Chromosomal and cytoplasmic analysis of heat shock resistance in natural populations of *Drosophila melanogaster*

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We evaluated genetic differences between two populations of *Drosophila melanogaster* that differed in thermal tolerance. Adults of one tropical population (Mali) survived heat shock (39.5°C for 30 min.) at 84 %. By contrast, those from a strain collected in Denmark survived at a rate of only 53 %. The greatest effect on variation was differences in cytoplasms, but variation in chromosomes 2 and 1 also played a role on tolerance. Heat shock proteins, however, reside on chromosome 3 and, therefore, variation at these sites is low or differences had little effect on results obtained from the methods employed.

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Heat shock resistance is a trait exhibiting genetic variability within and among populations of *Drosophila*. Different methods of estimating thermal resistance have been employed in different laboratories (knock-down resistance, sudden heat shock in a water bath or in a thermostatic chamber with or without heat hardening) that may be assessing different mechanisms of resistance (CAVICCHI et al. 1995; KREBS et al. 1996; GUERRA et al. 1997; LOESCHCKE et al. 1997; HOFFMANN et al. 1997). Independent of the specific assay used to characterize heat resistance, heritable variation in resistance to heat shock has usually been observed.

Differences in thermal resistance among related species and populations of *Drosophila* from climatically different regions (HOSGOOD and PARSONS 1968; PARSONS 1979; COYNE et al. 1983) suggest that the existing variation is the result of an evolutionary response to the thermal environment (HOFFMANN and PARSONS 1991; HUEY and KINGSOLVER 1993; LOESCHCKE et al. 1994). Evolution 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 are also present in natural populations from different climatic regions (KREBS et al. 1996; GUERRA et al. 1997).

Genetic analysis of thermal resistance has been performed by several methods: by direct or indirect selection, by the analysis of isofemale lines and by the assay of different chromosomes. Different methods

have been used to satisfy different purposes: indirect selection and the analysis of isofemale lines are devoted to the detection of genetic polymorphism present in a given population (MORRISON and MILKMAN 1978; KILIAS and ALAHIOTIS 1985; QUINTANA and PREVOSTI 1990; JENKINS and HOFFMANN 1994); direct selection adds the evaluation of the evolutionary potential of heat shock resistance (HUEY et al. 1992; KREBS and LOESCHCKE 1996; LOESCHCKE and KREBS 1996). The survey of chromosomal contributions is a powerful method to roughly locate the genes responsible for resistance, allowing one to distinguish between different genetic mechanisms at work.

Several previous experiments on *Drosophila melanogaster*, with the aim of locating genes contributing to heat shock resistance, were done applying the same procedure, that is exposing flies to a sudden heat shock in a water bath at 39–40°C for 25–30 minutes, so that they can be compared. By this method, gene(s) located on chromosome 2 (MORRISON and MILKMAN 1978; STEPHANOU and ALAHIOTIS 1983) and effects of the cytoplasm (STEPHANOU and ALAHIOTIS 1983) were found to be relevant for heat shock resistance of lines of *Drosophila melanogaster* subjected to indirect selection. However, genes located on all three major chromosomes of *Drosophila melanogaster* (STEPHANOU and ALAHIOTIS 1983; CAVICCHI et al. 1995) and, again, influences of the cytoplasm (STEPHANOU and ALAHIOTIS 1983) were found responsible for heat shock resistance of populations adapted to different temperatures in the laboratory. Interestingly, with the exception of the

cytoplasmic effect exhibited by populations from Greece, the contribution to heat shock resistance within or between populations seems to depend on different groups of genes.

These findings are consistent with the idea that heat resistance evolves as a correlated response to natural selection at non-extreme temperatures. However, the genes responsible for adaptation to intermediate temperature are located on chromosomes different from those controlling survivorship to extreme heat (CAVICCHI et al. 1989, 1995). In a recent paper we showed that stress resistance is related to climate: populations from warm regions were the most heat tolerant and those from cold regions were the most cold tolerant (GUERRA et al. 1997). As the average temperature at a given site is usually correlated with the extreme temperatures, natural populations have the opportunity to evolve resistance both as a consequence of a correlated response to natural selection at non-extreme temperatures and as a consequence of a direct response to extreme temperatures.

In the present paper we evaluate the relative contribution of different chromosomes and of the cytoplasm on survival to heat shock of two natural populations of *Drosophila melanogaster* (one from tropical and the other from temperate areas), with the aim to see whether their differential survivorship is the result of the evolution of specific and localized genes or of the whole genetic background.

MATERIALS AND METHODS

Origin of populations

The founder populations derived from a pool of ten isofemale lines of *Drosophila melanogaster* collected in an apple plantation near Hov, Denmark in late October, 1992 and from Bamako, southern Mali in December, 1993 (for details see GUERRA et al. 1997). Flies were maintained on a standard medium of yeast, sugar, cornmeal and agar at 25°C. The experiment was initiated in spring and concluded in summer 1994.

Assay of different chromosomes and cytoplasm

As the cytoplasm could have a role on heat resistance, we took care to evaluate the effect of the three major chromosomes of the two populations in a common cytoplasmic background of the balanced stock *Basc/Basc; SMl/bw^{Vl}; Sb/TM2* (Fig. 1 and 2). This stock carries large inversions on all three major chromosomes (chromosome 1, 2 and 3 in the order), preventing crossing over, and dominant markers on homologous chromosomes (LINDSLEY and ZIMM 1990).

As the balanced stock also showed a lower resistance than the two geographic populations, the assay of different chromosomes was done evaluating the effect on survivorship in flies of the balanced stock in which either chromosome 1, or chromosomes 1 and 3, or chromosomes 1 and 2 were replaced by wild type chromosomes, according to the scheme given in Fig. 1. The contribution of chromosome 4, if any, could not be detected by this design and has been randomised in all substitution lines as well as in the balanced stock and wild type populations (Fig. 1 and 2). The effect of the cytoplasm was evaluated comparing the survival rate of wild type flies carrying the cytoplasm of the balanced stock with those carrying the wild type cytoplasm. The initial cross was done with 20 flies from each population crossed in mass, which means that we evaluated a random pool of 40 chromosomes from each population.

Heat resistance

Flies were heat shocked using the procedures adopted in previous experiments (CAVICCHI et al. 1995; GUERRA et al. 1997). Males and females were collected using light ether anaesthesia and partitioned into about 50 flies per vial. Females and males were considered separately, though their survival rates were found to be similar in replicated experiments at different shock temperatures. Flies were restrained to the bottom of weighted plastic vials (without food) by sponge plugs and were shocked in a water bath at 39.5°C for 30 min. Care was taken to treat only 4–7 day-old flies, as resistance declines in older individuals (QUINTANA and PREVOSTI 1990; DAHLGAARD et al. 1995). During treatment humidity was not controlled within vials, but the water bath was a saturated humidity environment that minimized any desiccation effects (MAYNARD SMITH 1956; HOFFMANN and PARSONS 1989). Following heat shock, flies were transferred to new vials containing food, and survival was scored 24 h later as those individuals that reacted when touched with forceps.

Owing to the large variability between experiments, substitution lines for each population, including parental lines carrying the cytoplasm of the balanced stock, were treated simultaneously with two replicates (vials) in three to four independent experimental blocks, with the exception of the wild type lines carrying their own cytoplasm. The effect of the cytoplasm was then estimated independently by simultaneously treating wild type flies carrying the two different cytoplasms. We omitted to treat males of the balanced stock as we were not able to collect enough individuals due to the low viability of the stock. The within block variance was taken as a measure of experimental error.
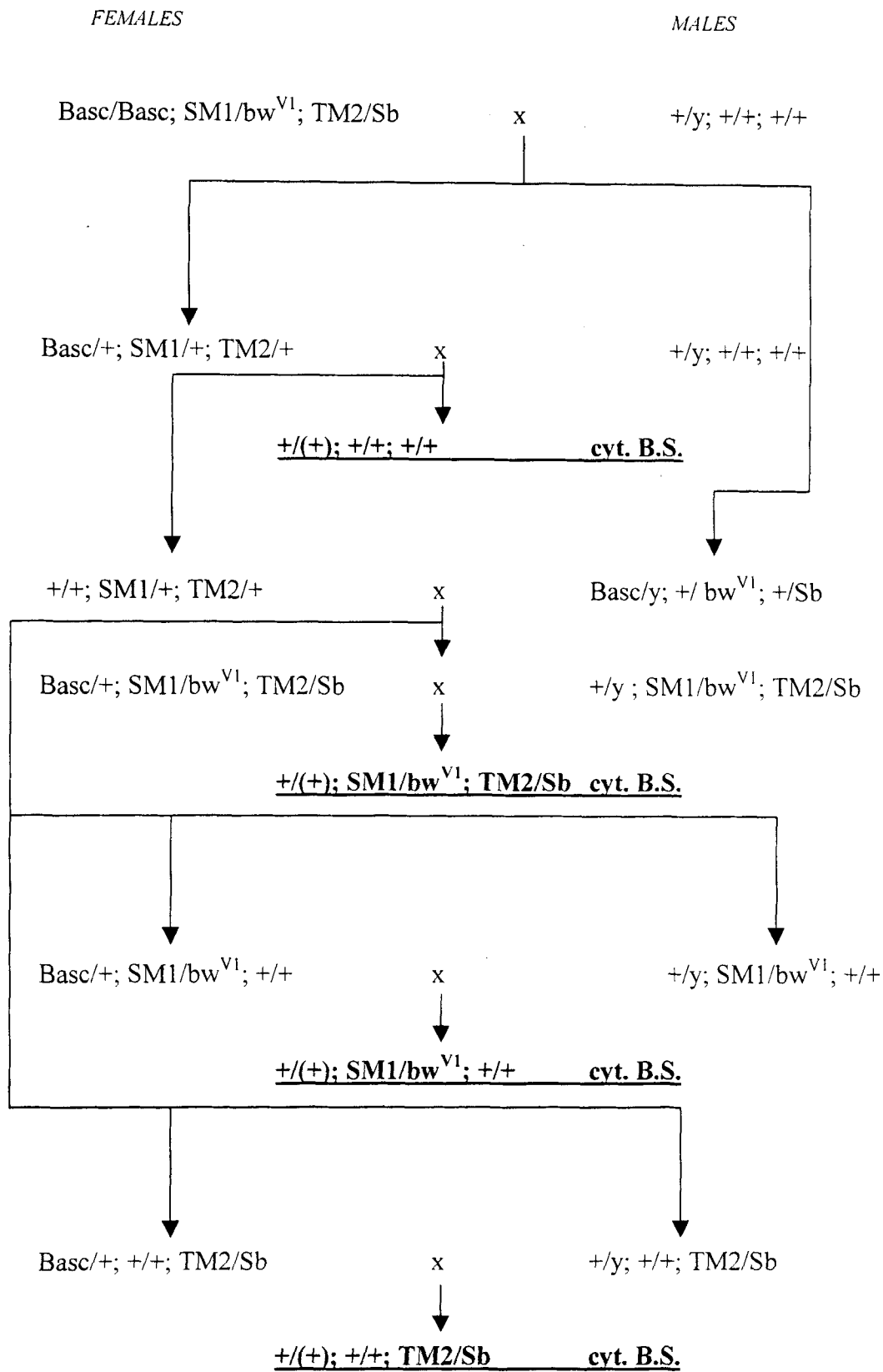
FEMALES   *MALES*

Basc/Basc; SM1/bw^{V1}; TM2/Sb   x   +/y; +/+; +/+

Basc/+; SM1/+; TM2/+   x   +/y; +/+; +/+

+/(+); +/+; +/+   **cyt. B.S.**

+/+; SM1/+; TM2/+   x   Basc/y; +/ bw^{V1}; +/Sb

Basc/+; SM1/bw^{V1}; TM2/Sb   x   +/y ; SM1/bw^{V1}; TM2/Sb

+/(+); SM1/bw^{V1}; TM2/Sb   cyt. B.S.

Basc/+; SM1/bw^{V1}; +/+   x   +/y; SM1/bw^{V1}; +/+

+/(+); SM1/bw^{V1}; +/+   cyt. B.S.

Basc/+; +/+; TM2/Sb   x   +/y; +/+; TM2/Sb

+/(+); +/+; TM2/Sb   cyt. B.S.

Fig. 1. Crossing scheme applied to construct different substitution lines. *Basc*; *SM1/bw^{V1}* and *TM2/Sb* are balancers of chromosome 1, 2 and 3 respectively. + are wild-type chromosomes. B. S. is the balanced stock.

RESULTS

The two natural populations obtained from the crosses given in Fig. 2 showed relative survival after heat shock to be very similar to that found previously (KREBS et al. 1996; GUERRA et al. 1997). The Mali population showed a female % survivorship of 83.7 ± 4.4 while the Danish population gave values of 52.5 ± 5.71. Females from the balanced stock were found far less resistant with a % survivorship of 9.15 ± 4.97.

Table 1 gives the differences between natural populations and each substitution line. All wild type chromosomes increased resistance, but chromosome 3 less than chromosome 1 and 2. Wild type chromosomes are less effective in increasing resistance in males than in females and in flies from Denmark than in those

from Mali but in both populations the largest difference was observed for line C followed by lines A and B.

The contributions of the different chromosomes, the cytoplasm and of chromosomal interactions are given in Table 2. The contribution of chromosome 1, and the sum of all chromosomal contributions, could not be computed for males of both populations, as it is obtained by the differences between lines D and C. For the other two chromosomes, the contribution is given for both sexes. The contribution of chromosome 2 is given by the difference between lines C and B, while that of chromosome 3 by the difference between lines C and A. In both populations, the largest contribution was that of chromosome 2, followed by that of chromosome 1. Chromosome 3 showed the smallest contribution and this was not
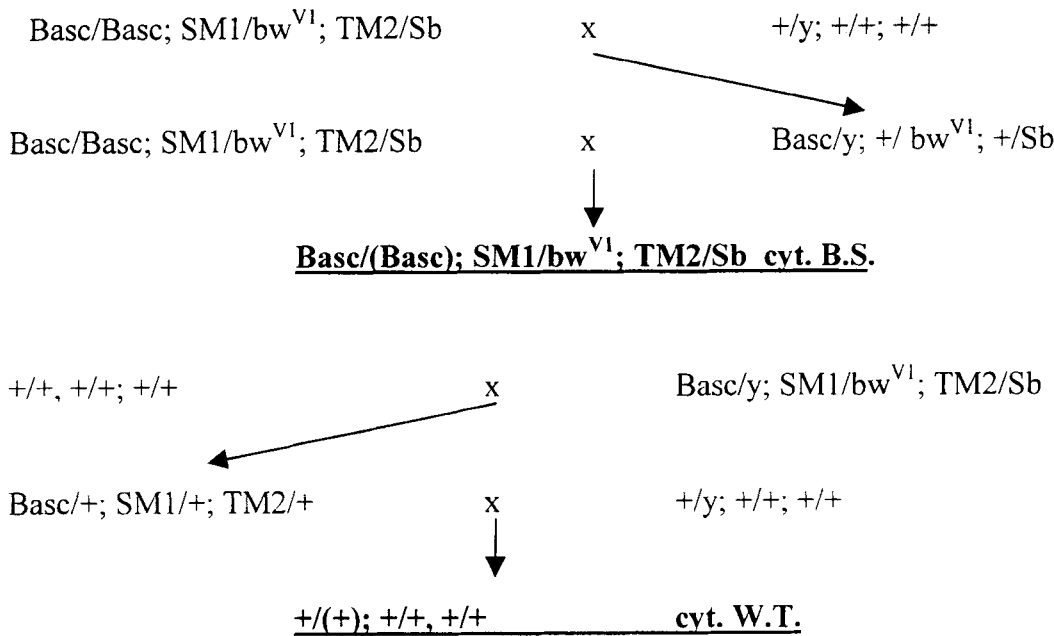
Basc/Basc; SM1/bw^{VI}; TM2/Sb   x   +/y; +/+; +/+

Basc/Basc; SM1/bw^{VI}; TM2/Sb   x   Basc/y; +/ bw^{VI}; +/Sb

Basc/(Basc); SM1/bw^{VI}; TM2/Sb cyt. B.S.

+/+, +/+; +/+   x   Basc/y; SM1/bw^{VI}; TM2/Sb

Basc/+; SM1/+; TM2/+   x   +/y; +/+; +/+

+/(+); +/+, +/+   cyt. W.T.

Fig. 2. Crossing scheme applied to recover the balanced (B. S.) and wild-type (W. T.) stocks. *Basc; SM1/bw^{VI}* and *TM2/Sb* are balancers of chromosome 1, 2 and 3 respectively. + are wild-type chromosomes.

Table 1. *Differences in survival (%) between lines carrying different chromosomes+ and cytoplasm of the balanced stock and wild type populations carrying the cytoplasm of the balanced stock*

	differences from			Mali		Denmark	
	+/(+); of	+/+;	+/+	females	males	females	males
A)	+/(+);	*SM1/bw^{VI}*;	+/+	47.9	36.1	42.1	27.9
B)	+/(+);	+/+;	*TM2/Sb*	29.2	23.8	18.0	22.2
C)	+/(+);	*SM1/bw^{VI}*;	*TM2/Sb*	57.1	49.6	50.2	33.1
D)	*Basc/Basc*;	*SM1/bw^{VI}*;	*TM2/Sb*	77.1	–	69.4	–
common s.e.				4.30	4.25	5.02	3.40

+ *Basc*; *SM1/bw^{VI}* and *TM2/Sb* are balancers of chromosome 1, 2 and 3 respectively

Table 2. *Contribution of different wild type chromosomes and of cytoplasms to survival after heat shock (%) and chromosomal interactions in populations from Mali and Denmark*

Contributions	Mali		Denmark	
	females	males	females	males
Chromosome 1	20.0	–	19.2	–
Chromosome 2	27.9	25.8	32.2	10.9
Chromosome 3	9.2	13.5	8.2	5.2
Cytoplasm	24.9	–	–14.8	–
All chromosomes	57.1	–	59.6	–
Overall Interaction	20.0	–	9.8	–
w.t. chromosome 1 in the presence of				
w.t. chromosome 3	38.7	12.6	33.9	22.7
w.t. chromosome 2	1.3	–2.0	–14.2	11.3
Differences	37.4	14.6	48.1	11.4
Balanced chromosomes 2+3	77.1	59.9	68.2	60.1
Balanced chromosomes 2&3	57.1	49.6	50.2	33.1
Differences	20.0	10.3	18.0	27.0
L.S.D. 95%	13.8	13.9	14.3	9.5

significant if compared with the 95 % least significant difference (L.S.D.).

The sum of all chromosomal contributions to survivorship was about 60 % in both populations, in spite of their different ability to survive heat shock. The cytoplasms of the two natural populations had a different impact on survivorship: the cytoplasm of the balanced stock was able to reduce the survivorship of Mali flies with about 25 % and to increase the survivorship of Danish flies with 14.8 %, indicating that the differences in thermal resistance between the two populations are of the same magnitude as the effect of the cytoplasm within populations.

The difference between the values of line D and the sum of the contributions of all chromosomes is a raw estimate of overall chromosomal interactions; it was significant for females from Mali but not for those from Denmark (Table 2). An overview of some two by two interactions could be obtained for both females and males. The contribution of chromosome 1 in the presence of chromosome 3 was obtained by subtracting the contribution of chromosome 3 (Table 2) from line A (Table 1), while the contribution of chromosome 1 in the presence of chromosome 2 was obtained by subtracting the contribution of chromosome 2 (Table 2) from line B (Table 1). 2 × 3 interactions could only be obtained for balanced chromosomes: lines A + B (Table 1) gave the sum of the effects of chromosomes 2 and 3 when they act independently, while line C gave their joint effect.

Chromosome 1 contributed less to survivorship in the presence of chromosome 3 (higher difference from wild type flies) than in the presence of chromosome 2. Chromosomes 2 and 3 of the balanced stock also showed a significant interaction (with the excep-

tion of the males from Mali), in that the sum of their effects was higher when they acted independently (balanced chromosomes 2 + 3) than when they acted jointly (balanced chromosomes 2&3, at the bottom of Table 2).

DISCUSSION

We compared the contributions of the three major chromosomes and of the cytoplasm on survival after heat shock of two natural populations of *Drosophila melanogaster*, one originating from a tropical and one from a temperate area. The dependence of heat resistance 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) and in the wild (KREBS et al. 1996; GUERRA et al. 1997). This trend suggests that natural selection in the wild at non-extreme temperatures presumably has led to a genetically correlated response in tolerance to extreme temperatures, though occasional direct selection for heat resistance cannot be excluded. Previous work on the relative chromosomal contributions to fitness components suggests that different groups of genes are involved in adaptation at intermediate temperatures (CAVICCHI et al. 1989) and resistance to extreme heat (CAVICCHI et al. 1995) and that the contribution of the cytoplasm can vary according to the studied population (STEPHANOU and ALAHIOTIS 1983).

The results of our analysis of natural populations are partially in accordance with those of previous studies on laboratory populations evolved at different temperatures (see above). We found a large effect of

the cytoplasm (but with opposite effects on survivorship of the two populations) attributable to heritable cytoplasmic factor(s) rather than to maternal effects since it was retained during the generations of chromosomal substitution (Fig. 1). The difference in survival between the two natural populations (about 30 %) seems completely dependent on the cytoplasm in the sense that a foreign cytoplasm (balanced stock) is able to reduce the survivorship of the tropical population (Mali) but to increase that of the temperate one (Denmark). Cytoplasmic factors seem therefore of primary importance for heat tolerance. CRILL et al. (1996) found cross generational effects on knock-down temperature, presumably implying a cytoplasmic effect; the role of the cytoplasm in heat tolerance was well documented by STEPHANOU and ALAHIOTIS (1983) but not found by CAVICCHI et al. (1995) when they analysed lines adapted to different temperatures in the laboratory. The difference in pattern between the last two studies might reflect differences in the selected temperatures (14°C and 25°C vs 18°C and 28°C) or stocks (Greece vs Oregon). Interestingly, in the present experiment, the cytoplasm of the balanced stock, that has experienced a constant temperature of 20°C for several years, shows a contribution to resistance that is almost intermediate to that of the two natural populations that have experienced in nature an average annual temperature of about 25°C (Mali) or 8°C (Denmark) (see GUERRA et al. 1997).

In a previous experiment we analysed the variation in the resistance of females from reciprocal crosses between the populations from Mali and Denmark (KREBS et al. 1996). In that experiment, however, we did not find any significant differences between reciprocal crosses and, in general, a tendency of higher survivorships when the mother came from the more resistant population was observed. Probably, the heterozygosity of the flies originating from the reciprocal crosses minimized the cytoplasmic effect if nucleous x cytoplasm and/or chromosomal interactions were present (see below).

Also chromosomes play an important role for heat resistance, particularly chromosomes 2 and 1, while chromosome 3 gave a poor contribution. Similar results were obtained in the previously cited work but with a more consistent contribution of chromosome 3. The fact that the contribution of chromosome 3, where the heat shock genes are concentrated, was not the most prominent one and the presence of high chromosomal interactions suggest that heat resistance is a property of genes spread over the whole genetic background giving functional stability (hardiness) at extreme high temperatures.

Populations held at warmer temperatures also may 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). The performances of different isofemale lines with or without conditioning correlated poorly (GUERRA et al. 1997) after a short and heavy shock or a high correlation (LOESCHCKE et al. 1997) after a more prolonged and less severe shock, suggesting that the role of heat shock genes is poor for heat tolerance when a population is rapidly subjected to a potentially lethal heat stress. Gene expression data support this observation, in that the maximal transcription level of a more inducible heat shock gene ($hsp70$) is reached about half an hour after a severe heat treatment, while for others ($hsp82$, 27) the maximum is observed after a longer time (DIDOMENICO et al. 1982a,b). However, the contribution of factors which regulate the expression of the hsp genes cannot be excluded. OTSUKA et al. (1997) showed that the level of total mRNA of the six hsp genes significantly varied between lines isogenic for the second chromosome in which all structural genes on the third chromosome were expected to be constant.

All these findings suggest that many genetic mechanisms are at work affecting survival after heat shock, some of which may be independent of the heat shock response as a general hardiness or weakness (GUERRA et al. 1997) or inbreeding (DAHLGAARD et al. 1995). Other mechanisms, though not strictly associated with the heat shock response, can have a relevant evolutionary impact such as the association between natural selection at different temperatures and variation of the kinetic parameters of enzymes (ALAHIOTIS 1982; HOFFMANN and PARSONS 1991; SOMERO 1995). All the experiments devoted to the assay of chromosomes and cytoplasm in populations of *Drosophila melanogaster* adapted to different temperatures (STEPHANOU and ALAHIOTIS 1983; CAVICCHI et al. 1995 and present work) probably refer to the last mechanisms.

Suggestions on how evolution in a population at intermediate temperatures may affect tolerance to extreme temperature stress are only speculative, but many findings indicate that heat resistance evolves as a correlated response to natural selection at non-extreme temperatures both in the laboratory or in the wild. Because heat resistance to a sudden heat shock appears to be a property of many genes spread over the genetic background, attempts to analyze the impact of single genes (e.g., *HSPs*) are unlikely to lead to a complete understanding of heat resistance.

ACKNOWLEDGEMENTS

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