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A comparison of behavioural change in *Drosophila* during exposure to thermal stress

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In order to understand how adaptive tolerance to stress has evolved, we compared related species and populations of *Drosophila* for a variety of fitness relevant traits while flies directly experienced the stress. Two main questions were addressed. First, how much variation exists in the frequency of both courtship and mating among *D. melanogaster*, *D. simulans*, and *D. mojavensis* when each are exposed to a range of temperatures? Second, how does variation in these same behaviours compare among four geographically isolated populations of *D. mojavensis*, a desert species with a well defined ecology? Our hierarchical study demonstrated that mating success under stress can vary as much between related species, such as *D. melanogaster* and *D. simulans*, as between the ecologically disparate pair, *D. melanogaster* and *D. mojavensis*. Strains of this latter desert species likewise varied in tolerance, with differences approaching the levels observed among species. The consequences of stress on male courtship differed markedly from those on female receptivity to courtship, as mating behaviours among species and among strains of *D. mojavensis* varied in subtle but significant ways. Finally, a comparison of variation in thermotolerance of F₁ hybrids between the two most extreme *D. mojavensis* populations confirmed that genetic variation underlying traits such as survival or the ability to fly after heat stress is completely different. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, **83**, 197–205.

ADDITIONAL KEYWORDS: courtship – diversity – *Drosophila mojavensis* – flight – genetic variation – mating behaviour – receptivity – thermotolerance.

INTRODUCTION

Temperature variation heavily impacts the distribution and abundance of many species and therefore the evolution of behavioural traits will vary along environmental gradients. Changes in behaviour will depend upon the manner and frequency with which populations encounter thermal stress (Parsons, 1979; David *et al.*, 1983; Hoffmann & Parsons, 1991). Species that inhabit a broad geographical area that spans many different environments may adapt to local conditions. Subsequently, they may express greater levels of variation in response to extreme stresses than those species whose range encompasses a more homogeneous set of environments (Hoffmann *et al.*, 2001). Extreme habitats, such as desert environments, pro-

vide particularly intense thermal stresses, and may be some of the best places to identify local adaptation.

To assess this variation, Bennett (1987a, b) has argued that multiple aspects of fitness should be studied because changes in physiological performance along a thermal gradient may vary by trait (e.g. Arnold, 1987; Koehn, 1987). In addition, a growing body of literature raises doubts as to whether selection for physiological tolerance of heat will increase adult survival in natural populations, or whether animals respond more strongly for traits such as mating behaviour that are more closely linked to fitness (Feder, 1996; Shine *et al.*, 2000; Hoffmann, Sørensen & Loeschcke, 2003).

In this paper, we ask whether there is a link between standard estimates of tolerance to high temperatures based on survival and other fitness-related characters, such as successful male courtship and female receptivity of males (mating frequency given

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successful courtship). We apply a comparative approach to assess why different species and populations of one desert species may vary in their response to changes in stress. To impart ecological relevance, we assessed behavioural responses during the application of a stress, rather than fitness consequences after a stress had been encountered. These comparisons were made using three *Drosophila* species, which differ in LD₅₀ for temperature given 1 h exposures; 38 °C for *D. simulans* Sturtevant, 39 °C for *D. melanogaster* Meigen, and 41 °C for *D. mojavensis* Patterson and Crow (Krebs, 1999). *Drosophila melanogaster* and *D. simulans* are closely related phylogenetically and ecologically, while *D. mojavensis* is an unrelated species that inhabits thermally diverse environments, including the deserts of south-western North America. Populations of this latter species vary significantly in allozyme frequencies (Zouros, 1973; Markow, Castrezana & Pfeiler, 2002), while *D. melanogaster* and *D. simulans* tend to be genetically more uniform in the Americas (Begun & Aquadro, 1993; Benassi & Veuille, 1995).

Using multiple populations of *D. mojavensis*, we tested three additional questions. (1) Do survival, courtship, mating and flight covary among populations? (2) Does variation relate predictably with the climate from which populations were collected? (3) Will mating success decline as a consequence of inhibiting a male's ability to court at high temperatures or are effects more pronounced in females? Imposing environmental stress can shift genetic trade-offs, whether assessed for resource availability (Messina & Fry, 2003) or temperature (Krebs & Loeschcke, 1999). Furthermore, identification of the conditions under which some thermal-response mechanisms, such as heat-shock proteins and/or shifts in metabolism, become important enhances opportunities to understand how physiology and behaviour evolve (Feder & Hofmann, 1999).

MATERIAL AND METHODS

The three species used for the interspecies comparisons were *Drosophila melanogaster*, *D. simulans* and *D. mojavensis*. Both *D. melanogaster* and *D. simulans* were obtained in September of 1998 at Patterson Farms in Chesterland, OH, USA (Patton & Krebs, 2001), with mass populations drawn from multiple lines of foundresses. Both of these sympatric species complete their life cycles in and around necrotic fruit. The *D. mojavensis* strain used in the interspecies comparison was obtained from Dr Teri Markow at the University of Arizona (SOSC0297); this population originated from flies aspirated directly from organ pipe cactus (*Stenocereus thurberi*) in the Sonoran Desert near San Carlos, Sonora, Mexico in 1997.

Therefore, each strain had been reared in the laboratory 3–4 years before use in these experiments in 2001.

Four additional populations of *D. mojavensis* were obtained for the intraspecific comparison. This set of laboratory strains collected by Dr Markow in 1999 originated from three localities: Santa Catalina Island (CI), 43 km west of Los Angeles, the one site where this species uses prickly pear cactus (*Opuntia*) as a substrate; Ensenada de los Muertos (EN), 35 km SE of La Paz, along the Cape Region of Baja California, Mexico, where agria cactus (*Stenocereus gummosus*) is the most common host plant; and Santa Rosa Mountains (SR) in southern Arizona, USA, another site dominated by organ pipe cactus (see Heed & Mangan, 1986; Markow *et al.*, 2002). We collected a fresh San Carlos (SC) population in January 2000 (Krebs, Patton & Fasolo, 2000).

The intraspecific comparisons were made in summer 2002. Several sets of experiments indicate that stress tolerance remains fairly constant in laboratory populations, at least for several years (Krebs & Loeschcke, 1999; Krebs *et al.*, 2001). All *Drosophila* strains were maintained at Cleveland State University on a standard cornmeal–yeast–molasses–agar medium containing tegosept and propionic acid, where they were reared either at room temperature (21–23 °C; *D. melanogaster* and *D. simulans*) or in a 25 °C incubator (*D. mojavensis*). Although rearing temperatures differed, this change was required because *D. mojavensis* larvae develop very slowly when laboratory temperatures dropped below about 22–23 °C, and small temperature differences have little effect on acclimation to heat (Bettencourt, Feder & Cavicchi, 1999). To obtain flies for experiments, all rearing bottles were cleared (adult flies removed and discarded) prior to collection to ensure that collected flies were virgins. For *D. melanogaster* and *D. simulans*, bottles were cleared in the late evening for early morning collection of adults or cleared in the morning for late afternoon collections, and never longer than 12 h after a bottle had been cleared. For *D. mojavensis*, adults were collected daily, because in this species there is a longer delay in sexual activity after emergence (Markow, 1982).

For collection, all flies were anaesthetized with CO₂ gas and separated by sex under a dissection microscope. Approximately ten flies were then placed into fresh glass holding vials containing roughly 2 mL of medium and a sprinkling of dry yeast. Once separated, *D. melanogaster* and *D. simulans* adults matured for 4–5 days before use in experiments. *Drosophila mojavensis* adults were held for 7–9 days before use to account for the greater time that the desert species needed to reach sexual maturity (Markow, 1982). Flies were transferred into fresh vials

midway through the holding period to prevent bacterial growth and to promote maximum health, and each species was kept at the same temperatures at which they were reared.

MATING EXPERIMENTS AMONG SPECIES

Mating experiments were conducted in the same general fashion for the comparison of three *Drosophila* species and for the comparison of four *D. mojavensis* populations. Each pair of flies was observed only once. The specific thermal conditions in an environmental room were controlled manually using two analogue thermostats. One controlled heat and the other an exhaust system to cool the room; together they maintained constant temperatures. Overhead fluorescent bulbs served as a light source. A small holding incubator set at 25 °C was used within the room to minimize the amount of time flies were exposed to high experimental temperatures. All vials were equilibrated to the predetermined experimental temperature, which was recorded as the average of readings from two physiological mercury thermometers placed within spare vials also containing medium. One female fly was aspirated into a vial followed by a single male, and the pair was observed for 30 min. No more than 20 pairs were observed concurrently. Because the flies are so small, and all mating vials were pre-warmed, flies should have equilibrated to the set temperatures before they had the opportunity to court and mate.

Temperatures used for the interspecific comparison ranged between 19.5 °C and 37.5 °C for *D. melanogaster*, between 19.5 °C and 35 °C for *D. simulans* and between 21 °C and 38.25 °C for *D. mojavensis*, with precise temperatures grouped into categories in 1 °C intervals (Fig. 1). Pooling within these intervals produced sample sizes sufficient to record a frequency of courtship and mating in the 30 temperature groups presented (382 pairs spread among ten groups in *D. melanogaster*; 278 pairs in eight group for *D. simulans* and 582 pairs in 12 groups for *D. mojavensis*), from which performance across varied temperatures was determined. Upper temperature limits varied by species because higher temperatures than those used would knock out the males.

TESTS OF SURVIVAL AMONG POPULATIONS

Extreme stress was applied by immersing glass vials holding seven-day-old *D. mojavensis* adults for 1 h in water baths (Polyscience) heated to 41.0 °C, and also monitored using two mercury thermometers. The water temperature did not vary during the time course of the experiment. Each vial of flies contained

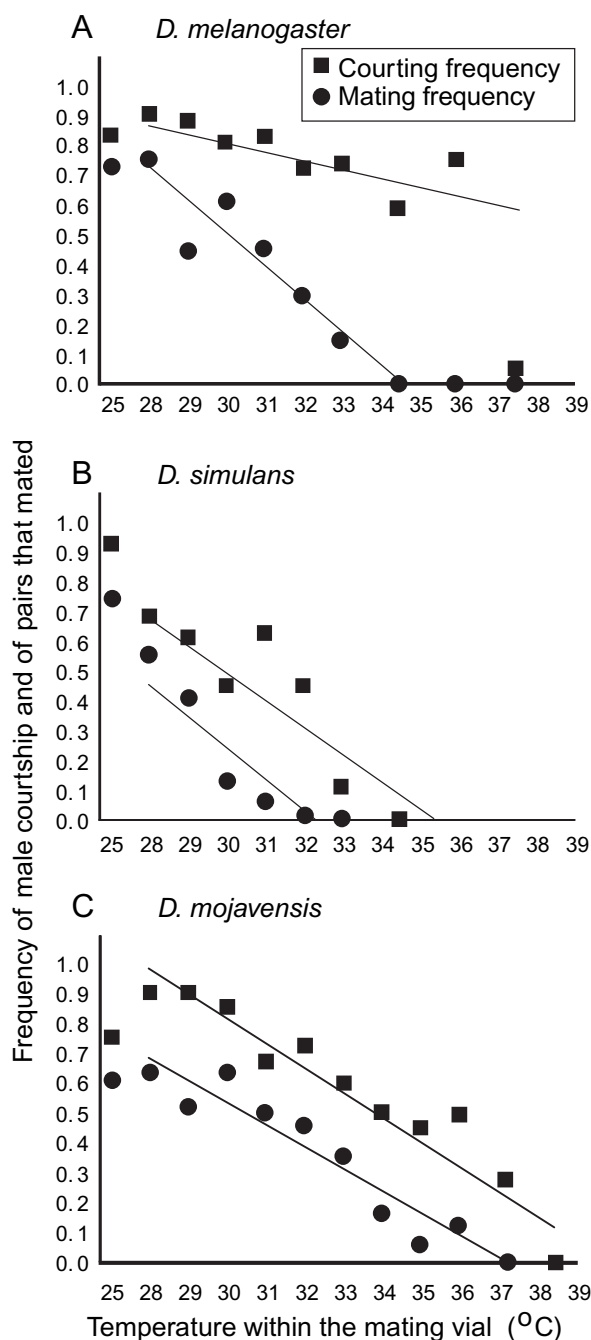


Figure 1. Linear performance curves for courtship frequency and mating frequency to describe the responses of three *Drosophila* species to heat stress. Linear regression coefficients are as follows (where $x \geq 28$ °C): A, *D. melanogaster*, court frequency = $1.71 - 0.030x$, mating frequency = $3.72 - 0.107x$; B, *D. simulans*, court frequency = $3.29 - 0.093x$, mating frequency = $3.37 - 0.104x$; C, *D. mojavensis*, court frequency = $3.17 - 0.078x$, mating frequency = $2.73 - 0.073x$.

an agar-based medium; they were stuffed with a cotton ball, sealed with a wet rubber stopper to ensure high humidity, and placed inverted within the baths. After exposure, the vials were lifted from the water and the rubber stoppers were removed. After a 24 h recovery time, fly survival was scored as the ability to walk.

MATING EXPERIMENTS AMONG POPULATIONS

For the intraspecies comparisons, adult *D. mojavensis* from each of the four populations were examined at pre-selected temperatures based on the performance curves generated for *D. mojavensis* in the interspecific test. The three temperature categories selected for this investigation were No Stress (27 °C), Low Stress (32 °C), and Moderate Stress (34.5 °C). As above, we considered extreme stress as the treatment that threatened survival.

Data collection involved scoring the frequency of both courtship and mating. A male courtship was scored where we observed wing waving, drumming on the female abdomen or persistent chasing of the female. Receptivity was scored where we observed a pair *in copula*. One hundred pairs of flies were observed for each population and stress level ($N = 1200$ pairs in total). To record the frequency, we separated each consecutive set of ten vials as a replicate group, and these were obtained for each population and temperature on different days to reduce any impact of day-to-day variation on group means.

ANALYSIS OF HYBRIDS IN *D. MOJAVENSIS*

The two strains of *D. mojavensis* that differed most in survival after exposure to extreme thermal stress were used to examine genetic variation for survival and the ability to fly: Catalina Island (CI) and Ensenada de los Muertos (EN). Virgin males and females of each strain were collected and used either to start pure strain cultures or reciprocal F_1 crosses between these lines. As new flies emerged, they were collected within 24 h of emergence and 10–20 males or females were placed in each glass vial. All experiments examined seven day-old flies.

For survival after heat shock, the water baths were again set to 41 °C for 1 h, and the ability to walk was scored after a 24 h recovery. For the test of flight, the heat shock was reduced to 38 °C for 1 h. These flies were allowed to rest for 1 h before flight was scored, based on the ability to take off and travel a distance 10 cm or more. If a fly would not take off, not fly when probed with a camel hair paintbrush, or would land less than 10 cm away, it was scored as no flight.

STATISTICAL ANALYSIS

Data were recorded as frequencies within each vial and arcsine transformed to increase the variance in the extremes of the distributions. Linear regression lines were generated from the interspecies comparisons using the REG procedure in SAS (SAS Institute, 1998). A model 1, two-way fixed-factor ANOVA for population, temperature and the interaction between these two effects, was used to compare courtship among populations. The design was completely balanced. Because receptivity is the product of the male courtship and the willingness of a female to accept a courting male, this frequency was analysed in two ways (1) by including male courtship frequency as a covariate in an analysis of mating frequency, and (2) by first regressing male courtship on mating frequency, and then analysing the differences among populations based on the residual variation.

RESULTS

INTER-SPECIFIC VARIATION IN THERMOTOLERANCE

Courtship and mating frequency were examined between 25 °C and 39 °C for *Drosophila melanogaster*, *D. simulans* and *D. mojavensis*. Courtship frequency declined at a lower temperature in *D. simulans* than in the other two species and was essentially eliminated by 34 °C (Fig. 1). *Drosophila melanogaster* and *D. mojavensis* males continued to court females until temperatures approached 38 °C, although courtship declined more slowly in *D. melanogaster*.

In contrast to courtship, mating in *D. simulans* began to decline at 28 °C and stopped at 32 °C, while in *D. melanogaster*, mating began to decline at 29 °C and stopped just below 34.5 °C (Fig. 1). In *D. mojavensis*, mating frequency remained high until between 31 °C and 32 °C, above which mating in this desert species declined slowly and finally stopped at 37 °C. Therefore, comparing these species, differences in temperature responses between courtship frequency and mating frequency were greatest in *D. melanogaster*, while in *D. simulans* and *D. mojavensis*, these traits declined in parallel but at very different temperatures (Fig. 1). The phylogenetic relatedness of *D. melanogaster* and *D. simulans* did not cause them to be any more similar in their behavioural response to heat than either were to *D. mojavensis*.

SURVIVAL VARIATION IN *D. MOJAVENSIS*

The four populations of *D. mojavensis* were studied for their stress tolerance as measured by survival, courtship and mating frequencies. A significantly greater proportion of flies from the Catalina Island population

(CI), which experiences the mildest environment, survived exposure to a severe heat stress than did flies from the three Sonoran Desert populations (Fig. 2). Survival in the two mainland populations, Santa Rosa Mts. (SR) and San Carlos (SC), was intermediate, while the lowest proportion of flies survived in the population from Ensenada de los Muertos (EN), near the southern end of the Baja Peninsula. No significant effects between males and females were observed for survival, and subsequent analysis pooling sexes indicated significance (Tukey's multiple range test, $P < 0.05$) among the three population groupings: CI > SR = SC > EN.

INTRASPECIFIC VARIATION IN *D. MOJAVENSIS* FOR COURTSHIP AND MATING

Courtship and mating frequencies were determined at 27 °C, at a low stress of 32 °C and at a moderate stress of 34.5 °C. The stress treatments greatly decreased ($P < 0.001$) the frequency of courtship and mating in all populations, as expected (Fig. 3A). There was significant variation among populations ($F_{3,108} = 2.78$, $P < 0.05$), as well as a significant population-by-stress effect ($F_{6,108} = 3.21$, $P < 0.01$). In the absence of stress (27 °C), a one-way fixed-factor ANOVA highlighted that all populations courted at a similar frequency. Under mild stress (32 °C), populations varied ($F_{3,36} = 4.25$, $P < 0.05$), as the courtship frequency of San Carlos males exceeded that for the other three populations ($P < 0.05$, Tukey's test). Under moderate stress (34.5 °C), variation among the populations again was significant ($F_{3,36} = 2.90$, $P < 0.05$). Ensenada males courted significantly more often than Santa Rosa males, which courted least often.

These four populations varied even more in mating success (Fig. 3B) than they did for courtship ($F_{3,108} = 10.2$, $P < 0.001$). However, the population-by-

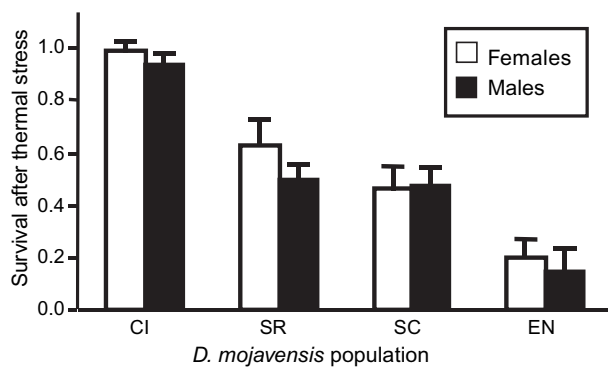


Figure 2. The proportion of females and males from four populations of *Drosophila mojavensis* that survived after exposure to a 41 °C heat stress for 1 h.

stress interaction only approached significance ($F_{6,108} = 2.05$, $P = 0.07$). To better gauge female responses to high temperatures, we repeated analyses on the residuals from a regression analysis of male courtship on mating frequency. This covariate removed most of the contribution of courtship frequency to the measurement of female willingness to mate. Figure 4 shows where females mated more or less often than could be predicted from courtship frequencies, and the deviations from zero reflect the magnitude of tendencies for females to accept courtship. Population effects remained large ($F_{3,108} = 10.2$, $P < 0.001$), and the interaction effect for stress-by-population was significant ($F_{6,108} = 2.62$, $P < 0.05$). Mating success in the Ensenada and Catalina Island populations fell below that predicted from courtship frequencies in these populations. Mating success in the two mainland Sonora populations, however, was higher than courtship frequency could predict both in the absence of stress and during mild stress, but not dur-

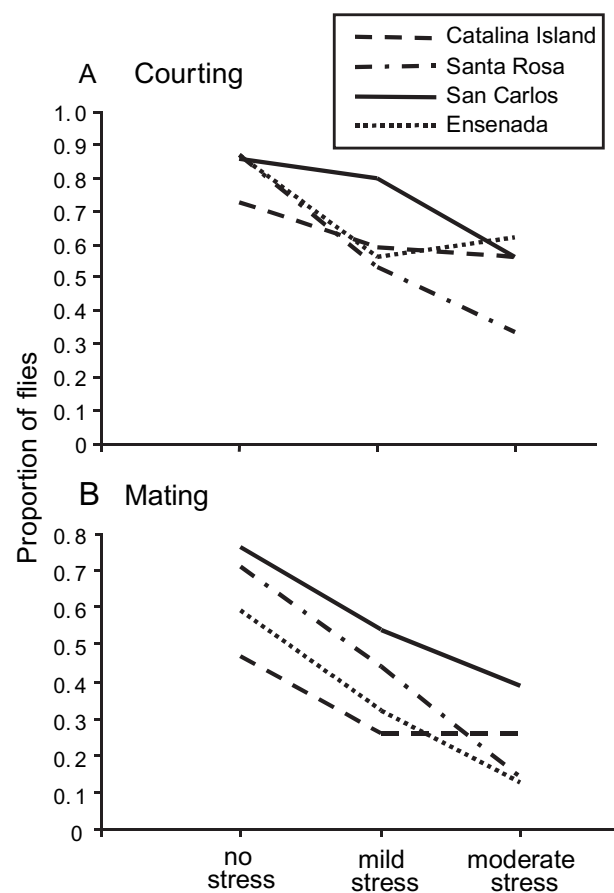


Figure 3. A, Courtship frequency means plotted for each of the four populations of *Drosophila mojavensis* under no stress (27 °C), mild stress (32 °C) and moderate stress 34.5 °C. B, Mating frequency means also plotted for each population under the three treatments.

ing moderate stress. The 34.5 °C treatment reduced female receptivity in all populations (Fig. 4).

SURVIVAL AND FLIGHT IN POPULATION HYBRIDS

Survival and the ability to fly after stress were measured in crosses between the two strains that varied most for survival. F₁ hybrid progeny derived from Catalina Island (CI) and the Ensenada de los Muertos (EN) populations survived thermal stress at a level similar to that of the more tolerant strain, CI (Fig. 5A). Significance of the variation ($F_{3,77} = 85.8$, $P < 0.001$) was due predominantly to the low survival of EN parental flies, a result that applied to both males and females when analysed separately (Tukey's multiple range tests, $P < 0.05$). However, gender differences occurred where males whose maternal parent was from the EN population survived the stress at an intermediate level (Tukey's test, $P < 0.05$). Population variation in the ability to fly after thermal stress differed greatly from results for survival (Fig. 5B). The EN and CI populations that differed so much in survival, tolerated heat similarly based on flight, while their reciprocal F₁ offspring exhibited much higher tolerance to heat stress ($F_{3,86} = 58.0$, $P < 0.001$, and Tukey's test, $P < 0.05$).

DISCUSSION

Above some optimum, biological performance declines with increasing temperatures. In this comparison of three very different members of *Drosophila*, variation in mating behaviours was assessed while flies experienced thermal stress. This variation in courtship and mate receptivity correlated with variation as measured previously by survival (Krebs, 1999). The same

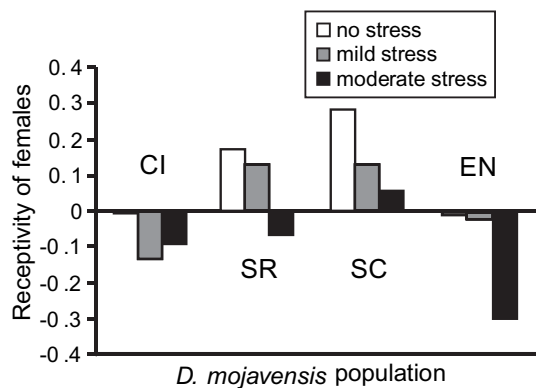


Figure 4. A plot of the residuals for mating frequency after regressing this trait on male courtship. Means are presented for each of the four populations of *Drosophila mojavensis* under no stress (27 °C), mild stress (32 °C) and moderate stress (34.5 °C).

pattern was shown for the ability for males to court and to mate following a stress exposure (Patton & Krebs, 2001). In each set of experiments, *D. simulans* died or stopped mating at lower temperatures than did its close relative, *D. melanogaster*, while some *D. mojavensis* adults successfully mated at temperatures that prevented mating in the two other species. These large differences in organismal responses to stress even among related species, indicate the potential for a general thermotolerance phenotype to evolve and to produce a consistent rank order of performance across a range of stress conditions. Such a consistent difference, however, was not observed at a population level in *D. mojavensis*.

The most striking difference among species was the large proportion of *D. melanogaster* males that continued to court females at temperatures approaching those that can 'knock out', or induce a paralytic state, in the flies (Huey *et al.*, 1992; Gilchrist & Huey, 1999). Therefore, males and females may respond very differently to thermal stress as is common in *Drosophila* (Hoffmann *et al.*, 2001), even among strains (Krebs & Loeschke, 1996; Guerra *et al.*, 1997).

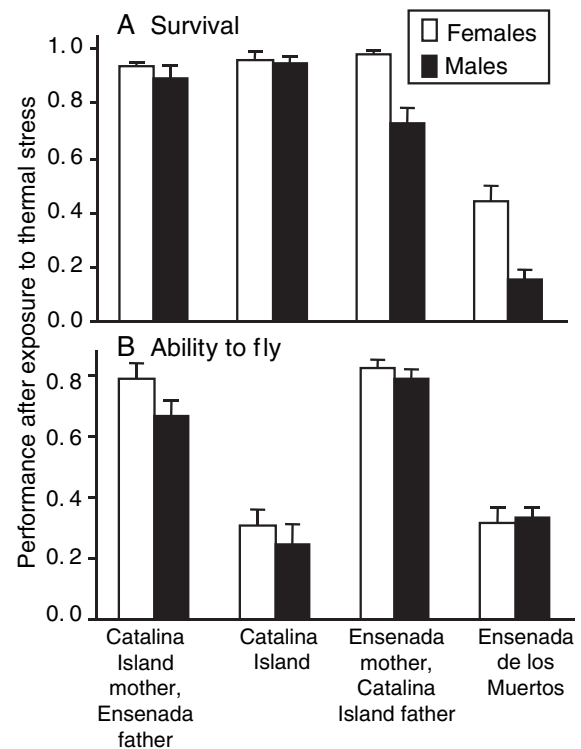


Figure 5. The proportion of female and male offspring from reciprocal crosses between the Catalina Island and Ensenada populations that (A) survived a 1 h exposure to 41 °C, and (B) maintained their ability to fly after a 1 h exposure to 38 °C.

The variation in survival, courtship, mating and flight among the populations of *D. mojavensis* exceeds that typically found in other better-studied species of *Drosophila*, notably *D. melanogaster* (Krebs *et al.*, 1996; Guerra *et al.*, 1997; Hoffmann *et al.*, 2001) and *D. buzzatii* (Krebs & Loeschcke, 1995), a cactophilic species related to *D. mojavensis* (Durando *et al.*, 2000). The strain most tolerant of an extreme stress, one that may kill, was from Santa Catalina Island, but this strain was only intermediate in tolerance based on ability to maintain courtship. The strain that courted most often under moderate stress was the one that suffered the highest mortality. Thus, by analysing a suite of traits, all strains can be considered different.

These differences probably possess a genetic basis, as the crosses between the two more extreme populations indicated. Survival variation between CI and EN after exposure to a high stress suggested largely dominant effects in the autosomes with an additional role of the X-chromosome. A mitochondrial effect is less likely because reciprocal-hybrid daughters tolerated heat similarly. For flight after exposure to a low stress, hybrids were far more tolerant to heat than were either parent, which is best explained by a heterozygote advantage. Whether the differences were caused by inbreeding in these strains, which could have fixed a deleterious recessive in each strain, or whether the differences originated from local genetic variants in the natural populations cannot be determined from the present results. Both genetic background and inbreeding alter thermotolerance, and the effects can interact with the type and/or intensity of a stress (Dahlgaard & Hoffmann, 2000; Kristensen, Dahlgaard & Loeschcke, 2003). Clearly, the variation observed here that promoted hybrid vigour is very specific to mechanisms for flight, as the inheritance of flight differed so much from that for survival.

Overall, the traits and the thermal conditions chosen by an experimenter can give a very different picture of variation in thermotolerance, as suggested by Bennett (1987a) and later demonstrated by Hoffmann *et al.* (1997). They found that knockdown tolerance and survival of adult *D. melanogaster* are only weakly correlated. In addition, by studying multiple traits, performance can be compared over a stress-level range of 9 °C, a much more ecologically relevant range of conditions than is generally considered. Huey (1982) argued previously that tests of thermal stress should include sublethal effects, and that those traits related to mating may be a more direct target of selection (Shine *et al.*, 2000).

The second question posed was whether variation in stress tolerance correlates with differences in the climate from which populations were collected. Because relative performance varied among the different traits, any test of environmental correlation becomes

ad hoc, especially with only four populations. Nonetheless, results for one population stood out as unusual. Survival tolerance to heat of the Santa Catalina Island flies greatly exceeded that of the three strains from the Sonoran Desert. According to the National Center for Climate Data, mean daily maximums for July 2002 averaged 75.7 °C on the island, while average temperatures in the desert averaged 98.0 °C in Santa Rosa, Arizona, USA, and daily maxima for San Carlos and Ensenada were only slightly lower (Fasolo, 2002). Performance in the CI strain was not the lowest for any trait. High temperatures are predicted to select for stress tolerance in Sonoran desert *Drosophila* at all developmental stages (Gibbs, Perkins & Markow, 2003). The only clear difference that we found between flies from the CI strain and the others was during a small-scale analysis of variation in the 16S rRNA gene; all of the southern strains predominantly possessed the same sequence for a 500 bp fragment, while the CI strain differed at three DNA sites (GenBank accession numbers AY515016–19, $N = 4$ flies per strain). The differences between CI and the other strains also are supported by allozyme variation (Markow *et al.*, 2002).

Finally, we investigated whether the cause of a breakdown in mating success results from inhibition of males to court at high temperatures or from females' response to courting. If mating declined primarily because males would not court, then mating frequency in all strains should have followed change in courtship frequency unless females respond negatively to temperature effects on male courtship quality. Behavioural variation among *D. mojavensis* strains enables discrimination between these possibilities. In the absence of stress, females from the Ensenada and the Catalina Island strains require little courtship prior to accepting a male (Krebs & Markow, 1989; R. Krebs, pers. observ. on the Catalina Island flies). However, females of the two Sonoran mainland populations require longer courtship, presumably to discriminate against males of a sympatric sibling species present only in mainland Sonora (Wasserman & Koepfer, 1977; Markow, 1991). Because courtship declined in all strains as temperatures were increased, and because the greatest decline in mating after courtship was observed in the Catalina and Ensenada strains, high temperature stress must have reduced mating propensity in both males and females.

Such consequences of heat stress may change the mating system of a species. *Drosophila mojavensis* expresses two mating peaks during the day, one in the morning and a smaller peak at sunset (Markow, 1982), suggesting that heat will reduce mating success. Around mid-day, the hottest period, few flies can be found (Markow & Toolson, 1990). Even late in the

morning mating period, mating leks rotate to the shaded sides of cactus arms as the desert, and hence the plant itself, rapidly warms (Krebs & Bean, 1991). Similar responses to stress may influence the timing of oviposition in *D. buzzatii* (Dahlgaard, Hasson & Loeschcke, 2001).

Although selection may first affect behaviour, the ability to survive still must impart a huge effect on fitness (Huey, 1982). *Drosophila mojavensis* adults tolerate temperatures up to 40 °C before death is likely and, while such conditions may not be frequent, they do occur in the Sonoran desert (Gibbs *et al.*, 2003). Where both population structure and temporally consistent environmental differences occur, strong divergence in stress tolerance may evolve among *Drosophila* populations (Dahlgaard *et al.*, 2001; Michalak *et al.*, 2001; Sørensen, Dahlgaard & Loeschcke, 2001). Depending on how the environment and physiology interact, changes will impact survival and mating behaviours differently.

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