Original article

Resistance to heat and cold stress in *Drosophila melanogaster*: intra and inter population variation in relation to climate

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Summary – Genetic variation for resistance to high and low temperature stress and wing size was examined within and among four *Drosophila melanogaster* populations from temperate (Denmark and Italy) and subtropical areas (Canary Islands and Mali). The temperature of induction of the heat shock response was examined by conditioning flies to different high temperatures in the range 34 to 40°C prior to exposing them to heat shock (41.5°C for 0.5 h). Stress resistance appeared to be related to climate: populations from warm regions were the most heat tolerant and those from cold regions were the most cold tolerant. This trend suggests that natural selection in the wild at non-extreme temperature can lead to a correlated response in tolerance to extreme temperature. Wing size varied significantly, and generally was larger for flies from more northerly populations. Populations varied genetically in all traits measured. Among traits, a positive correlation was present between heat-shock resistance with conditioning and resistance to cold, and the correlation was suggestive between heat-shock resistance with and without a conditioning treatment, but no correlation was indicated between cold resistance and heat resistance of non-conditioned individuals. Wing size was not correlated with any stress type. The results suggest that different groups of genes are involved in the resistance at extreme temperature ranges.

acclimation / heat shock resistance / cold shock resistance / wing size

Résumé – Résistance au stress de chaleur et de froid chez *Drosophila melanogaster* : variation entre et intrapopulations en fonction du climat. *La variation génétique pour la résistance au stress à haute ou basse température et la taille de l'aile ont été examinées*

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dans quatre populations de Drosophila melanogaster *provenant des régions tempérées (Danemark et Italie) et subtropicales (îles Canaries et Mali). La température d'induction de la réponse au choc thermique a été examinée après conditionnement à températures différentes (de 34 à 40 °C) avant le traitement proprement dit (41.5 °C pendant 30 min). La résistance au stress est en relation avec le climat : les populations des régions chaudes montrent la plus grande résistance à la chaleur et celles des régions froides, la plus grande résistance au froid. Ce résultat suggère que la sélection naturelle dans un milieu tempéré peut amener à une réponse corrélée pour la tolérance au stress thermique. On a observé une variation significative de la taille de l'aile, qui augmente avec la latitude. Une variabilité génétique pour tous les caractères considérés a été aussi mise en évidence dans toutes les populations. La résistance à la chaleur après conditionnement a été en corrélation positive avec la résistance au froid et une corrélation presque significative a été trouvée entre mouches conditionnées et non pour la résistance au choc thermique. D'un autre côté, on n'a pas trouvé de corrélation entre la résistance à la chaleur et la résistance au froid chez les mouches non conditionnées. La taille de l'aile n'a été corrélée avec aucun stress thermique. Les résultats suggèrent que des groupes différents de gènes contrôlent la résistance à différentes températures extrêmes.*

climatisation / résistance à la chaleur / résistance au froid / taille de l'aile

INTRODUCTION

Variation in resistance to environmental stress has been observed among related species and populations of *Drosophila* from climatically different regions, particularly for heat (Hosgood and Parsons, 1968; Parsons, 1979; Coyne et al, 1983), and cold shock resistance (Jefferson et al, 1974; Tucic, 1979; Marinkovic et al, 1980; Kimura, 1982; Fukatami, 1984; Heino and Lumme, 1989; Hoffmann and Watson, 1993), and this variation appears to be an evolutionary response to the environment (Hoffmann and Parsons, 1991; Loeschcke et al, 1994). Success in selecting for stress resistance indicates that a significant additive genetic component also is present within populations (Morrison and Milkman, 1978; Kilias and Alahiotis, 1985; Quintana and Prevosti, 1990 b; Jenkins and Hoffmann, 1994; Krebs and Loeschcke, 1996).

Maintenance of *Drosophila* populations at different temperatures in the laboratory indicates that adaptation to non- extreme temperatures may yield correlated responses to tolerance to extreme high temperatures (Stephanou and Alahiotis, 1983; Huey et al, 1991, Cavicchi et al, 1995), and that these correlated effects include changes in the induction of the heat shock response (Cavicchi et al, 1995). Conditioning individuals with a short exposure to high temperatures before heat shock increases resistance relative to that without a conditioning treatment, and multiple treatments may increase survival more than a single treatment (Loeschcke et al, 1994; Krebs and Loeschcke, 1995). The molecular basis of the regulation of the heat shock response (Maresca and Lindquist, 1991; Morimoto et al, 1990, 1994), which occurs across all kingdoms of life (Landry et al, 1982; Vierling, 1991; Parsell and Lindquist, 1994), provides a link between conditioning treatments that induce heat shock protein production and those increasing survival under thermal stress or other stress types (Landry et al, 1982; Lindquist, 1986; Brown, 1991).

Here, we investigated heat and cold resistance and the induction of thermotolerance in populations of *D melanogaster* from temperate and subtropical areas.

Our aim was to identify if this resistance relates to the climate of the localities of origin. If so, natural selection in the wild at non-extreme temperature has led to a genetically correlated response in tolerance to extreme temperature. The question of general interest is: does selection for increased fitness at a given range of temperature lead to a genetically correlated response in the resistance to extreme temperature close to the optimum? If so, are the same or different groups of genes involved in the adaptation to the optimum and/or to either hot or cold temperature extremes (Huey and Kingsolver, 1993)? Our previous works on chromosomal analysis of laboratory populations of *Drosophila* adapted to different temperatures (Cavicchi et al, 1989, 1995) showed that the genes responsible for adaptation to intermediate temperature are located on chromosomes different from those controlling survivorship at extreme heat, although heat resistance evolved as a correlated response to natural selection at non-extreme temperature. Does the same relationship occur in natural populations from different climatic areas? Here we analysed the survivorship of genotypes from different populations at high and low temperature extremes. The correlation between performances of different isofemale lines could be a useful tool to assess whether the same or different evolutionary mechanisms are at work in the laboratory and in the wild.

Because phenotypic differences in body size may have impact on resistance to temperature extremes (Quintana and Prevosti, 1990a; Loeschcke et al, 1994), wing size variation among populations was compared and the correlation with stress resistance analysed. Size variations may not be easily separated from variation in resistance, as geographical clines for body size follow temperature gradients in several *Drosophila* species (Stalker and Carson, 1947; Prevosti, 1955; Misra and Reeve, 1964; David et al, 1977; David and Capy, 1988; Capy et al, 1993; Imasheva et al, 1994). A genetic and phenotypic relationship between body size and temperature also has been shown in the laboratory (Anderson, 1973; Cavicchi et al, 1985, 1989), where adult body size negatively correlated with temperature (Starmer and Wolf, 1989; Thomas, 1993), except at temperatures approaching the limit for development (David et al, 1994).

MATERIALS AND METHODS

Origin of populations

The founder populations derived from 50–100 females of *D melanogaster* collected in nature from Hov, Denmark in late October, 1992; from Bologna, Italy in October, 1993; from southwestern Teneriffe, Canary Islands; and from Bamako, southern Mali in December, 1993. Table I describes differences in thermal extremes for each region.

Single females were put in vials. From those identified as *melanogaster*, ten isofemale lines for each population were established. The lines were reared in bottles with discrete generations, avoiding overcrowding. Mass populations were obtained by pooling lines of each population in cages with overlapping generations. Flies were maintained on a medium of yeast, sugar, cornmeal and agar at 25°C. Experiments were initiated in the spring of 1994.

Table I. Mean minimum and maximum temperature in the colder (winter) and warmer (summer) months at different localities.

	Mali	Canary I	Italy	Denmark
Winter				
min	20	16	−1.0	−1.5
max	25	21	6.1	3.9
Summer				
min	24.4	19	17.7	8.9
max	31.4	26	30.9	19.5

Heat resistance and induced thermotolerance

Flies were heat shocked using the procedures adopted in previous experiments (Cavicchi et al, 1995). Males and females were collected using ether anaesthesia and partitioned into about 50 flies per vial. Females and males were considered together because, under our experimental conditions, they survived similarly in replicated experiments at different shock temperatures. Flies were restrained at the bottom of weighted plastic vials (without food) by sponge plugs and were shocked in a water bath at 41.5°C for 30 min. Care was taken to treat only 4–7-day-old flies as resistance declines in older individuals (Quintana and Prevosti, 1990b; Dahlgaard et al, 1995). During treatment, humidity was not controlled within vials, but the water bath was a saturated humidity environment that minimised any desiccation effects (Maynard Smith, 1956; Hoffmann and Parsons, 1989). Following heat shock, flies were transferred to new vials containing food, and survivorship was scored 24 h later. As almost all individuals were knocked-down, survivorship was taken as the proportion of individuals that reacted when touched with forceps. Heat shock was applied both on mass populations and on the individual isofemale lines. For comparing populations, three replicate measurements were obtained in each of two independent blocks. For isofemale lines, two replicates were subjected to the heat treatment, but, owing to the bath size, at different times for various populations. Data were arcsin transformed before statistical analysis.

To determine differences among the four mass populations for the threshold temperature that induces thermotolerance, two replicates of 50 flies each in one or two independent blocks were conditioned for 5 min at one of a graded series of temperatures ranging from 34 to 40°C, returned to 25°C for 0.5 h and then heat shocked as described (Cavicchi et al, 1995). As only two conditioning temperatures could be tested at any one time, non-conditioned control flies from each mass population were also simultaneously heat shocked. Therefore, induction of thermotolerance was measured for each population as the difference between the proportion of flies that survived heat shock with conditioning in each replicate and the mean for each population that survived without conditioning. A total of 44 vials were non-conditioned (12 for Mali and Denmark, 10 for Canary Islands and Bologna) while 78 vials were conditioned (18 for Denmark and Italy, 20 for Canary Islands and 22 for Mali).

For isofemale lines, a treatment condition was chosen prior to heat shocking lines that maximally induced thermotolerance for each population. Individuals of

the Canary Island and Danish populations therefore were first exposed to a slightly lower temperature (36°C) than those from Mali or Bologna (38°C). In this case also, the preconditioning and heat shock treatments were performed separately for each population.

Cold resistance

Flies both from mass populations and isofemale lines were subjected to cold treatment of 0°C for 48 h in a thermostatic chamber with saturated humidity. The initial temperature was 20–22°C, and the temperature declined to 0°C in about 15 min. As for heat shock, about 50 flies were placed in empty plastic vials. Two replicates in three blocks were treated for comparing populations. For comparing lines, two replicates for each isofemale line were cold shocked at the same time, while, as for heat shock, various populations were treated at different times. Again, resistance was scored as the proportion of flies reacting when touched with forceps and the data were arcsin transformed before statistical analysis.

Wing size

After rearing individuals of each isofemale line in uncrowded conditions, the right wing of five females per line was removed and mounted on slides, from which wing area was measured (MTV3 program of Data Crunch Corporation, South Clemente, CA). The overall mean was taken as the population mean size.

Statistical analysis

In the first experiment, significance of population differences for heat resistance, cold resistance (after arcsin transformation) and wing size was tested by Anova and a posteriori hypotheses of pair-wise differences were examined using Tukey's multiple comparisons test. Significance of differences among populations and different acclimatization treatments in the second experiment was tested in a two-way fixed effects Anova (SAS, 1989).

Intraclass correlations (t) were derived from Anova only for wing size. For survivorship, which is a threshold trait, we followed the method proposed by Robertson and Lerner (1949) in which:

$$t = [\chi^2 - (N - 1)]/n_0$$

where χ^2 is the heterogeneity in the $2 \times N$ table, as flies can be classified only as dead or alive, N is the number of isofemale lines and

$$n_0 = \sum n_i - \left(\sum n_i^2 / \sum n_i \right) - (N - 1)$$

where n is the number of flies heat or cold shocked for each isofemale line. In our experiment, $N = 10$ isofemale lines and $n \geqslant 50$ flies for each line, averaging two replicates of more than 50 flies, as they cannot be assigned to different experimental blocks.

The observed variance in binomial data is correlated with the mean. Hence correction for comparing intraclass correlations from different treatments and populations can be made by transforming t on the probit scale by multiplying t by

$$[\bar{p}(1 - \bar{p})]/z^2$$

where $\bar{p}$ is the fraction which survives (or dies) and z is the ordinate of the normal curve at the point where the tail area is equal to $\bar{p}$.

Standard errors of intraclass correlations were computed following Falconer (1989) for wing size and Fisher (1941) for survivorships.

Overall t values were reported on the basis of a pooling procedure both for stress resistances and wing size.

Standard parametric correlation coefficients among the four traits were obtained for each population using the mean stress resistance (after arcsin transformation) or size of each line. Overall correlations also were reported on the basis of the pooled variance–covariance matrix.

RESULTS

Interpopulation analysis

Mean survivorship (%) for each population following either a heat or cold treatment, and mean wing size of females are presented in table II (rows identified by No 1). For cold resistance, variation among blocks was significant ($P < 0.01$). For neither heat nor cold shock was the population by block interaction significant. Variation due to the origin of populations was highly significant for all three traits ($P < 0.001$), and two by two comparisons (Tukey's multiple comparisons test) revealed significant differences between geographic areas (table II). Heat resistance was higher for flies from the Canary Islands and from Mali than for flies from Denmark and Italy; while cold resistance was highest for flies from Italy, followed by those from Denmark, Mali and the Canary Islands, respectively, although significance levels overlapped between some populations. Wing size was significantly larger for flies from Italy and Denmark than for those from the Canary Islands population, and wing size of flies from Mali was significantly smaller than that of all other populations.

Mean survivorship differences between flies heat shocked with and without conditioning at temperatures from 34 to 40°C are presented in table IIIA. The two populations subjected to higher summer temperatures in nature (Mali and Italy) showed a larger induction of thermotolerance at higher temperatures than the other two (38 versus 36°C). The increase in survival was not significantly different among the four populations conditioned with any of the temperatures. Similar results for all conditioning treatments enabled us to pool across temperatures and test differences in survival among populations either with or without conditioning (table IIIB).

Conditioning significantly increased survival, and as before, the populations varied in survival after thermal stress, while the population by treatment interaction was not significant. Survival of flies from the Canary Islands population and from Mali was significantly higher than that for flies from Denmark, and flies from the Italy population had the lowest survival (table II, rows identified by No 2). All

Table II. Survivorship (%± se) of mass populations and lines after heat-shock at 41.5°C for 0.5 h with and without conditioning, after cold treatment at 0°C for 48 h and wing size (in mm^2), in experiment 1, 2 and 3.

Treatment	Mali	Canary I	Italy	Denmark
Heat				
1. mass (not conditioned)	85.5 ± 2.5 a	75.2 ± 8.1 a	55.3 ± 6.5 b	57.7 ± 3.8 b
2. mass (conditioned)	61.8 ± 5.7 a	69.5 ± 6.8 a	37.6 ± 5.1 c	51.4 ± 5.6 b
2. mass (not conditioned)	46.1 ± 5.9 a	45.0 ± 3.4 a	24.7 ± 4.4 b	32.6 ± 3.3 b
3. lines (conditioned)	71.4 ± 4.2	88.4 ± 3.6	53.6 ± 3.0	60.7 ± 4.8
3. lines (not conditioned)	37.8 ± 5.2	19.6 ± 4.2	21.6 ± 4.8	20.9 ± 5.2
Cold				
1. mass	48.5 ± 2.9 bc	44.3 ± 2.2 c	58.4 ± 5.3 a	53.0 ± 3.7 ab
3. lines	46.8 ± 1.7	46.6 ± 4.1	63.6 ± 4.2	47.0 ± 2.1
Size				
wing size	1.42 ± 0.01 c	1.49 ± 0.02 b	1.64 ± 0.01 a	1.59 ± 0.01 a

Comparisons are given only between comparable groups. Equal letters denote groups not statistically different based on Tukey's multiple comparisons test. For mass populations, three replicates of about 50 flies in two independent blocks were considered for heat and two replicates in three blocks were considered for cold shock (experiment 1); in experiment 2, a total of 44 vials were not conditioned (12 for Mali and Denmark, 10 for Canary Islands and Bologna) while 78 vials were conditioned (18 for Denmark and Italy, 20 for Canary Islands and 22 for Mali). For lines (experiment 3), two replicates of about 50 flies for 10 isofemale lines were exposed to thermal stress. Wing size refers to five female right wings from ten isofemale lines.

values are lower than those of the previous experiment (No 1) owing to a slight increase (less than 0.5 of a degree) of the water bath temperature.

Intrapopulation analyses

From analyses on individual isofemale lines, mean values (table II, rows identified by No 3) and intraclass correlations (table IV) were obtained in each population for heat shock resistance with and without conditioning, for cold resistance, and for wing size.

Comparisons among populations were not given as each population also represents a different experimental block. In spite of that, with the exception of the Canary Islands population subjected to heat shock without conditioning, the inter-population differences were comparable to those of the previous experiments.

Intraclass correlations were not different among stress types, but those for wing size were consistently larger. The Mali population, for heat shock resistance and wing size, and the Italian population, for cold resistance, showed the lowest intraclass correlations.

Table III. A) Survivorship differences (% ± se) for adult flies of four populations either first conditioned at different temperatures and then exposed to a thermal stress or flies not conditioned before exposure to stress (N for all means = 2 or 4 vials). B) Anova for the test of differences between populations, treatments and their interaction.

A)

Conditioning temperature (° C)	Mali	Canary I	Italy	Denmark
34	7.3 ± 2.1	9.6 ± 12.3	10.1 ± 7.1	−2.0 ± 4.9
35	20.1 ± 2.5	22.5 ± 11.0	9.8 ± 4.5	21.5 ± 9.4
36	15.4 ± 6.1	40.0 ± 4.3	4.8 ± 3.8	29.8 ± 2.1
37	13.1 ± 3.6	25.6 ± 8.8	19.4 ± 7.1	23.0 ± 13.8
38	32.6 ± 1.0	28.4 ± 10.0	27.4 ± 2.2	16.4 ± 5.7
39	11.1 ± 10.0	20.1 ± 5.0	14.6 ± 1.4	26.9 ± 6.3
40	10.9 ± 6.9	25.6 ± 1.2	4.5 ± 2.7	16.2 ± 1.8

B)

Sources of variation	df	MS	F	P
Populations	3	0.454	10.5	0.001
Conditioned versus not conditioned	1	1.108	25.7	0.001
Interaction	3	0.031	0.7	NS
Residual	114	0.043		

Table IV. Intraclass correlations (± se) of isofemale lines for survivorship after exposure to thermal stress either without or with a conditioning treatment, survivorship after a cold treatment and of wing size for four populations.

Trait	Mali	Canary I	Italy	Denmark	overall
Heat (not conditioned)	0.055 ± 0.035	0.114 ± 0.058	0.218 ± 0.092	0.141 ± 0.068	0.140 ± 0.068
Heat (conditioned)	0.081 ± 0.045	0.102 ± 0.054	0.219 ± 0.093	0.097 ± 0.052	0.125 ± 0.063
Cold	0.198 ± 0.086	0.139 ± 0.067	0.045 ± 0.030	0.106 ± 0.055	0.124 ± 0.062
Wing size	0.156 ± 0.153	0.789 ± 0.098	0.420 ± 0.174	0.464 ± 0.171	0.567 ± 0.149

Correlations among stress types and wing size

Table V gives correlation coefficients between each pair of traits separately for each population and the overall correlations. At the population level, a significant correlation is observed between cold and heat shock resistance without conditioning in the Canary Islands population and between cold, wing size and heat shock

Table V. Correlations (r) between survivorship of different isofemale lines after exposure to thermal stress either without or with a conditioning treatment, survivorship after a cold treatment and wing size (df = 8). Overall correlations are also given (df = 32).

		Heat (conditioned)	Cold	Wing size
Heat (not conditioned)	Ma	0.194	−0.046	−0.446
	CI	0.488	0.640*	−0.544
	It	0.517	−0.094	0.195
	Dk	0.199	−0.081	0.404
Overall		0.379*	0.104	−0.077
Heat (conditioned)	Ma		0.249	0.214
	CI		0.467	−0.295
	It		0.365	−0.121
	Dk		0.726**	0.626*
Overall			0.417**	−0.142
Cold	Ma			−0.280
	CI			−0.039
	It			0.369
	Dk			0.269
Overall				−0.053

* $P < 0.05$; ** $P < 0.01$.

resistance with conditioning in the Danish population. The analysis of covariance showed homogeneity among populations for the correlations between any pair of traits. The overall correlations, based on the pooled variances–covariances within populations, revealed that body size is not correlated with any stress type. Heat shock resistance with conditioning and cold shock resistance were correlated significantly and positively. A positive correlation between heat shock resistance with and without conditioning approached significance.

DISCUSSION

We investigated heat and cold resistance and the induction of thermotolerance in four populations of *D melanogaster*, two from temperate and two from subtropical areas. Our aim was to evaluate i) the amount of genetic variability for different resistance traits and ii) their correlations; to identify whether iii) this resistance relates to the climate of the localities of origin and to determine whether iv) body size, which varies latitudinally, correlates at an intrapopulational level with resistance to temperature extremes.

Both within and among natural populations of *D melanogaster*, genetic variation for survival at extreme temperatures is present, as well as for wing size, as also

shown in the same and other Drosophila species by the authors quoted in the introduction to this work (Morrison and Milkman, 1978; Stephanou and Alahiotis, 1983; Quintana and Prevosti, 1990b; Jenkins and Hoffmann, 1994;. Tucic, 1979; Heino and Lumme, 1989 for temperature stresses; David and Capy, 1988; Capy et al, 1993, 1994 for size).

Intraclass correlations for isofemale lines estimate the genetic component of variance in a broad sense, including the additive, dominance, interaction and maternal components. When the additive variance is the prevailing component, the intraclass correlation includes half the heritability (Parsons, 1983). Direct estimates of heritability for survivorship under temperature stress in D $melanogaster$ are reported for cold shock by Tucic (1979) after long-term artificial selection on a population captured near Belgrade. He reported an estimate of 14% on adult flies, slightly lower than the value we obtain by averaging our four populations (25%), but similar to the average of the two populations from temperate climates (15%). For heat resistance we found heritability estimates of 25–28%. Other direct estimates of heritability in this species are available only for knockdown temperature (28%; Huey et al, 1992). Experiments of indirect selection for heat survivorship (Stephanou and Alahiotis, 1983) confirmed that D $melanogaster$ possesses genetic resources to survive heat shock. For wing size, our estimates are similar to those reported by Capy et al (1994), with the exception of the Canary Islands population whose heritability exceeded 1 ($t = 0.789$). Wings of one isofemale line were consistently 20% shorter than the population mean. A single mutational event rather than quantitative variation may have caused this result. In the absence of this line, the intraclass correlation reduces to 0.49, which is in line with other estimates.

For heat resistance in the Mali population and cold resistance in the Italian population, the lowest level of genetic variation and the maximum performance for these traits were observed. Also, the highest levels of genetic variation were found for the reverse comparison, cold resistance in the Mali population and heat resistance in the Italian population, where minimal performance was observed. Populations subjected to novel stress conditions often exhibit genetic variance at the highest levels (Hoffmann and Parsons, 1991). In general, the low level of variation in the Mali population, may reflect a relatively homozygous population following continuous directional selection for adaptation to heat extremes in nature (Parsons, 1983), though, for morphological traits, tropical populations show phenotypic variability larger than that exhibited by temperate populations and genetic variability that is almost the same (Capy et al, 1993).

Relative performance under heat and cold stress seemed related to the mean summer maximum temperature and the mean winter minimum temperature, respectively, for the four areas from which the flies were collected (comparing tables I and II). Clear differences were present only between very separate geographic regions. For most traits, differences between Mali and the Canary Islands or between Italy and Denmark were small, although wing size of Mali flies was smaller than that of flies from the Canary Islands. Perhaps behavioural traits that enable escape from unfavourable climatic conditions (Jones et al, 1987) are possible within a given temperature range and these reduce physiological differences between populations. Migration by fruit trading also could be relevant and give a reason for the relatively small size and resistance to cold of the Danish flies. Independent samples from each

locality would have given more information for a comparison of relative thermal resistance in relation to the climatic conditions at the sample sites. However, we chose to keep up the number of geographic populations and traits instead of increasing sample number per locality.

The populations differed much more for heat than for cold resistance, a result that could depend either on the kind of treatment performed or upon different reaction norms to hot or cold temperature extremes.

The dependence of heat tolerance on the temperature at which a given population evolves has been well documented for populations adapted to different temperatures in the laboratory (Stephanou and Alahiotis, 1983; Huey et al, 1991; Cavicchi et al, 1995). Populations held at warmer temperatures may also show genetic differences for induction of thermotolerance, expressing the heat shock response at a higher temperature than those adapted to cold (Cavicchi et al, 1995). This trend suggests that natural selection in the wild at non-extreme temperature has led to a genetically correlated response in tolerance to extreme temperature, but that adaptation to one part of the thermal performance curve reduces adaptation at temperature extremes farther away. Previous work on relative chromosomal contributions to fitness components suggests that different groups of genes are involved for adaptation at intermediate temperature (Cavicchi et al, 1989) and resistance to extreme heat (Cavicchi et al, 1995). The present results, though not concerning intermediate temperatures, suggest that different groups of genes are important at the two extremes.

However, the correlation between heat shock resistance with conditioning and cold shock resistance in lines derived from natural populations was significant, suggesting that similar groups of genes may affect resistance at the two temperature extremes. Perhaps this relationship is due to a general hardiness or weakness of some lines that is independent of the shock response. Inbreeding is expected within isofemale lines and uncontrolled genetic drift or inbreeding may lead to positive associations among fitness traits (Dahlgaard et al, 1995).

The performances of different isofemale lines with or without conditioning show a low correlation, suggesting that the role of heat shock genes is unimportant for heat tolerance when a population is rapidly subjected to a potentially lethal heat stress (41.5°C for 0.5 h without conditioning). Molecular data support this observation, in that the maximal transcription level of a more inducible heat shock gene (hsp-70) is reached after about half an hour after a severe heat treatment, while for others (hsp-82, -27) the maximum is observed after a longer time (DiDomenico et al, 1982 a,b).

Suggestions on the mechanistic basis underlying how evolution in a population at intermediate temperature may affect tolerance to extreme temperature stress are, however, speculative. Nevertheless, studies of enzymes suggest that natural selection at different temperatures can be associated with variation in their kinetic parameters (Alahiotis, 1982; Hoffmann and Parsons, 1991; Somero, 1995) in such a way that enzymes show a greater efficiency under the conditions an organism normally encounters.

For the minimum temperature for induction of thermotolerance, the four *D melanogaster* populations, which come from very different regions, were similar. This was not expected on the basis of our previous observations on laboratory

populations adapted to different temperature optima (Cavicchi et al, 1995). These response types may be a general rule across species, and relate to the activation of heat shock genes (Lindquist and Craig, 1988; Huey and Bennett, 1990). The heat shock response is a physiologically plastic response to deal with stress, and in natural variable environments, induction temperatures may change little. Therefore, above some threshold temperature, the level of acclimation that occurs may be similar.

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