference in precipitation between Tempe, Arizona, in the Sonoran Desert, and the other localities from which these two species were sampled. It is possible that flies of the two mesic species studied here disappear from desert areas during the most stressful times of the year, in which case collections from less stressful times may not permit accurate testing of predictions about regional variation in desiccation resistance. Of the three *D. mojavensis* localities, Catalina Island has the most mild climate, which would predict that flies from this location would be the least resistant to desiccation. Since the opposite is observed, other factors, such as wind, may be important.

Resource microhabitat and use could have a major effect on exposure to desiccation stress. Among the cactophilic species, for example, the various cacti differ significantly in water content[13] and in patch duration.[12] Organpipe (*Stenocereus thurberi)* necroses last on average 4.5 months, and are more abundant and smaller than saguaro necroses which last on average 6 months.[12] Although no data on the longevity of *Opuntia ssp.* necroses are available, given the small size of the cactus cladodes relative to an organpipe's arm it can be assumed that they will last for substantially less time than necrotic organpipe stems. If the *Opuntia* cactus pads used by *D. mojavensis* on Catalina Island dry out more rapidly than do the arms of columnar cacti used by flies from Sonora or Baja California, *Drosophila* may move around more looking for hosts on Catalina than in the desert, experiencing greater exposure than conspecifics from other localities. Other factors that could prevent Drosophila from remaining in more moist microhabitats might include the presence of predators or competitors in those microhabitats.[13] The observation that in some cases only one sex exhibited significant geographic differentiation (Tables 1 and 3) may reflect differences in the microhabitats in which they spend their time. A similar pattern was reported earlier by Coyne et al.[7] Drosophila species do differ in the distribution of males and females on their resources[24] perhaps leading to one sex being more exposed to local environmental stress than the other. Additionally, selection does not necessarily have to be on desiccation resistance. For example selection for increases in glycogen[25] or cuticular hydrocarbon length[17,26] would also have a correlated desiccation resistance response. Another potentially important difference between the sexes is that the smaller size of males increases their cuticular surface area to volume ratio and therefore their relative water loss rate.

In some species, such as *D. nigrospiracula,* populations have been found to exhibit almost no genetic differentiation across their range.[27-29] This observation, coupled with the fact that they are relatively strong dispersers, may mean that strong gene flow prevents the build up of any local adaptation in this species. Populations of *D. mojavensis* and *D. pseudoobscura* are characterized

Table 4 **Intraspecific ANOVA analysis for LT_{50}/mass**

	df	SS	F
D. melanogaster			
Sex	1	1.20	12.91***
Pop	1	20.32	218.39***
Sex × Pop	1	0.64	6.89**
Error	121	11.26	
D. pseudoobscura			
Sex	1	153.74	916.15***
Pop	3	7.41	14.73***
Sex × Pop	3	5.50	10.92***
Error	251	42.12	
D. nigrospiracula			
Sex	1	39.21	95.27***
Pop	1	3.83	9.32**
Sex × Pop	1	0.70	1.70 ns
Error	140	57.62	
D. mojavensis			
Sex	1	74.13	96.41***
Pop	2	70.45	45.81***
Sex × Pop	2	3.47	2.26 ns
Error	119	91.50	

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns not significant.

by considerable genetic differentiation across the ranges used in this study,[30-33] consistent with the finding of significant differences in a phenotypic character such as desiccation resistance.

The observation that populations of a given species can differ significantly in desiccation resistance has important implications for interspecific comparisons. Species comparisons that employ observations on only one population for a given species run the risk of making an inaccurate generalization about that species. This would be especially true when attempting to correlate species performance with a climatic variable, as can be seen from the data reported here. For example, if the only population of *D. mojavensis* tested were the one from CI, we would have concluded that this species was significantly more desiccation resistant than *D. nigrospiracula*. If, on the other hand, we had used only the ENMU population, we would not have found any difference.

Finally, the degree to which body size influences desiccation resistance may reflect the action of other physiological mechanisms and it will be important to identify them. This interpretation is supported by the nonlinear relationship between LT_{50} and dry mass. An obvious one is starvation resistance, since desiccating flies also are not receiving food. If starvation resistance were measured on a different set of the same species or strains, its relative contribution to desiccation resistance, especially via body weight, could be assessed. The fact that the influence of body mass on desiccation resistance differs among species suggests that resistance to desiccation possibly involves multiple mechanisms.

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