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Heat-shock resistance in *Drosophila* populations: analysis of variation in reciprocal cross progeny

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Genetic variation for resistance to high temperature stress was studied in populations of *D. melanogaster* and *D. buzzatii* from different geographic regions. *Drosophila melanogaster* individuals were presented with either a direct short exposure to a high temperature or exposure to high temperature after receiving a pretreatment, which increased resistance. Heat-stress resistance varied among populations, with one much more resistant than all others under both treatments. Another possessed low stress resistance when exposed without the heat pretreatment; but with pretreatment, resistance increased relative to the other populations. Evidence from reciprocal crosses suggests that the X chromosome of the more resistant population carries alleles that greatly increase resistance, and that one or more factors on the autosomes also affect resistance. Non-additive interaction effects among the three less resistant populations, were suggestive that all differ for various elements that contribute to stress resistance, and that some clearly change inducible resistance more than basal levels. In *D. buzzatii*, the two least resistant populations were genetically very similar. Crosses to the more resistant population gave results suggesting that the low resistance to heat is dominant. A small X-chromosome effect that increased resistance, and a dominant enhancer of male resistance also may have contributed to variation in resistance.

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Variation in resistance to high temperature has two major sources. One is genetic variation among species and populations (HOFFMANN and PARSONS 1991; PARSELL and LINDQUIST 1994; WHITE et al. 1994). The other is phenotypic adjustments in relation to thermal history, such as conditioning, acclimation or acclimatization (HUEY and BENNETT 1990; LOESCHCKE et al. 1994; KREBS and LOESCHCKE 1994a), and the heat-shock response (LINDQUIST 1986; PARSELL and LINDQUIST 1993). The former, genetic variation, has received relatively less attention. Comparison of distinct lines from the same population, as well as selection techniques, have been used to show significant levels of genetic variation for thermotolerance within populations (HOSGOOD and PARSONS 1968; WHITE et al. 1970; MORRISON and MILKMAN 1978; KILIAS and ALAHIOTIS 1985; QUINTANA and PREVOSTI 1990; HUEY et al. 1992; LEROI et al. 1994; LOESCHCKE et al. 1994; KREBS and LOESCHCKE 1996).

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Crosses among genetically distinct lines that vary in thermotolerance could be used to begin analyses of the genetic basis of line differences, but only rarely has this been done. Following successful selection to reduce tolerance, MORRISON and MILKMAN (1978) crossed lines to map heat susceptibility effects to the second chromosome of *D. melanogaster*, and similarly OUDMAN (1991) identified a natural variant that reduced thermotolerance, at a locus on this chromosome. KREBS and LOESCHCKE (1996) crossed selected thermotolerance lines of *D. buzzatii* to show that different genetic loci affect variation in survival between individuals exposed directly to a high temperature stress or first pretreated before exposure. Many systems should be affected by heat, but those contributing most of the genetic variation may be fewer. Identifying heritable patterns for stress resistance may help focus attention on specific physiological mechanisms and their role in the response of organisms to environmental change.

Here we examine variation in thermotolerance of reciprocal cross progeny among four populations of *D. melanogaster* and three of *D. buzzatii* to

compare patterns of genetic variation related to resistance. The specific populations differ in thermotolerance, and thus are well suited to elucidate how genetic differences interact and/or separate among autosomes and the X chromosome, and whether differences among populations are due to one or several factors. Crosses also may identify genetic differences among populations with similar resistance levels.

Materials and methods

Drosophila melanogaster. — Ten isofemale lines of *D. melanogaster* were obtained from collections near the following localities: Hov, Denmark in October, 1992; Bologna, Italy in October, 1993; southwestern Tenerife, one of the Canary Islands, in December, 1993; and southern Mali, in December, 1993. Similar numbers of adults from each set of 10 lines were pooled to form mass populations in spring, 1994. These populations were maintained 4–5 generations, after which experiments were begun in July, 1994.

Reciprocal F_1 crosses were made among each of the four populations. Virgin females and males were collected under CO_2 anesthesia from each. Twenty females of one population were placed with the same number of males in each of two bottles that contained yeast-sucrose-agar medium and live yeast sprinkled on the surface. In total, there were 6 population pairs × 2 reciprocal crosses (2 bottles each), and 4 bottles were made for each of the four parental populations. Flies in these 40 bottles were transferred to new food bottles after two and four days to be sure ample progeny of the same age were available for the experiments.

For analysis of resistance to high temperatures, emerging males and females were separated each day, and 30 individuals were placed in each vial, which also contained yeast-sucrose-agar medium and live yeast. Two replicate vials were collected for males and females of the parental lines, and one replicate was collected for each reciprocal cross between populations. This procedure was repeated over 12 consecutive days to produce separate blocks of replicates. When 4–5 days old, these adults were heat shocked in a water bath either at 39.5°C for 30 min in the non-pretreated treatment group, or at 39.9°C for 30 min in the pretreated group, with six blocks of replicates assigned to each group. Flies were pretreated by exposure to 38.7°C for 10 min, 4 h prior to exposure to the higher temperature. All treatments were performed in plastic vials with a foam pad placed at the bottom (see CAVICCHI et al. 1995, for details). Humidity was not controlled during heat treatments, but desiccation effects are probably small over the short treatment time. After heat shock, flies were transferred to new vials containing food. Survivorship was scored 24 h later by counting the proportion of individuals that responded when touched with forceps.

Drosophila buzzatii. — Crosses also were made among three *D. buzzatii* populations that varied in heat resistance. These were the three more extreme populations of KREBS and LOESCHCKE (1995a). A high resistance line was obtained from Tenerife, Canary Islands, and two low resistance lines were from Metz Gorge, NSW, Australia, and from Cordoba, Spain. These populations also were produced from pooling 10 isofemale lines, except for the Canary Islands population, which was formed from 22 lines. All three had been maintained at 25°C for more than one year. Virgin females and males were separated under CO_2 anesthesia from which reciprocal F_1 crosses between each pair of the three populations were prepared (3 × 2 crosses in total). For each cross, 20 males of one population were placed with 20 females of another on instant *Drosophila* medium (Carolina Biological Supply). Prepared were four bottles for each parental population cross and three for each between population cross. Flies in these bottles were transferred to new food after two and four days. Emerging males and females were separated under CO_2 anesthesia and partitioned to 20 per vial (containing yeast-sucrose-agar medium, and live yeast on the surface). Each of the five blocks of replicates were prepared with 4 vials from crosses between males and females of the same parental line and with 2 vials for crosses between different parental lines.

Adults were heat shocked at 4–5 days of age in glass vials containing a 2% agar base in an air incubator set to 41.5°C, with an exposure time of 100 min. Twenty-four hours before this exposure, all individuals were pretreated to high temperature for 75 min in an incubator set to 38°C. Vials in air incubators heat up more slowly than those in water baths, and the final temperatures within vials tend to be slightly below that of the set temperature (KREBS and LOESCHCKE 1994a). During treatment, humidity was controlled within vials by moistening stoppers and inserting them flush with

the vial top. Vials then were inverted. Following heat shock, flies recovered in these inverted vials. Survivorship was scored 24 h later by counting the proportion of individuals that could walk when touched with a brush.

Species differences are apparent in the much greater reduction in female fecundity and male fertility of *D. buzzatii* individuals surviving thermal stress (KREBS and LOESCHCKE 1994b) than of *D. melanogaster* survivors (KREBS and LOESCHCKE 1994a,c). Although scoring techniques differed for the experiments with *D. melanogaster* and *D. buzzatii*, our experience suggests that scoring methods have little effect on relative line differences. Additionally, whereas *D. melanogaster* tends to die or to be very alive following stress, *D. buzzatii* individuals may be damaged much more severely while remaining alive.

Statistics. — The measured response variable was the proportion surviving in each vial, which was arcsine-square-root transformed. Initial analyses compared parental types only, using ANOVA. Fixed effects were population, sex, block, and the interactions between population and sex. Residuals from these analyses were not significantly different from a normal distribution. Differences in mean resistance with or without the pretreatment were tested separately. Separate *t*-tests were run to examine differences between each of a pair of parental populations and their F_1 offspring. These comparisons were not independent, as means for parental populations were used several times, once for each comparison with another population, and means for F_1 crosses were used in tests with each parental line. These cross-comparisons using the same data do not meet the requirements for a sequential Bonferroni analysis (RICE 1989). Therefore, significance levels are presented for the individual comparisons and not at a table-wide level. The large number of comparisons, especially given the necessity of splitting results for males based on the origin of the X chromosome, likely led to some "significant" results that were spurious. Conclusions therefore focused on consistent patterns in variation across population comparisons. Care was also taken because an unusually low or high result could affect several comparisons.

Results

Block variation. — For both species, block variation between different sets of vials that were placed at high temperature at different times was examined using parental population data. For the experiments on *D. melanogaster*, the effect of block was significant only where individuals were not pretreated, but interaction effects with block, which were tested in preliminary analyses, were not significant. Therefore, variance due to interaction effects that included block were maintained as a component of the error. Likewise for *D. buzzatii*, the mean proportion of surviving adults varied in different blocks of replicates, but interaction effects including block were not significant.

D. melanogaster — parental populations. — Populations varied in survival following both heat-shock treatments, exposure of individuals to high temperature without any prior experience, and for exposure with pretreatment (Table 1). Individuals of one population, Mali, had significantly higher survival under both treatments (Table 2, Tukey's multiple comparisons test). For basal tolerance, survival of another population, Italy, was significantly lower than for individuals from either Denmark or the Canary Islands. With pretreatment, survival of these three populations after heat stress was similar.

Overall, female survival was higher than that of males under both treatment conditions. A population × sex interaction was significant only for the comparison of pretreated individuals (Table 1). A higher proportion of males than females of one population survived heat shock when pretreated, but in the others, and for all populations when individuals were not pretreated, survival of females was higher than that of males (Table 2).

D. melanogaster — population crosses. — As each population was crossed to three other populations, results were separated to examine significance of differences between reciprocal crosses of each pop-

Table 1. Analysis of variance for survival of *D. melanogaster* males and females exposed to a high temperature stress of 39.5°C without any acclimation treatment or acclimated by exposure to 38.7°C for 10 min, and 4 h later exposed to a stress of 39.9°C for 30 min

Source	df	Acclimated mean square	Not acclimated mean square
Block	5	0.037	0.445***
Population	3	1.280***	1.675***
Sex	1	1.058***	1.340***
Population × sex	3	0.226***	0.050
Error	83	0.019	0.030

*** $P < 0.001$

Table 2. Survival after stress (in percent ± SE) of *D. melanogaster* males and females from four populations (part A) and the F_1 progeny between these populations (part B). Individuals were exposed to a high temperature stress of 39.5°C without any acclimation treatment or acclimated by exposure to 38.7°C for 10 min, and 4 h later exposed to a stress of 39.9°C for 30 min. Different letters in part A denote significant differences among parent strains (Tukey's multiple comparisons test), and asterisks in part B indicate significance of differences between reciprocal cross progeny from that pair of parental strains

Source populations		Not acclimated		Acclimated	
females	males	females	males	females	males
A. Parental populations					
Mali	Mali	84.9 ± 2.6	71.8 ± 5.8a	83.3 ± 2.4	88.0 ± 2.2a
Denmark	Denmark	58.7 ± 5.5	30.0 ± 7.6b	65.5 ± 2.6	39.9 ± 4.7b
Canary Isl	Canary Isl	52.7 ± 6.2	36.3 ± 5.8b	50.3 ± 4.3	25.6 ± 3.9b
Italy	Italy	33.3 ± 5.2	14.6 ± 4.5c	69.6 ± 3.8	35.1 ± 3.4b
B. Reciprocal cross progeny					
Mali	Denmark	70.1 ± 7.5	61.3 ± 11.7*	88.6 ± 2.7	90.3 ± 2.8*
Denmark	Mali	73.6 ± 5.7	16.9 ± 6.1	91.4 ± 2.0	68.6 ± 6.9
Mali	Canary Isl	70.2 ± 5.3	73.2 ± 6.1*	89.9 ± 1.9	86.7 ± 2.5*
Canary Isl	Mali	70.9 ± 6.8	22.2 ± 6.0	93.0 ± 1.8	62.3 ± 8.7
Mali	Italy	87.6 ± 5.0	66.1 ± 14.3*	82.6 ± 4.4	85.5 ± 4.3*
Italy	Mali	76.5 ± 5.4	31.7 ± 4.1	89.6 ± 3.8	62.3 ± 4.3
Denmark	Italy	31.6 ± 9.5	13.4 ± 8.7	44.9 ± 4.3	18.6 ± 5.7
Italy	Denmark	30.1 ± 3.9	8.1 ± 4.0	46.1 ± 9.6	25.5 ± 12.6
Canary Isl	Italy	37.8 ± 7.8	27.7 ± 15.5	62.0 ± 6.3	20.3 ± 5.4*
Italy	Canary Isl	48.3 ± 4.3	16.3 ± 4.2	71.2 ± 5.1	40.3 ± 9.6
Denmark	Canary Isl	50.1 ± 8.9	21.2 ± 5.4	57.3 ± 5.3	24.2 ± 4.6
Canary Isl	Denmark	33.0 ± 4.2	22.6 ± 3.9	58.3 ± 7.3	22.1 ± 6.7

* $P < 0.05$

Table 3. For the four *D. melanogaster* populations, a summary of tests of significant differences between F_1 cross progeny and their parental strains. Results for reciprocal crosses were pooled unless significant differences occurred between reciprocal-cross progeny. Otherwise, results for reciprocal-cross progeny for males are separated based on the origin of the X chromosome (from the parental strain with which means are compared or from the other strain used to produce the F_1 progeny)

Parental strain	F_1 cross	Not acclimated				Acclimated			
		females all progeny	males			females all progeny	males		
			all progeny	X-same strain	X-other strain		all progeny	X-same strain	X-other strain
Mali	Mali/DK	*	**	ns	***	*	ns	ns	*
Mali	Mali/C.I.	***	*	ns	***	**	0.051	ns	**
Mali	Mali/Italy	ns	0.053	ns	***	ns	*	ns	***
DK	DK/Mali	*	ns	0.061	*	***	***	***	***
DK	DK/C.I.	*	ns			ns	**		
DK	DK/Italy	***	***			**	**		
C.I.	C.I./Mali	**	ns	0.058	***	***	***	***	***
C.I.'	C.I./DK	ns	*			*	ns		
C.I.	C.I./Italy	ns	ns	ns	**	**	ns	ns	0.051
Italy	Italy/Mali	***	**	*	**	**	***	***	***
Italy	Italy/DK	ns	ns			**	*		
Italy	Italy/CI	ns	ns	ns	ns	ns	ns	ns	**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

ulation pair (Table 2), and to compare F_1 progeny with the parental lines from which they were produced (Table 3). Where X-chromosome effects were indicated, results for F_1 males were further separated based on the source of the X chromosome. Both for pretreated and basal resistance levels, differences between female offspring originating from different reciprocal crosses were not significant, and therefore female offspring were always analyzed combining reciprocal-cross progeny.

Results in Table 2 can be considered in three groups. First are comparisons of crosses between the most resistant population, Mali, and all others. Second, are comparisons of crosses between the least resistant population, Italy, and the populations from Denmark and Canary Islands. Third, are comparisons of crosses between Denmark and Canary Islands, the two populations that did not show significant differences in mean survival.

(1) For all crosses that included the most resistant population, Mali, survival of male F_1 offspring was significantly higher for individuals carrying the Mali X chromosome than for those carrying an X chromosome from another population (Table 2). For F_1 males that carried the Mali X, survival was not significantly different from that of males of the parental Mali genotype, while survival of male offspring carrying an alternate X chromosome differed significantly from the parental Mali individuals (Table 3). This pattern was observed for heat-shock resistance of non-pretreated and pretreated males. However, differences between reciprocal cross progeny were somewhat larger in the non-pretreated group. An unexpected result was reduced survival in crosses between Denmark or Canary Islands females and Mali males. Basal resistance of male progeny was significantly less than the survival of the parental individuals. With pretreatment, survival of F_1 male progeny from these crosses was intermediate and significantly different to survival of both parent populations.

For female offspring of Denmark and Canary Islands individuals crossed to Mali, survival was significantly different from the Mali individuals (Table 3). The F_1 progeny had *lower* survival than had the Mali individuals without the pretreatment, but *higher* survival than Mali where individuals were pretreated (Table 2). This result may have been due to an aberrant low score for resistance in pretreated Mali females. Survival of female offspring from the cross between Mali and Italy was significantly higher than that of the Italy parental individuals and not significantly different from that of the Mali parental individuals either with or without pretreatment (Table 3).

(2) For male offspring from crosses between the low surviving population, Italy, and intermediate ones, basal resistance was lower where males carried the Italy X chromosome than if they possessed the X chromosome from either Denmark or the Canary Islands. However, resistance was higher for pretreated males possessing the Italy X chromosome (Table 2). Specific comparisons between reciprocal crosses showed significance only for pretreated males of the Italy–Canary Islands cross (Table 3), providing evidence for differences between the Canary Islands and Denmark populations that is not expressed in analyses of the parental individuals. Basal resistance of offspring from crosses between Denmark and Italy was similar to that of the Italy population (means significantly different from Denmark); and with pretreatment, resistance was significantly below that of both parental populations (Table 3). Survival of F_1 female offspring from crosses between the Canary Islands and Italy populations were intermediate and not significantly different from that of either parental population.

(3) Basal resistance levels below that of both parental populations were observed for F_1 progeny between the Canary Islands and Denmark populations, except for pretreated females. Differences were significant between F_1 and parental Denmark females and between F_1 and parental Canary Island males. The lower resistance of the F_1 progeny also was significant between pretreated F_1 progeny and the Denmark parental individuals.

D. buzzatii — parental populations. — Populations varied significantly in survival to heat shock with pretreatment (Table 4), with resistance of individuals from the Canary Islands populations being significantly higher than for those from either Cordoba or Metz Gorge (Table 5, Tukey's multiple comparisons test). Differences in results between the lower surviving populations were not significant. Overall, the population × sex interaction was highly significant. Survival was significantly higher for males than for females of the high-resistance population (by 16.4 %), with sex differences small and not significant for one low-resistance population; and for the other, survival of females was 12.5 % higher than that of males. None of these differences were individually significant.

Table 4. Survival after stress (in percent ± SE) of *D. buzzatii* males and females from three populations and the F_1 progeny between these populations. Individuals were acclimated to high temperature by exposure to 38.0°C for 75 min, and 24 h later they were exposed to 41.5°C for 100 min. Different letters in part A denote significant differences among parent strains (Tukey's multiple comparisons test) and asterisks in part B indicate significance of differences between reciprocal cross progeny from that pair of parental strains

Source population		Percent surviving of acclimated flies	
females	male	females	males
A. Parental populations			
Canary Isl	Canary Isl	48.4 ± 6.1a	64.8 ± 5.4a
Cordoba	Cordoba	26.3 ± 4.1b	23.0 ± 5.0b
Metz Gorge	Metz Gorge	30.3 ± 5.9b	17.8 ± 4.2b
B. Reciprocal cross progeny			
Canary Isl	Cordoba	25.7 ± 8.5	44.1 ± 9.3
Cordoba	Canary Isl	33.3 ± 6.1	36.5 ± 9.3
Canary Isl	Metz Gorge	36.8 ± 9.1	63.4 ± 10.4*
Metz Gorge	Canary Isl	38.6 ± 4.6	40.4 ± 10.0
Metz Gorge	Cordoba	23.0 ± 6.7	24.7 ± 4.8
Cordoba	Metz Gorge	21.6 ± 5.8	27.4 ± 8.4

Table 5. For *D. buzzatii*, summary of tests of significant differences between F_1 cross progeny and parental strains, pooling results for reciprocal crosses for females. Results for reciprocal-cross progeny for males are separated based on the origin of the X chromosome (from the parental strain with which means are compared or from the other strain used to produce the F_1 progeny)

Parental	F_1 cross	Females	Males		
		all progeny	all progeny	X-same strain	X-other strain
C.I.	C.I./COR	***	***	**	***
C.I.	C.I./MET	*	*	ns	ns
COR	COR/C.I.	ns	**	*	*
COR	COR/MET	ns	ns	ns	ns
MET	MET/C.I.	ns	***	***	***
MET	MET/COR	ns	ns	ns	ns

D. buzzatii — population crosses. — For offspring of individuals from both low surviving lines, Metz Gorge and Cordoba, crossed to the high surviving Canary Islands individuals, resistance tended to be intermediate (Table 4). Survival of F_1 female progeny differed significantly only to resistance of the high line and not to survival of either low-resistance parental population (Table 5). Survival of male offspring from crosses with the high line was intermediate, and differences were significant from parental populations. Although resistance of F_1 males carrying the high-line X chromosome tended to be higher than that of males carrying the X chromosome from another population, resistance was higher than for the low parental line regardless of which X chromosome was possessed, and differences between reciprocal cross progeny were significant only for the Canary Islands–Metz Gorge cross. Survival of offspring from crosses between the low resistance populations was not significantly different from that of either parental population. Neither were there differences between males and females or between reciprocal cross progeny between these populations.

Discussion

Genetics of D. melanogaster. — The four *D. melanogaster* populations differed in resistance to thermal stress as has been observed for other populations (HOFFMANN and WATSON 1993) and related species (YAMAMOTO and OHBA 1982, 1984). Variation in reciprocal cross progeny among these populations indicated that larger amounts of genetic variation in resistance are present than is evident from analyses of parental populations alone. Even where survival after stress was similar for two populations, the underlying genetic variation for resistance may have varied. Resistance of the F_1 offspring between Canary Islands and Denmark populations, was reduced relative to the parental lines, and the origin of the X-chromosome altered resistance of pretreated F_1 offspring between Canary Islands and Italy. Differences between these population pairs were not apparent from comparisons of the parental lines.

High resistance, as observed for the Mali population, was due predominantly to factors on the X chromosome, based upon higher survival of male F_1 progeny that had Mali population mothers. Female offspring were similar with either Mali or non-Mali origins for cytoplasm, suggesting that differences among populations are due to loci on the X chromosome. Because this X-chromosome effect on males was present both with pretreated and non-pretreated individuals, the genetic variant(s) present on the X-chromosome will likely influence basal resistance rather than a heat inducible response. Basal effects obviously would increase resistance with, as well as without, a pretreatment. Further, resistance of non-pretreated F_1 female offspring with Mali parents increased nearly to that of pure Mali females, indicating that these factors are either partially or completely dominant in action.

Autosomally based genetic differences among populations were observable after pretreatment. Survival after stress for pretreated male offspring that lacked a Mali population X chromosome was intermediate between the parental populations, while pretreated F_1 female offspring with Mali individuals as one parent were not significantly different from parental Mali females. With the Mali X, survival of pretreated F_1 male offspring was as high as that of Mali parental males. Therefore, we believe the variant(s) present in the Mali autosomes to be dominant in action, as a single copy of these variants improves resistance to equal that of the Mali population, provided that the Mali X chromosome is present. However, an epistatic effect between autosomal and X-linked factors cannot be excluded.

Chance variation may have confounded simple interpretation of variation in some instances. Unclear is why survival of F_1 progeny between the most and least resistant populations, without pretreatment, was equal to or greater than that of progeny between the higher and two intermediate populations. Implied is greater expression of the Mali genotype in crosses with the Italy population, namely, that the presence of recessive variants contributes to low resistance in the Italy derived strain. However, epistatic effects may have been present, as crosses between the low population and others gave offspring with very low resistance. Apparently, variants on the X chromosome of the Denmark population, and to some degree the Canary Islands population, are not compatible with the autosomes of the other populations, including Mali, at least with respect to expressing high thermotolerance.

An X-chromosome effect also was suggested from crosses with the population from Italy. F_1 males that possessed the Italy X had lower basal resistance than males with an alternate X chromosome (excluding crosses with Mali). With pretreatment, the Italy X provided significantly greater resistance. No simple genetic explanation can be given for this pattern, and its explanation requires at least two separate loci.

Genetics of D. buzzatii. — As for *D. melanogaster*, variation among *D. buzzatii* populations was expected from differences among related species (HUEY and BENNETT 1990) and previously identified population variation (KREBS and LOESCHCKE 1995a). For these *D. buzzatii* populations, results followed relatively simple genetic patterns, although only the pretreated treatment condition was used. LOESCHCKE et al. (1994) found that variation among three other *D. buzzatii* populations is similar for adults that either are exposed directly to stress or are pretreated to high temperatures. F_1 progeny from crosses between the two populations of lower resistance were similar to their parental populations, suggesting that low resistance populations possess similar allelic variants. Likewise, F_1 progeny from crosses between each of the lower surviving populations and that from the Canary Islands, which had higher survival to heat stress, were similar and like the low populations. Consequently, dominant negative effects appeared to be the best explanation for the population differences between high and low resistance populations. However, as survival tended to be slightly higher for female F_1 progeny, the possibility of some additive effects cannot be excluded. Neither can we exclude the possibility of recessive gene variants favoring resistance in the parental Canary Islands population. However, the former explanation may be the more likely because YOST and LINDQUIST (1988) provide a plausible model for dominant-negative effects, via the translation of unspliced protein precursors in lines that fail to cut off transcription rapidly in response to increased temperatures. Useless proteins would be produced, reducing the fitness of heat-exposed individuals (LINDQUIST 1993).

As for the *D. melanogaster* populations, recessive X-chromosome effects that increase resistance are a probable explanation for differences in survival of reciprocal male progeny between high and low resistance lines. F_1 male offspring also had higher survival than their low resistance parent populations even without the Canary Islands population X chromosome, but survival of the F_1 females was similar to the low resistance population. These results suggest that loci responsible for low resistance are dominant in females, but recessive in males. A male-specific dominant enhancer of resistance also was identified following selection for high resistance with pretreatment, and those lines were derived from this same Canary Islands population (KREBS and LOESCHCKE 1996). Overall, the variation observed for reciprocal crosses among these populations can be explained by simple dominance across the genome for negative effects on resistance, with at least one locus on the X chromosome and the dominant male-specific factor in the autosomes. The almost complete dominance of low resistance in females is sugges-

tive that few other loci may affect variation in heat stress resistance in *D. buzzatii*.

General discussion. — HUEY and BENNETT (1990), KREBS and LOESCHCKE (1994a), and FEDER (1996) propose a need to differentiate effects in heat-conditioned individuals from basal levels of resistance to high temperature. Individuals that have an opportunity first to acclimate before exposure to stress are much more likely to survive than those that are exposed directly to the high temperature. The underlying genetics of these mechanisms therefore should differ. Resistance to high temperature increases in most organisms following exposure to temperatures 10–15°C above those for normal growth (ASHBURNER and BONNER 1979; LINDQUIST 1986) at least partly through accumulation of heat-shock proteins (WELTE et al. 1993; FEDER et al. 1995; CAVICCHI et al. 1995), although cell chemistry may change in many ways as the thermal environment varies (PARSELL and LINDQUIST 1993). Because so many systems may be affected by heat, high temperature resistance is predicted to have an additive genetic basis (FEDER 1996). The potential role of stress response systems, however, may create a picture of variation where a small number of loci have large physiological effects.

The task of linking mechanisms of resistance to specific genetic inheritance patterns has just begun. Dominant-negative effects, as could occur via the YOST and LINDQUIST (1988) model, can account for only a small proportion of the variation observed here. Effects varied across autosomes and sex chromosomes, and among dominant, additive, and perhaps epistatic actions. Potentially complex and multilocus characteristics of thermotolerance in *Drosophila* adults were suggested from this straightforward analysis of F_1 progeny. Perhaps, as biochemical mechanisms for heat tolerance become better understood, these phenotypic differences may be linked to genetic interactions. Possible follow-up analyses would be to isolate lines differing only for specific chromosomes or physiological differences, or to identify stress protein differences among lines under the two treatment conditions.

From an ecological perspective, we note that all populations differed in their resistance to thermal stress after long maintenance in the laboratory under similar conditions. At 25°C, the temperature of maintenance, resistance to stress is not important, and therefore the measured resistance variation probably reflects differences within the natural populations from which the lines were derived. Divergence among these original populations may have been influenced by selection or other factors. However, the variation among populations in their response to thermal stress cannot be inferred from the climatic conditions of the original localities, except in a very broad sense. The most resistant line of each species originated from the hottest region from which populations were collected. Adaptive divergence among populations may be more pronounced for stages other than adults (COYNE et al. 1983). Accordingly, preadult stages are well worth considering in future studies, although population variation for resistance to thermal stress is higher in adults than in juvenile stages both in *D. buzzatii* (KREBS and LOESCHCKE 1995b) and *D. pseudoobscura* (COYNE et al. 1983).

Variation in thermotolerance is much greater between than within species, as are differences in the climates to which related species and populations are exposed. *Drosophila buzzatii* is one of the cactophilic *Drosophila* that breed in very warm habitats, at least for part of the year. Comparitively, resistance of *D. buzzatii* was much higher than that of *D. melanogaster* despite that maximum constant temperatures at which one can maintain populations of the two species are very similar, about 31°C.

Additionally, many more factors are necessary to explain genetic variation in thermotolerance in *D. melanogaster* than in *D. buzzatii*, where all variation could be explained by a minimum of three factors. Perhaps, adaptation to the much hotter environment has led to fixation of variants that favor high temperature resistance in *D. buzzatii*, and only a few genetically variable systems remain. Such hypotheses are only conjectures at this point, but with the accumulation of information linking hsps and thermotolerance in *D. melanogaster*, the possibility of comparing mechanisms for stress resistance among populations or species soon may be available.

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