RESEARCH ARTICLE

Sexual conflict and environmental change: trade-offs within and between the sexes during the evolution of desiccation resistance

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Abstract

Intralocus sexual conflict occurs when males and females experience sex-specific selection on a shared genome. With several notable exceptions, intralocus sexual conflict has been investigated in constant environments to which the study organisms have had an opportunity to adapt. However, a change in the environment can result in differential or even opposing selection pressures on males and females, creating sexual conflict. We used experimental evolution to explore the interaction between intralocus sexual conflict, sexual dimorphism and environmental variation in *Drosophila melanogaster.* Six populations were selected for adult desiccation resistance (D), with six matched control populations maintained in parallel (C). After 46 generations, the D populations had increased in survival time under arid conditions by 68% and in body weight by 20% compared to the C populations. The increase in size was the result of both extended development and faster growth rate of D juveniles. Adaptation to the stress came at a cost in terms of preadult viability and female fecundity. Because males are innately less tolerant of desiccation stress, very few D males survived desiccation-selection; while potentially a windfall for survivors, these conditions mean that most males' fitness was determined posthumously. We conjectured that selection for early maturation and mating in males was in conflict with selection for survival and later reproduction in females. Consistent with this prediction, the sexes showed different patterns of age-specific desiccation resistance and resource acquisition, and there was a trend towards increasingly female-biased sexual size dimorphism. However, levels of desiccation resistance were unaffected, with D males and females increasing in parallel. Either there is a strong positive genetic correlation between the sexes that limits independent evolution of desiccation resistance, or fitness pay-offs from the strategy of riding out the stress bout are great enough to sustain concordant selection on the two sexes. We discuss the forces that mould fitness in males under a regimen where trade-offs between survival and reproduction may be considerable.

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Introduction

Sexual conflict arises when the fitness interests of males and females differ. There are two distinct forms of conflict, depending upon whether there is direct physical coercion (interlocus) or genetic constraint on sex-specific evolution (intralocus). Interlocus sexual conflict occurs when a locus expressed in one sex reduces the fitness of the opposite sex through direct physical interaction (e.g., harassment and toxicity). This selects for the expression in the opposite sex, at other loci, of a counteracting mechanism that will reduce the fitness costs of sexual interaction. This form of conflict may contain the ingredients for an arms race (or evolutionary chase) between the sexes driven by sexually antagonistic coevolution (Parker 1979; Rice 1998). An apparent example of this dynamic is seen in the evolution of elaborate grasping (males) and anti-grasping (females) structures in the gerrid water striders (Rowe and Arnqvist 2002). Numerous other examples have recently been catalogued, sug-

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gesting that interlocus sexual conflict is both taxonomically widespread and occurs through a variety of different organs of expression (Arnqvist and Rowe 2005).

The second form of conflict, intralocus sexual conflict, is likely to prove equally pervasive but is less apparent. Intralocus sexual conflict arises because males and females experience sex-specific selection on a shared genome. This creates conflict when the same allele or expression pattern has opposite effects on the relative fitness of each sex, leading to a kind of tug-of-war over gene expression between males and females (Rice and Chippindale 2001). Without the evolution of mechanisms to isolate female selection from male selection, the outcome is genotypes that are optimized for neither sex and a reduction in average fitness (Rice and Chippindale 2002). Chippindale *et al*. (2001) and Prasad *et al*. (2007), showed evidence for intralocus sexual conflict in *Drosophila melanogaster* in a strong and negative intersexual genetic correlation for adult fitness. The genotypes that code for high fitness females tended to code for low fitness males and vice versa. Since then, a methodologically and taxonomically broad canon of results has come to light, including further data from *D. melanogaster*, other insects, reptiles and amphibians, and mammals, including humans (Bedhomme and Chippindale 2007).

Because the sexes themselves represent different environments for gene expression, intralocus sexual conflict suggests a mechanism through which genetic variation for fitness traits may be maintained in populations, even under constant external environmental conditions (Rice 1984; Gibson *et al*. 2002). However, the interactions between sexual conflict, sexual dimorphism and external environmental variation may have important consequences that have been largely overlooked until now. A change in the environment can result in differential or even opposing selection pressures on males and females, and this is particularly likely when the sexes are dimorphic. This is because extant sexual dimorphism may not dictate the same best response to the same new external selective agent by both sexes. For example, an environmental stress that makes it beneficial to migrate for one sex—the more mobile sex—may not create conditions that favour migration in the other sex, where the cost of movement is greater, along with the odds of survival *in situ* (Perrin and Mazalov 2000; Munshi-South 2008). Numerous examples could be imagined, particularly involving sexual size dimorphism (SSD) and environmental change. While sex-specific expression and dimorphism represent cures for intralocus sexual conflict, a changing environment has the potential to induce new forms of sexual antagonism.

D. melanogaster is a good model to study the interplay between sexual conflict and adaptation to a novel environment. The species is promiscuous and sexually dimorphic, with males being smaller and slower growing than females, and they are legendarily responsive to selection. In one of the few studies linking sexual conflict to adaptation in a new environment, Chippindale *et al*. (1998) discussed sex-specific selection on the life-history and physiology of *D. melanogaster* selected for desiccation tolerance. They noted that the sex-specific patterns of adaptation to stress were linked to initial sexual dimorphism in body size and development time, along with different resource acquisition and allocation priorities. Different and opposing selection pressures for males and females apparently resulted from the same environmental challenge.

As is typical of small organisms, drosophilids are inherently sensitive to desiccation because of a high surface area to volume ratio. However, they have undergone multiple adaptive radiations in arid regions and their adaptation to desiccation has been widely studied in both nature (e.g., Marron *et al*. 2003; Matzkin *et al*. 2007) and the laboratory (e.g., Hoffmann and Parsons 1989; Gibbs *et al*. 1997; Chippindale *et al*. 1998). In the laboratory, it has been found that the evolution of desiccation resistance in *Drosophila* can result in a complex and extensive reshaping of their phenotype and life-history. Prasad and Joshi (2003) noted that desiccation resistance is reliably associated with changes in two adult life-history traits, longevity and fecundity. Previous studies showed that adult populations of *D. melanogaster* selected for desiccation resistance showed increased mean longevity and reduced early fecundity (Hoffmann and Parsons 1989; Rose *et al*. 1990, 1992; Leroi *et al*. 1994a,b), suggesting that the strong genetic correlations between these traits generate trade-offs and influence the response to selection.

Chippindale *et al*. (1998) found similar age-related patterns of desiccation resistance and resource management in males and females of control populations, but not in males and females of desiccation-selected populations. In the latter populations, females showed greater desiccation resistance associated with markedly higher rates of carbohydrate and bulk water storage as larvae and young adults than both control populations and desiccation-selected males. To understand the sex-specific selection pressures underlying this differentiation, a closer look at the experimental protocol is necessary. Young adult flies were subjected to an intense desiccation period (low to zero relative humidity) every generation, after having grown and lived in relatively rich food, with standard conditions of humidity and temperature until this time point. The desiccation period was terminated when 90% of the initial population had died, after which eggs for the next generation were collected. Because of sexual dimorphism in desiccation tolerance, few, if any, males survived the desiccation period and no mating was observed during the time the flies were under the desiccation stress. Females necessarily used stored sperm to produce offspring. The authors suggested that females were under strong selection to acquire resources to resist stress, while males were selected to expend energy on early maturation and mating.

We adopted an experimental protocol similar to Chippindale *et al*. (1998) to evolve populations of *D. melanogaster* resistant to intense desiccation stress and further explore the relationship between sex and adaptation. We focussed the

analysis on a direct characterization of the new costs, and consequently new selection pressures generated by the environmental change, and the differences in response between the sexes. We paid particular attention to the evolution of sexual dimorphism for traits that were suspected to be under divergent selection pressures in males and females.

Materials and methods

Selection treatments

In 2005, the experimental populations used in this study were derived from two long-term, outbred laboratory-adapted populations, the wild-type red-eyed LH_M (see Chippindale *et al*. 2001 for a description of the LH_M population) and mutant brown-eyed LH_M-*bw*. The LH_M-*bw* flies carry a recessive eye colour marker in the LH_M genetic background. Three populations selected for desiccation resistance (D) and three matched control populations (C) were derived from each of the LH_M (DR_{1-3}; CR_{1-3}) and LH_M-*bw* (DB_{1-3}; CB_{1-3}). In the D treatment, flies were desiccated, where both food and water were absent for a certain period of time. In the C treatment, flies were mildly starved since the proper control for the effects of desiccation alone should permit access to water, but not food.

Selection occurred on day 12 post egg lay in every generation. In the D treatment, approximately 5000 individuals per population were transferred from 8-dram vials to a cage ($20 \times 20 \times 14$ cm^3) with a bag containing 100 g of Drierite® desiccant (anhydrous calcium sulphate). A second desiccant bag was added if the indicators in the initial desiccant bag detected an increase in humidity. The cage was sealed with a cloth sleeve and plastic autoclave bag to maintain very low humidity. Selection was terminated when the population reached approximately 75% mortality, as estimated by the main experimenter (LK). The C populations were maintained in parallel to the D populations. In the C treatment, approximately 1250 individuals per population were introduced into the cage and supplied with a nonnutritional agar plate as a source of water. The cages used in the C treatment were also sealed with cloth sleeves and plastic autoclave bags. Starvation stress in a C population was terminated once selection in the corresponding, same-numbered, D population was terminated. With this protocol, populations bearing the same subscript were paired by handling, and the D and C populations wound up with approximately the same number of individuals after selection. We note, however, that the sex ratio in the D-selected cages was strongly female-skewed, potentially affecting the effective population size. Starvation stress in the C populations was too mild to result in significant mortality, even among males. Once selection on all populations was terminated, survivors were supplied with fresh cornmeal–molasses food and live yeast for two days, and then allowed to oviposit for 12 h. On day 16, 125–150 eggs were introduced into eight-dram vials (> 5 ml of food per vial) to start the next generation. The larvae and adults were reared at 25°C (±0.5°C), 50% relative humidity, and 12L:12D cycle.

Prior to all experimental assays, approximately 1250 individuals per population per treatment were reared for two generations with relaxed selection to remove residual non-genetic (i.e., parental and grand-parental) effects.

Desiccation resistance

Sex-specific desiccation resistance was assayed periodically to follow the direct response to selection, and the evolution of sexual dimorphism in desiccation resistance. Desiccation resistance assays were performed on the D and C populations at generations 5, 10, 15, 31, 37 and 46. On day 12 post oviposition, 2–3 day old adults were lightly anesthetized with CO_2, separated by sex, and placed in temporary holding vials containing food. From each population, 50 individuals per sex were collected and split into five vials. In each vial, a thin sponge stopper separated the flies at the closed end from the desiccant (~6 g) at the open end, which was sealed with parafilm to prevent humidity from entering. Mortality was examined hourly until all individuals died.

Female fecundity

At generation 48, the fecundity of females was assayed to evaluate the effects of desiccation-selection on fitness (i.e., survival and productivity) and potential costs of adaptation. The fecundity of females was measured under two treatments, unstressed and stressed. On day 12 post egg-lay, 112 and 224 sexually mature females were collected for the unstressed and stressed treatments, respectively, from each population. In the unstressed treatment, females were kept in vials containing food. In the stressed treatment, females were subjected to desiccation stress, as explained above in 'desiccation resistance'. Stress was terminated once half of the females died (i.e., we administered an LD_{50}), and the duration of stress was recorded. The rationale for this treatment was to induce a severe stress that was in some sense equivalent in intensity between the D and C populations, but still preserve a reasonable cross-section of genotypes from each type of population. In both treatments, females were supplied with food and live yeast for two days after the stressed treatment reached LD_{50}, and then allowed to oviposit in eight-dram vials (> 5 ml of food per vial) for 12 h, corresponding to their normal selection protocol. Progeny from these vials were counted 12 days later.

Male fitness

As previously discussed, desiccation-selection may result in different selection pressures on males and females. The selection treatment is stringent enough to kill all, or all but a few males, and mating has never been observed under zero-humidity conditions (Chippindale *et al*. 1998; Kwan L., Bedhomme S., Prasad N. G. and Chippindale A. K. unpublished data). We observed that most males achieve fertilization by

mating prior to selection because females are capable of storing sperm that can be used to fertilize eggs after the stress bout. However, the few males that survive the stress, if they remain fertile, may have a significant advantage because of high last-male sperm precedence (between 80% and 90% in *D. melanogaster*, e.g., Björk *et al*. 2007). We were therefore interested in quantifying the proportion of males that survived the desiccation stress, as well as their postselection fitness contributions to the next generation.

At generation 52, a male fitness assay was performed (figure 1). The assay took advantage of the recessive brown-eyed colour mutation carried by half of the replicate populations to track preselection and postselection paternities. Eggs from both the brown-eyed ($DB_{1,3}$; $CB_{1,3}$) and red-eyed ($DR_{1,3}$; $CR_{1,3}$) populations were collected. On day 12 post egg lay, sexually mature individuals were lightly anesthetized with CO_2 and separated by sex. In the brown-eyed populations, males were discarded and exact numbers of females were collected and placed in temporary holding vials containing food. The progeny sired at this time (i.e., prior to selection) could be quantified since both parents were homozygous for the recessive eye colour marker and thus, produce only brown-eyed progeny. The brown-eyed females were then transferred into cages with equal numbers of same age red-eyed males from the matched 'R-population'. The flies were immediately placed under desiccation stress in the D combinations (combinations 5–8) and mildly starved in the C combinations (combinations 1–4) (table 1). Although there is no pairing of numbered replicates between the DR and DB treatments, for logistical reasons we only set up four D combinations and four C combinations. The postselection fitness contribution of the red-eyed males could be quantified since their progeny, resulting from matings with brown-eyed females, would have red-eyes, the dominant eye colour marker. In the D combinations, there were 500 brown-eyed females and 500 red-eyed males per cage. At about 75%

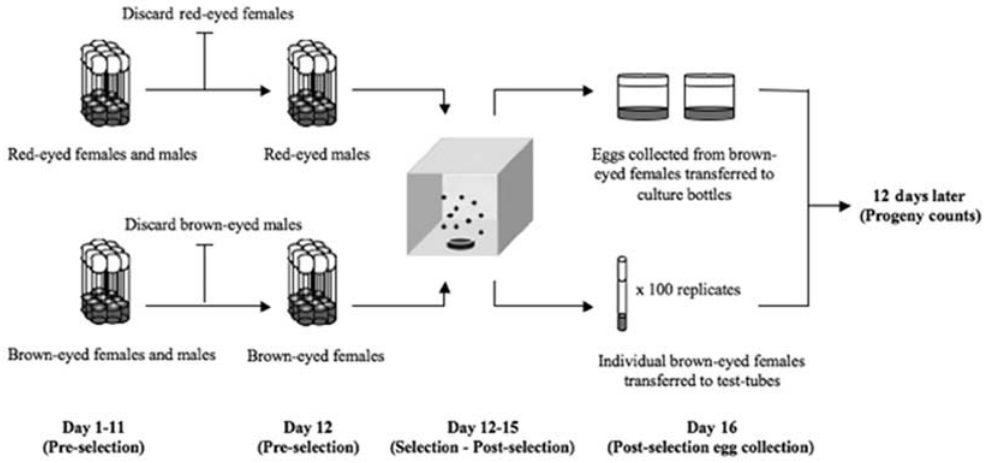

Figure 1. Male fitness assay (generation 52). For each combination (table 1), brown-eyed females were reared with brown-eyed males and red-eyed females were reared with red-eyed males in vials prior to selection (preselection). On day 12, brown-eyed females and red-eyed males were collected and transferred into cages for selection, and held together afterwards (postselection). On day 16, surviving females were allowed to oviposit on food plates, which were transferred to culture bottles, and in test-tubes. Progeny were counted 12 days later.

Table 1. Different combinations used in male fitness assay (generation 52): four C combinations (combinations 1–4) and four D combinations (combinations 5–8). See figure 1 for technical details.

Combination	Preselection (days 1–11)	Selection to postselection (days 12–16)
1	$CB_1 \times CB_1$	$CB_1 \times CR_1$
2	$CB_1 \times CB_3$	$CB_1 \times CR_3$
3	$CB_3 \times CB_1$	$CB_3 \times CR_1$
4	$CB_3 \times CB_3$	$CB_3 \times CR_3$
5	$DB_1 \times DB_1$	$DB_1 \times DR_1$
6	$DB_1 \times DB_3$	$DB_1 \times DR_3$
7	$DB_3 \times DB_1$	$DB_3 \times DR_1$
8	$DB_3 \times DB_3$	$DB_3 \times DR_3$

mortality, desiccation stress was terminated and deceased individuals were removed. The C combinations were run in parallel with 125 brown-eyed females and 125 red-eyed males per cage. Starvation stress was terminated once the corresponding D combination reached 75% mortality. In both the D and C combinations, survivors were supplied with food and live yeast for two days once selection was terminated. The cages were also checked daily for additional mortality and if present, deceased individuals were removed, counted, and sexed.

Flies were then allowed to lay eggs on food plates in the cages, corresponding to their normal selection protocol. Sectors of the plates were transferred to 150 ml culture bottles containing abundant food. For a more accurate measure of individual fitness, 100 females were transferred from each cage to 100 test-tubes (13 mm × 100 mm) containing abundant food. In both protocols, females were allowed to oviposit for 12 h and progeny were counted 12 days later. After egg-laying, all survivors were counted and sexed. There were three replicates per combination and all replicates were run simultaneously.

Cost of social and sexual interactions

Previous studies have found that increased sexual activity reduces longevity (Partridge and Farquhar 1981) and immunity (McKean and Nunney 2001) in *D. melanogaster* males. There is also evidence that mating is costly for females, with mated females having a higher mortality and lower oxidative stress resistance than virgin females (Salmon *et al*. 2001). Therefore, for females about to face severe desiccation stress any trade-offs between survival characters and mating are likely to be exacerbated. On the other hand, our observations and direct measurements in the male fitness assay suggest that the vast majority of male fitness was achieved prior to selection (see Results: male fitness). The present experiment was designed to explore this potential conflict over optimal mating pattern for the D populations, by assessing the cost of social and sexual interactions in terms of desiccation resistance and body weight.

An experiment was conducted at generation 37. For each population, 50 recently emerged and 50 sexually mature individuals of each sex were assayed for desiccation resistance, while 25 recently emerged and 25 sexually mature individuals of each sex were assayed for body weight. As adults emerged, 10 to 11 days after egg lay, virgin flies (< 8 h old) were collected, separated by sex, and held in vials containing food for an additional 6 h to harden their cuticles prior to desiccation stress or dry weight measurements. The socially inexperienced flies were compared to controls allowed to eclose and interact until day 12 post egg lay, as per the usual selection protocol; these sexually mature and relatively socially-experienced individuals were similarly collected and assayed for desiccation resistance and body weight. Desiccation resistance was assayed as described above. For body weight measurements, flies of the same sex were placed in groups of five in 1.7 ml plastic vials and frozen at −18°C (±0.5°C). All individuals were later dried for 36 h at 65°C and weighed in groups of five using a Cahn C-33 microbalance (Thermo Fisher Scientific, Waltham, USA) to the nearest 0.001 mg.

Juvenile growth and survival

Based on current and past data, and logical arguments, we suspected that D females were under strong selection to increase their body weight to store more water and nutrients, while it may be more important for males to maximize mating opportunities before selection. Consequently, there may be selection for early reproductive maturation and mating in males. To shed light on these hypotheses, we evaluated the body weight, development time, and preadult viability of D and C males and females during the evolutionary history of these populations. More precisely, we wanted to determine if the sexes had the opportunity to evolve independently, each responding to the new selection pressures created by the desiccation stress, or if the genetic correlations between the sexes were too strong to allow such an independent evolution.

Body weight assays were performed on the D and C populations at generations 10, 15, 31, 37 and 46. For each population, 25 individuals per sex were lightly anesthetized with CO_2 and placed in groups of five in 1.7 ml plastic vials and frozen at −18°C (±0.5°C) on day 12 post egg-lay. All individuals were later dried for 36 h at 65°C and weighed in groups of five using a Cahn C-33 microbalance to the nearest 0.001 mg.

Development time, preadult viability, and growth rate of the D and C populations were also examined at generations 31 and 46. To measure development time, exactly 150 eggs were introduced per vial. For each population, seven vials were created, with the D and C populations alternating in the same experimental rack. To control for the effects of potential differences in temperature and light, the racks were randomly distributed, repositioned, and rotated daily. The larvae and adults were reared at 25°C (±0.5°C), 50% relative humidity, and 12L:12D cycle. Checks for emergence were started shortly after the pupae darkened (eight days post egg lay) and made at 07:30, 14:30 and 22:30 daily. Each check consisted of counting and sexing all emerged individuals. Checks were terminated 15 days post egg-lay, at which time almost all individuals had emerged. All populations' egg-to-adult development times were measured concurrently. Preadult viability (total flies emerged/total eggs collected) was also calculated for each population. After each check, individuals from each vial and each sex were pooled into a corresponding vial frozen at −18°C (±0.5°C). On day 15 post egg lay, five individuals were sampled from each holding vial and weighed as outlined above. Mean growth rate (mean body weight/mean development time) was calculated for each population and sex.

Statistical analysis

All statistical analyses were implemented using JMP 7.0 statistical software (SAS Institute 2007). All values are presented as means ± standard error (s.e.). At first, eye colour was introduced as a factor in the statistical models to account for a potential fitness cost of the *bw* marker. However, because the eye colour effect was not significant in any of the analyses, it was removed from the models and red-eyed and brown-eyed populations were considered as equivalent replicates within each selection treatment (D_{1-6}; C_{1-6}). On the other hand, 'population' (replicate number), which was introduced as a nested random factor (within selection), generally had a significant effect and was therefore featured in all analyses.

For desiccation resistance and preselection body weight, an analysis of variance (ANOVA) was performed with selection, generation and sex as fixed factors, and population nested within selection as a random factor. Further, a regression of each of the variable over time for each sex and population was performed and the obtained slopes were used as data subjected to an analysis of variance, where selection and sex were fixed factors.

Results

Desiccation resistance

Desiccation survival time responded rapidly to the selection protocol in the D populations relative to controls (table 2; figure 2). This was confirmed by a significant difference

Table 2. An analysis of variance on the mean desiccation resistance (generatin 5, 10, 15, 31, 37 and 46), preselection body weight (generation 10, 15, 31, 37 and 46), development time (generation 31 and 46), and growth rate (generation 31 and 46).

	Desiccation resistance			Body weight			Development time			Growth rate		
Effect	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Selection	1, 758	332.37	< 0.01	1, 560	22.10	< 0.01	1, 270	26.84	< 0.01	1, 270	28.05	< 0.01
Generation	5, 758	221.36	< 0.01	4, 560	52.74	< 0.01	1, 270	233.39	< 0.01	1, 270	6.68	0.01
Selection × generation	5, 758	104.04	< 0.01	4, 560	9.83	< 0.01	1, 270	8.70	< 0.01	1, 270	1.03	0.31
Sex	1, 758	1746.94	< 0.01	1, 560	2561.35	< 0.01	1, 270	37.65	< 0.01	1, 270	402.52	< 0.01
Selection × sex	1, 758	106.35	< 0.01	1, 560	21.23	< 0.01	1, 270	0.02	0.90	1, 270	0.59	0.44
Generation × sex	5, 758	15.96	< 0.01	4, 560	9.44	< 0.01	1, 270	0.40	0.53	1, 270	4.06	0.04
Selection × generation × sex	5, 758	1.80	0.11	4, 560	6.52	< 0.01	1, 270	1.07	0.30	1, 270	0.11	0.75
Population (selection) and random	10, 758	3.76	< 0.01	10, 560	5.15	< 0.01	10, 270	18.78	< 0.01	10, 270	3.91	< 0.01

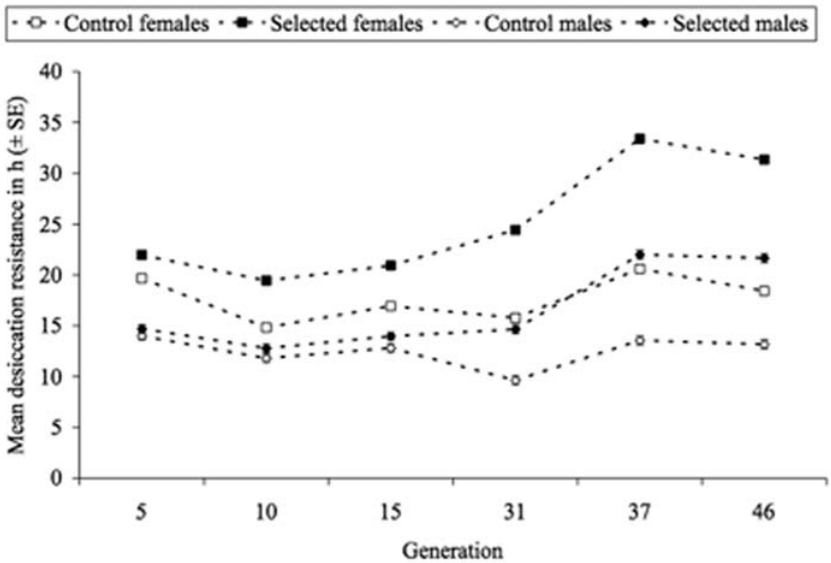

Figure 2. Desiccation resistance (± s.e.) after 5, 10, 15, 31, 37 and 46 generations of selection. There was no change in the degree of sexual dimorphism in desiccation resistance ($P = 0.17$; table 3)

Table 3. An analysis of variance on the slopes of the mean desiccation resistance (generation 5, 10, 15, 31, 37 and 46) and preselection body weight (generation 10, 15, 31, 37 and 46).

	Desiccation resistance			Body weight		
Effect	df	*F*	*P*	df	*F*	*P*
Selection	1, 20	160.25	< 0.01	1, 20	12.74	< 0.01
Sex	1, 20	14.66	< 0.01	1, 20	1.26	0.28
Selection × sex	1, 20	1.99	0.17	1, 20	4.03	0.06

between the D and C populations for the slope of the regression of desiccation resistance over time (table 3). The ANOVA on the desiccation resistance data revealed a significant selection × sex interaction (table 2; figure 2), however this interaction did not have a significant effect on the slope of the regression (table 3), probably due to a lower sensitivity of the slope analysis. This indicates that the rate of stress resistance evolution was not detectably different for males and females in the D treatment.

Female fecundity

For the stressed treatment, the mean duration to reach LD_{50} was calculated for each population. An ANOVA was employed, with selection as a fixed factor and population nested within selection as a random factor. D females endured stress significantly longer than C females (D = 31.18 ± 0.69 h; C = 18.79 ± 0.69 h) (ANOVA, $F_{1,10} = 159.52$, $P < 0.01$), which reflects their higher desiccation tolerance.

Alongside the duration of desiccation stress, the progeny of D and C females were examined with an ANOVA, with selection and treatment as fixed factors and population nested within selection as a random factor. D females were significantly less fecund (ANOVA, $F_{1,154} = 35.17$, $P < 0.01$; figure 3), and the stress significantly reduced fecundity (ANOVA, $F_{1,154} = 35.56$, $P < 0.01$; figure 3). Moreover, the selection × treatment interaction was significant (ANOVA, $F_{1,144} = 15.64$, $P < 0.01$; figure 3), with D females suffering a larger decrease in fecundity than C females as a result of the stress.

Male fitness

For the D combinations (combinations 5–8), survivors of the desiccation stress were counted and sexed. The sex-ratio (proportion of males) immediately prior to selection was 0.5 since the exact same number of males and females were stressed in each combination. The mean sex-ratios after selection were calculated. For the C combinations (combinations 1–4), all individuals survived the mild starvation stress. All proportions were arcsine-squareroot transformed, and the statistical analysis (not presented here) yielded the same results as when performed on the untransformed proportions. A *t*-test of grand means, at the combination level,

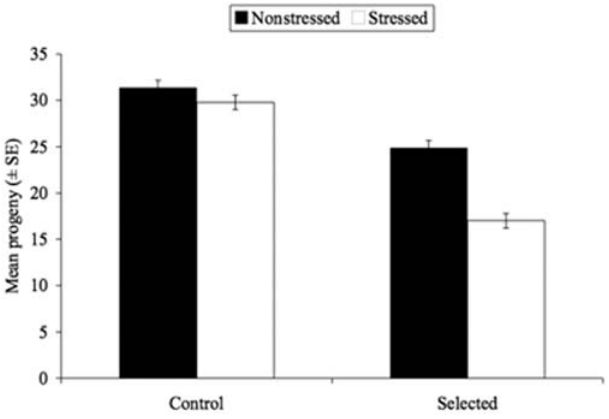

Figure 3. Fecundity (± s.e.) of control and selected females in nonstressed and stressed treatments (generation 48). Control females were more fecund than selected females in both treatments.

revealed that the preselection sex ratio of 0.5 was significantly different from the postselection sex ratio of 0.03 ± 0.01 ($t_{3df} = -46.63$, $P < 0.01$). Thus, as expected, desiccation stress was much deadlier for males, but a small fraction (2.3%) survived.

The postselection fitness of surviving males was examined. Recall that brown-eyed females were first permitted to mate with brown-eyed males prior to selection (preselection male fitness), and then we swapped in red-eyed males upon the imposition of stress (postselection male fitness). We were interested in the relationship between the mean proportion of surviving D males and their fitness contribution to the next generation, which would depend upon their condition as well as female receptivity to mating after the bout of desiccation stress. A paired *t*-test, with 'combination' as replicate unit, revealed that the mean proportion of red-eyed offspring was significantly greater than the mean proportion of surviving males by 0.04 ± 0.00 in test tubes (paired $t_{3df} = 8.37$, $P < 0.01$) and 0.03 ± 0.01 in culture bottles (paired $t_{3df} = 3.59$, $P = 0.04$). This positive difference indicates that the genes of surviving males are overrepresented in the next generation; thus, males that survived were fertile and capable of securing matings after selection. In fact, based on the assumption of isometry between male frequency and fertility, the fact that 2.3% of males sired 5.7% of total offspring, on average (figure 4), suggests a substantial reward for survival of the stress; a reward which is additional to offspring that may have been sired prior to the imposition of stress (nonestimable due to the one-way swapping in the experiment). For the C combinations, the mean proportion of red-eyed progeny was 0.90 ± 0.02 in test-tubes and 0.89 ± 0.02 in culture bottles, suggesting that late mating is the predominant mode of fertilization under mild starvation stress.

Cost of social and sexual interactions

Desiccation resistance and body size of recently emerged and sexually mature flies were each subjected to an ANOVA,

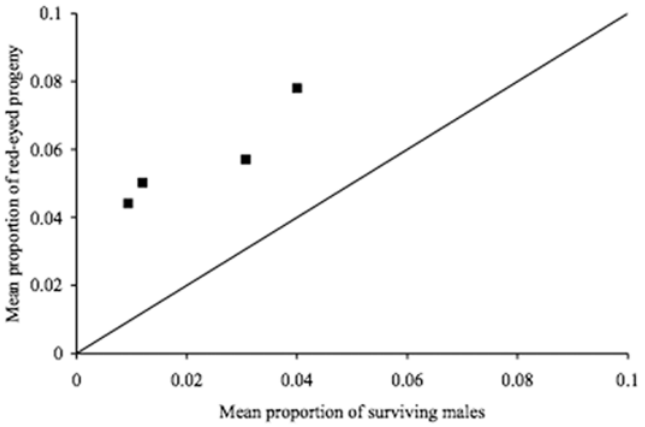

Figure 4. Poststress fitness (mean proportion of red-eyed progeny) as a function of proportion of surviving males (generation 52). Each point represents one of the four combinations (combinations 5–8; see table 1). The diagonal line represents isometry between survival probability and postselection fitness. The small fraction of surviving males sired 2.5 times more offspring than predicted, suggesting a potential fitness advantage to surviving stress. Such an advantage would be countered by the low odds of survival and any costs to preselection fitness incurred by survivalist males.

with selection, treatment (recently emerged versus sexually mature), and sex as fixed factors and population nested within selection as a random factor. Because of a strong selection × sex interaction (desiccation resistance: ANOVA, $F_{1,220} = 85.48$, $P < 0.01$; body weight: ANOVA, $F_{1,222} = 6.85$, $P < 0.01$), the sexes were examined separately (table 4). Interestingly, there appears to be a cost to social or sexual interactions since sexually mature males and females were significantly less resistant to desiccation than recently emerged flies were (table 4; figure 5,a). While males lost body weight, females in the same treatment significantly gained body weight (table 4; figure 5,b), which is consistent with sex differences in acquisition and allocation of resources early in life. However, the interaction between selection and treatment was not significant in any of the analyses, which suggests that D flies have not evolved to mitigate the costs of social and sexual interactions early in life.

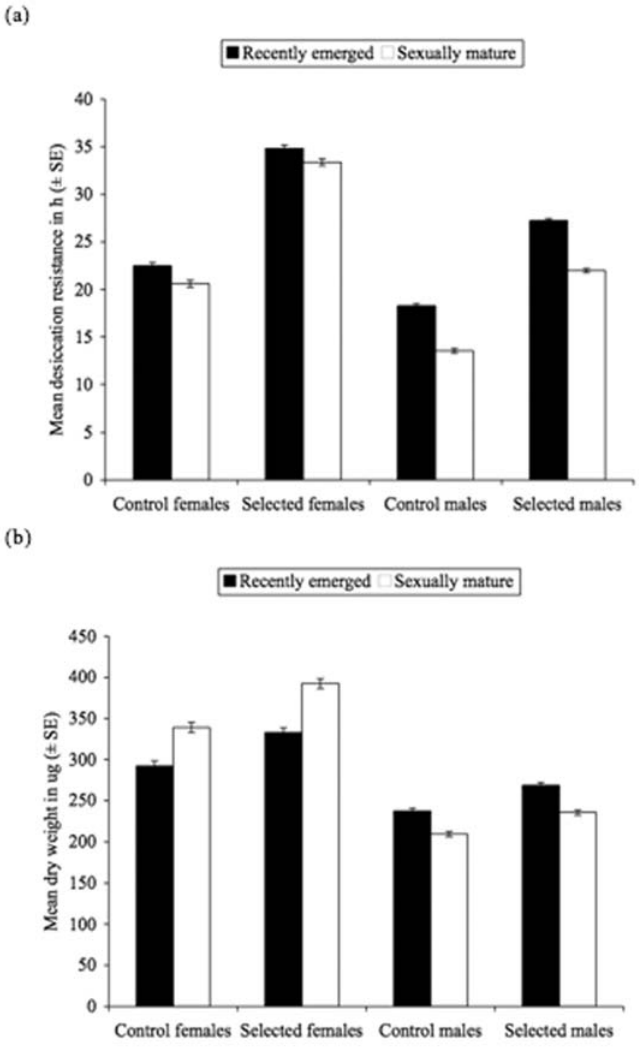

Figure 5. (a) Young adult changes in desiccation resistance (± s.e.) (generation 37). Flies lost resistance with age in both sexes and selection treatments, with male resistance dropping faster. However, no significant interactions were revealed between the selection, treatment, and sex. (b) Young adult changes in weight (± s.e.) (generation 37). Females gained weight after eclosion, while males lost weight. However, there was no significant interaction between selection and treatment.

Table 4. An analysis of variance on the mean desiccation resistance and preselection body weight of recently emerged and sexually mature flies (generation 37).

	Desiccation resistance						Body weight					
	Female			Male			Female			Male		
Effect	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Selection	1,105	478.97	< 0.01	1, 105	210.86	< 0.01	1,105	36.65	< 0.01	1,102	23.18	< 0.01
Treatment	1,105	21.85	< 0.01	1,105	490.15	< 0.01	1,105	79.02	< 0.01	1,102	89.92	< 0.01
Selection × treatment	1,105	0.37	0.54	1,105	1.28	0.26	1,105	1.15	0.29	1,102	0.61	0.43
Population (selection) & random	10,105	2.59	< 0.01	10,105	7.07	< 0.01	10,105	1.68	0.09	10,102	3.47	< 0.01

Juvenile growth and survival

D flies significantly increased in body weight relative to C flies (table 2; figure 6). This was confirmed by a significant difference between the D and C populations for the slope of the regression of body weight over time (table 3). The ANOVA on the body weight data revealed a significant selection × sex interaction (table 2; figure 6), which reflects a growing difference in weight between males and females in the D populations (females heavier). This interaction showed up in the analysis of regression slopes (table 3) as near statistical significance ($P = 0.06$), suggesting a relationship we could not detect with reduced degrees of freedom in the slope analysis. It appears that sexual size dimorphism was slowly increasing in the D selection treatment.

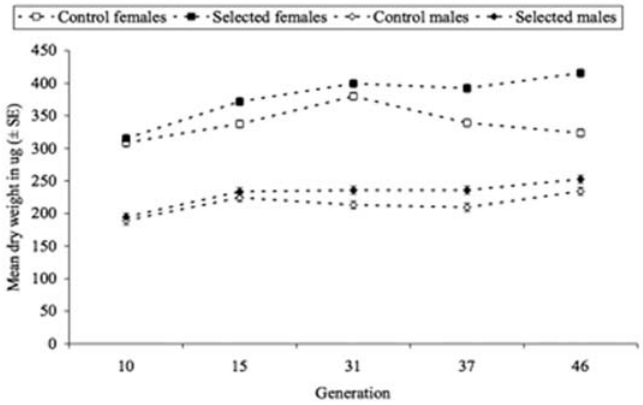

Figure 6. Changes in body weight (± s.e.) after 10, 15, 31, 37 and 46 generations of selection. In the selected treatment, both sexes increased in dry body mass, relative to controls. There was a trend towards greater female-biased sexual dimorphism in body weight ($P = 0.06$; table 3).

D flies had a significantly extended egg-to-adult development (D = 254.21 ± 0.77 h; C = 248.56 ± 0.77 h) and faster growth rate (D = 1.16 ± 0.02 μg/h; C = 1.02 ± 0.02 μg/h) (table 2). A significant generation × selection interaction was revealed for development time (D_{G31} = 251.89 ± 0.23 h; D_{G46} = 256.54 ± 0.28 h; C_{G31} = 246.98 ± 0.23 h; C_{G46} = 250.13 ± 0.28 h), but interestingly not for growth rate (table 2). Further, the selection by sex interaction was not significant.

Finally, preadult viability was examined with an ANOVA, with selection and generation as fixed factors and population nested within selection as a random factor. Desiccation-selection slightly but significantly lowered survivorship (D = 0.82 ± 0.01; C = 0.84 ± 0.01; ANOVA, $F_{1,140} = 5.73$, $P = 0.02$). Viability for D and C flies was not significantly different between generations 31 and 46.

Discussion

Wright (1932) envisioned adaptation occurring on multidimensional fitness landscapes generated by epistasis. The existence of sexual dimorphism in a species suggests that the adaptive landscape may be different for males and females, with the two sexes selected towards different fitness peaks. Where loci are shared between the sexes, there may be sexual conflict over optimal alleles or gene-expression patterns, imposing a load on populations by moving each sex off its fitness peak. This form of conflict can be offset by the evolution of modifiers and sex-specific gene-expression through a variety of mechanisms. However, an environmental change can create a new landscape for fitness, with potential for the sexes to be drawn apart at novel loci, not previously in conflict. Here, we discuss a simple scenario observed during laboratory selection for stress resistance in fruit flies: the same selective agent—desiccation stress—appears to introduce different (opposite) pathways of best response for males and females with respect to development, behaviour, and fertility schedules, but the genetic correlation between the sexes for these characters may have limited the evolutionary response towards different optima.

Drosophila have undergone multiple adaptive radiations into arid regions and respond readily to laboratory selection for desiccation resistance (e.g., Hoffmann and Parsons 1989; Gibbs *et al*. 1997; Chippindale *et al*. 1998). Here, after 46 generations, the desiccation-selected (D) populations had increased in survival time under desiccation by 68% compared to their controls (C populations; table 2; figure 2). The physiology of desiccation tolerance inevitably relates to water balance. To increase survival time, the organism may (i) store more water in advance of the drought, (ii) reduce its water loss rate, or (iii) decrease the critical threshold of water content to increase dehydration stress. Previous work with a different set of *D. melanogaster* populations implicated the first two mechanisms, showing changes throughout the juvenile and adult life-history and physiology to augment water storage of both metabolic and bulk water, and to limit water loss (Gibbs *et al*. 1997; Chippindale *et al*. 1998). Importantly, that research also found substantial differences between the sexes in their evolved responses to desiccation stress. Selected males lost weight, water, and key metabolites at an accelerated rate (relative to controls) as young adults, whereas that pattern was reversed in females. The results were consistent with selection for 'live fast, die young' males and hardy, drought-tolerant females under the stress imposed. Our research follows up on the inference of sexually antagonistic selection under desiccation stress, particularly by characterizing the nature of selection upon males. Based upon the previous research and pilot experiments, we predicted that desiccation-selection would affect a wide range of traits differentially between the sexes, exaggerating extant sexual dimorphism if it is not constrained by genetic correlations.

As a first step in contrasting our results with those from the earlier research at UC Irvine (Gibbs *et al*. 1997; Chippindale *et al*. 1998), it is important to note some of the differences between the protocols. In the earlier selection experiment, selection was more intense (90% mortality versus

75% mortality) and both the growth media and timeline for selection differed. The cornmeal–molasses medium used in the present study is both drier and harder for larvae to 'work' than the banana–agar medium used in the earlier selection experiment at UC Irvine. At the same time, the Irvine protocol allowed 14 days in the same vial prior to desiccation-selection, whereas our animals were put into the stress treatment at 12 days from egg. This combination of differences is certain to place a heavier emphasis on rapid development, higher larval resource acquisition, and early maturation and mating in our D lines than the Irvine D lines. Such factors may help to explain why the earlier experiment saw a more rapid response to selection, with survival time more than doubling over the first 45 generations of selection. However, the source population used in our experiment also differed (LH_M versus Ives), and it is possible that there was less standing genetic variation for desiccation resistance at the outset. A final possibility is that the response has been constrained by sexual conflict, a possibility we explore here.

Evolution of development

Among the many potential mechanisms employed to combat desiccation stress (Gibbs *et al*. 1997; Chippindale *et al*. 1998; Marron *et al*. 2003), increasing water intake (in the form of bulk or metabolic water) and decreasing water loss rate are the most obvious. These two mechanisms are particularly compatible because an increase in water and metabolites will increase body size, producing a smaller surface area to volume ratio at the same time. In the current study, after 46 generations, D flies had increased their dry body weight, which is highly correlated with volume, by 20% relative to C flies (table 2; figure 6).

Further insight into the link between desiccation resistance and body weight is provided by development time and growth rate. To develop into a larger adult, an individual has two options: (i) extend development time or (ii) increase growth rate. The larger body size of D flies is explained by a combination of these two possibilities. At generation 46, D flies showed extended egg-to-adult development compared to C flies (table 2). Extended egg-to-adult development may permit more resources to be acquired and stored during the larval stage, or create a bigger compartment for the adult to do so. The positive genetic correlation between development time and body size we observed (table 2) has been widely reported in *D. melanogaster* (e.g., Zwaan *et al*. 1995; Nunney 1996; Prasad *et al*. 2000; Chippindale *et al*. 2003). In addition to their longer period of development, D flies grew 14% faster than C flies did (table 2). Because growth rate was estimated by mass at eclosion divided by total development time, which includes nongrowing stages like late L3 and the pupal stage (about half of total development time), this further suggests intense pressure on the feeding larvae to maximize size at eclosion.

Costs to adaptation

Adaptation to desiccation came at a cost in terms of both preadult viability and female fecundity. C flies had greater preadult viability than D flies did. Although the difference was slight (2.5%), it should be noted that juvenile mortality represents an absolute fitness cost exposed to the full force of natural selection. C females also produced more offspring than D females in both the nonstressed and stressed treatments (figure 3). The fact that C females produced more progeny than D females in the nonstressed treatment suggests that selection for investment in stress resistant characters leads to reduced fecundity because of a resource trade-off. Energy sequestered for survival of the stress bout may be irreversibly dedicated to survival, taking away from reproduction (Chippindale *et al*. 1998). Considering both body size and fecundity, the lowered reproductive output of D females despite their size advantage underscores the apparent trade-off between stress tolerance and reproductive activity.

That D females were also less fecund in the desiccation stress treatment is at first counterintuitive. However, the metric of stress applied was akin to an LD_{50}: equal stress to D and C females was determined by 50% mortality, which required 13 h (+66%) of additional stress exposure for D females. Because the treatments differed, with D females receiving a longer stress bout and C females having more time to recover, we are reluctant to interpret the results as evidence of a generic decline in female fecundity. We also consider it unlikely that the fitness differences observed are the result of differential inbreeding because we employed large populations and attempted to match selection treatments for effective population size.

Sex and fitness

On first consideration, similar responses by the two sexes would be expected because they share the same gene pool, to a large extent, and were exposed to the same ecological stress. However, sex-specific responses might arise because D males infrequently survived selection, and mating has never been observed during selection (Chippindale *et al*. 1998; Kwan L., Bedhomme S., Prasad N. G., Chippindale A. K. unpublished data). We expected the desiccation protocol to create dissimilar fitness interests between the sexes, with strong selection for early maturation and mating in males, and opposing selection for resource acquisition and survival in females. On the other hand, the C populations should experience coordinated evolution because both males and females survive the mild starvation stress and females are promiscuous and likely to remate.

Observation of the recovered flies after selection in routine culture indicated that it was common for all males to perish under selection, leaving females to reproduce with only stored sperm. Our formal estimate showed that about 2% of males survived selection and that these males sired 2.5 times as many progeny as predicted based on their frequency in the

cages (figure 4). Because this is in addition to any progeny sired prior to selection, survival of stress may have paid off by as much as quadrupling fitness. Hence for males there was a substantial benefit to surviving the stress to mate with sexually receptive females, but this strategy may have been risky if investing in survival was deleterious to early mating success. To fully evaluate the costs and benefits of being a survivor male, we would require more quantitative data on the early fitness impact of the extended development and larger body size associated with stress survival, and further census to estimate survival probabilities.

The idea that optimal strategies differed between the sexes under desiccation-selection is reinforced by our data on age-specific stress resistance and weight. After 37 generations, recently emerged flies withstood stress longer than sexually mature flies, with the loss of resistance being substantially (22%) greater in males than females (6%) (table 4; figure 5,a). Over the same time period, females gained, and males lost weight (table 4; figure 5,b). These responses suggest that social and sexual interactions prior to selection increased stress susceptibility, with males paying a higher cost. Males appear to expend more net energy early in adult life, likely pursuing and courting mates at the expense of feeding and 'bulking up' for selection they are unlikely to survive; this confirms earlier evidence that *D. melanogaster* males hardly eat, and generally lose weight over the first several days of adult life (Chippindale *et al*. 1998). For females, conservation of resources early in life may contribute to both survival of stress, and fecundity thereafter. However, the absence of a significant interaction between selection and treatment (recently emerged versus sexually mature) indicates that D and C weight and desiccation time changed in similar ways from eclosion to application of selection (table 4; figure 5). This result was surprising because increased resistance to the costs of social and sexual interactions in D females might be predicted after 37 generations of selection. If such a response was impeded by opposite selection on males, this would be a manifestation of sexual conflict.

We expected D males and females to move towards divergent fitness optima if not constrained by genetic correlation. Here, sexual size dimorphism (SSD) is of particular interest because an increase in body size is associated with resistance to desiccation stress. Our data suggest the gradual evolution of female-biased SSD under desiccation-selection, although the result was marginally nonsignificant ($P = 0.06$; table 3). It is likely that the positive genetic correlation between the sexes for body size is strong, impeding independent evolution in each sex. Reeve and Fairbairn (1996, 1999) have shown weak and inconsistent outcomes of selection to modify SSD. Given these constraints, the inferred forces of selection on males may be limiting to the evolution of female desiccation tolerance. Similarly, male desiccation resistance may have been pulled along with female resistance because of tight linkage between the sexes for developmental characters, body size and other factors influencing desiccation resistance. Among these other factors would be the indirect benefit of having resistant daughters.

Sexual conflict will arise when characters that are positively genetically correlated across the sexes have opposite influences on relative fitness. For example, Long and Rice (2007) recently characterized locomotor activity as a sexually antagonistic character in the same (LH_M) population used to found the D and C lines described herein. Interestingly, locomotion is exactly the sort of character we would expect to be under divergent selection in stress selected populations because one of the most spectacularly obvious adaptations we see is a loss of panic response to stress and adoption of the opposite, sit and wait, strategy by females under stress. It is possible for new differential or even opposing selection on the sexes to arise from a change in the environment, and when this is constrained by genetic correlation it will generate sexual conflict (Bedhomme and Chippindale 2007). We follow the earlier study by Chippindale *et al*. (1998) in suggesting that desiccation stress places the two sexes under divergent selection pressures for optimal mating schedule and patterns of development. But while selection resulted in the extensive remodelling of a suite of traits related to stress resistance, here we found only weak evidence of different adaptive solutions for males and females in remediation of sexual conflict. We interpret this as evidence that the intersexual genetic correlations underlying characters involved in stress resistance are positive and have limited evolutionarily lability over this micro-evolutionary time scale. It is unclear whether the differences between studies have arisen because of differences in the selection protocols themselves, where our protocol was more restrictive with respect to changes in pre-adult and early-adult characters, or if they reflect differences in the genetic architecture of the LH_M and Ives populations. In either case, we submit that our data support a view that a changing environment can remodel fitness in a sex-specific manner, with potential to foster intralocus sexual conflict.

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