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Genetic and environmental factors in the resistance of *Drosophila subobscura* adults to high temperature shock

2. Modification of heat resistance by indirect selection

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Summary. We have carried out two equivalent selection experiments to increase and decrease heat shock resistance of *Drosophila subobscura* adults, using an indirect selection method that avoids excessive consanguinity. Heat shock was 33 ± 0.5 °C at saturation humidity. Control lines showed a rapid change of the physiological trait as a consequence of laboratory culture conditions, expressed as a decrease both in heat shock resistance and in the initial population variability for heat shock resistance. Thus, this reduction of variability seems to consist in the loss of those combinations of genes that confer high resistance to heat shock. After eight generations of selection, the selected lines were differentiated from their respective control lines, and the selection response obtained was similar in "resistant" and "sensitive" lines. Differences in survival of progeny of reciprocal crosses between selected lines suggest that inheritance of heat resistance may depend in part on the origin of egg cytoplasm.

Key words: Heat shock resistance – Indirect selection – Laboratory population evolution – *Drosophila subobscura*

Introduction

Information on the adaptation of *Drosophila subobscura* to high temperatures is of special interest because this species has been reported in very different climatic conditions and habitats, which points to a high adaptive flexibility. Moreover, the probable influence of temperature on adaptive variability is known for some characters such as chromosomal polymorphism (Prevosti et al. 1988) and wing length (Prevosti 1955), which show a clinal N-S distribution in natural populations.

Preliminary analysis of the influence of environmental factors on heat shock resistance in *Drosophila subobscura* adults (Quintana and Prevosti 1990) showed an important influence of culture temperature and larval density and suggested that their degree of influence depended on age and sex of adults. It is thus necessary to control these factors in order to ensure correct interpretation in studies of heat shock resistance. It is clear that the next step in the study of this physiological trait is to determine what kind of genetic factors control the resistance of a population to heat stress. One technique used to reveal the underlying genotype of a probable quantitative trait has been the development and analysis of extreme strains following a period of directional selection (Parsons 1986). Successful selection for heat shock resistance was not, to our knowledge, reported until 1978, when Morrison and Milkman succeeded in increasing heat resistance and heat sensitivity within an isofemale line of *Drosophila melanogaster*, using an indirect selection method. These authors confirmed the considerable potential for genetic variability in isofemale lines of this species, and predicted greater response in both directions if more genetic variability was available. Later, Stephanou and Alahiotis (1983) also succeeded in increasing heat resistance and heat sensitivity within an isofemale line of *Drosophila melanogaster*, using an indirect selection method similar to that used by Morrison and Milkman (1978); they pointed out that genetic analysis of heat-sensitive lines revealed that survival rate was chiefly determined by cytoplasmic inheritance, but also depended to some extent on the nucleus.

This paper presents the results obtained in performing indirect selection to increase and decrease heat shock resistance in *Drosophila subobscura* adults. Two equivalent selection experiments have been carried out, together with their respective control lines. Moreover, considering

the possibility of obtaining greater response if more genetic variation is available (Morrison and Milkman 1978), our selection method used a number of isofemale lines, so that excessive consanguinity is avoided.

Materials and methods

Sixty isofemale lines were started from wild, inseminated females caught in June 1984 in Tibidabo (Barcelona) and cultured at 17±0.25°C and 75–80% relative humidity (RH). These isofemale lines were separated at random into two sets of 30, so that two equivalent selection experiments could be performed. Indirect selection was started at once in one of these sets (experiment 1 = E1). The other 30 isofemale lines were maintained in mass culture for 3 months before starting the second selection experiment (experiment 2 = E2). Indirect selection was carried out as follows. From each isofemale line, 30 virgin males and 30 virgin females, 4–8 days old, were subjected to a heat shock of 33±0.5°C for 7 h (in the first generation of selection) at saturation humidity. These adults had previously been maintained at 17±0.25°C and 75–80% RH and were then transferred to empty tubes immediately before heat shock. This was performed by introducing the adults into a climatic chamber (50 × 40 × 40 cm) that maintained temperature and humidity at the values required during treatment. Temperature and relative humidity during treatment were checked by means of a hygro-thermograph. After heat shock, flies were transferred to a new fresh food bottle and placed at 17±0.25°C and 75–80% RH for 24 h, and the percentage mortalities were then calculated. Untreated siblings were separated daily until a minimum of 50 males and 50 virgin females were obtained from each isofemale line. From the isofemale lines, the six with the highest and the six with the lowest tolerances to the heat shock treatment were chosen. This represented a selection pressure of 20%. To obtain the second generation of selection, the 30 crosses needed to carry out all the possible reciprocal combinations between the six selected isofemale lines (except the crosses between individuals of the same isofemale line) were performed. Each of these crosses was performed with five males and five females of the corresponding lines. The progeny were subjected to the indirect selection method described above and the selection was continued for seven generations, both in "resistant" (R) and "sensitive" (S) lines. The initial isofemale lines were maintained in mass culture at 17±0.25°C and 75–80% RH, avoiding severe crowding and overlapping of generations, and were used as "control" lines (C). The same heat shock times were used in lines selected in the same direction. The time was increased or decreased when survival/mortality in selected bottles was >60%. Thus, in R lines, time was increased by 30′ each generation until the fourth generation of selection, after which the increase was 15′, and in S lines heat shock exposure was decreased by 30′ each generation.

The heat shock resistance was estimated in the initial (generation 0 = G0), fourth (G4), and eighth (G8) generations of selection. The estimation of heat shock resistance was performed from the progeny of the generations studied, as this permitted the use of a standard method for controlling larval density, an environmental factor that affects heat shock resistance (Quintana and Prevosti 1990). For this estimation, a male and a virgin female were taken from each bottle, giving 30 males and 30 virgin females for each generation. This process was carried out three times. Eight days after emergence these adults were placed together in bottles and 4 days later they were transferred to plastic boxes, where eggs were collected by introducing fresh petri dishes with ethanol-acetic acid agar medium seeded with live yeast. One hundred eggs were seeded in each culture bottle containing 25 cm^2 cornmeal sugar-agar food medium and kept at 17±0.25°C and 75–80% RH. Adults emerging during the period of greatest emergence were transferred to fresh food bottles on the day of emergence, in groups of 50 males or females. These adults were also kept at 17±0.25°C and 75–80% RH until the heat shock. Their ability to withstand a high temperature shock was measured as the median lethal dose, LD50 (Finney 1971). A sample of 500 8-day-old adults of each sex was tested, using five different exposure times (5, 6, 7, 8, and 9 h) and two replicates/time. The heat shock was performed as indicated above. Heat shock resistance of progeny from each reciprocal cross between R and S lines was tested after the eight generations of selection, using the same method.

The median lethal dose, LD50, was estimated by calculating the probit regression line, using the maximum likelihood estimation by the iterative method (Finney 1971). Another parameter used was the slope of this line as an estimator of $1/\sigma$ (Finney 1971), where σ is the resistance variability in the population, i.e., the variability in the population of the maximum dose value that an individual can survive. ANOVAs were carried out using "BMDP Statistical Software" (Dixon et al. 1983), specifically the P4V program ["General Univariate and Multivariate Analysis of Variance and Covariate, including repeated measures (URWAS)", Michael Davidson and Jerome Toporek], and using the angular transformation of mortality frequencies for the different doses.

Results

The median lethal doses (LD50) and their 95% fiducial limits, for C, R, and S lines in the generations studied (G0, G4, G8) of each selection experiment (E1, E2), are given in Tables 1 and 2. The graphic representation of these LD50 values is shown in Figs. 1 and 2, respectively, for each selection experiment.

Control lines of both experiments show a rapid decline in the ability to withstand the heat shock, measured as LD50, when the number of generations in laboratory conditions increases ($P<0.001$), and this is true in both sexes (Tables 1 and 2, Figs. 1 and 2). On the other hand, LD50 values of E2 are lower than those of E1 ($P<0.05$), except for males in the eighth generation (see Tables 1 and 2). E2 was started when isofemale lines had been maintained for 3 months in laboratory conditions. This suggests a rapid change of the physiological trait as a consequence of laboratory culture conditions, expressed as a decrease of heat shock resistance of the population. However, this effect seems to decrease or even disappear as the laboratory culture time increases, since LD50 differences between C lines in E1 and E2 are practically nonexistent in G8 (Tables 1 and 2).

Slope values of the regression lines showing the relation between log.dose-mortality percentage probit of C lines are given in Table 3. There is a significant influence of laboratory culture time on slope values ($P<0.001$). Slopes are normally higher in both sexes and in both experiments when culture time in laboratory increases. Slope is an estimator of the variability in the resistance of the individuals of the population; slope and variability

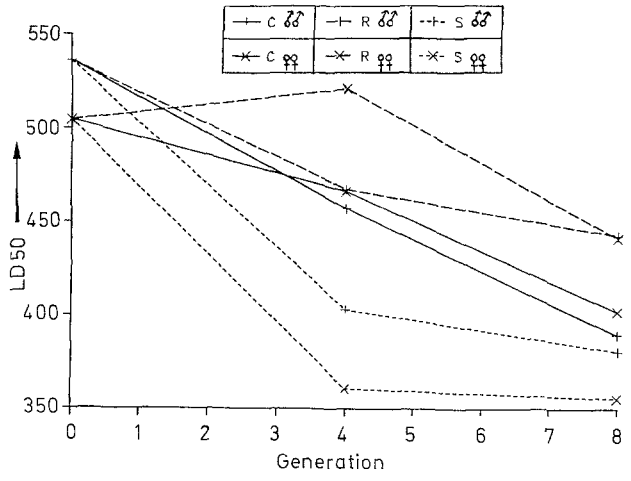

Fig. 1. Graphic representation of LD50 values (in minutes) for males (♂♂) and females (♀♀) of C, R, and S lines of E1 in generations analyzed (G0, G4, G8)

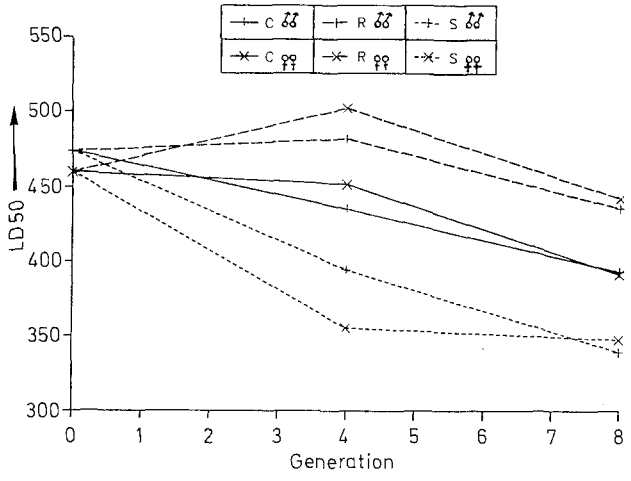

Fig. 2. Graphic representation of LD50 values (in minutes) for males (♂♂) and females (♀♀) of C, R, and S lines of E2 in generations analyzed (G0, G4, G8)

are inversely related (Finney 1971). This indicates that variability decreases as culture time in laboratory increases. The decreases both in heat shock resistance and resistance variability seem to suggest that the reduction of variability affects those combinations of genes that confer high resistance to heat shock. It is evident that LD50 values of selected lines should be compared to respective values of control lines, if the selection response obtained is to be interpreted correctly.

From the indirect selection experiments, four lines have been derived: two "resistant" (R) and two "sensitive" (S). These lines exhibit differences in survival rate when their progeny are subjected to heat shock (Tables 1 and 2, Figs. 1 and 2). R and S lines exhibit between 9.4 and 13.6% increase or decrease of heat shock resistance in relation to their C line after the eight generations of

Table 1. LD50 (in minutes) for males (♂♂) and females (♀♀) of C, R, and S lines in generations studied (G0, G4, G8) of experiment E1 and of reciprocal crosses between R and S lines (♀♀ × ♂♂) of the same experiment. Their 95% fiducial limits are given in parenthesis

	G0	G4	G8
E1			
R ♂♂		468 (432–522)	443 (426–461)
♀♀		521 (479–625)	441 (425–458)
C ♂♂	536 (495–621)	457 (420–506)	390 (360–417)
♀♀	504 (483–534)	466 (421–539)	402 (384–419)
S ♂♂		403 (364–441)	381 (357–401)
♀♀		361 (331–385)	355 (326–380)
R × S ♂♂			435 (415–456)
♀♀			407 (385–428)
S × R ♂♂			360 (350–370)
♀♀			364 (354–372)

Table 2. LD50 (in minutes) for males (♂♂) and females (♀♀) of C, R, and S lines in generations studied (G0, G4, G8) of experiment E2 and of reciprocal crosses between R and S lines (♀♀ × ♂♂) of the same experiment. Their 95% fiducial limits are given in parenthesis

	G0	G4	G8
E2			
R ♂♂		482 (438–570)	436 (420–452)
♀♀		502 (461–597)	442 (422–462)
C ♂♂	473 (458–491)	435 (395–486)	393 (376–411)
♀♀	460 (443–479)	452 (419–493)	392 (383–400)
S ♂♂		395 (337–445)	340 (330–349)
♀♀		356 (342–368)	348 (339–356)
R × S ♂♂			352 (313–380)
♀♀			415 (403–426)
S × R ♂♂			301 (263–326)
♀♀			378 (343–408)

Table 3. Slopes of regression lines (log. dose-probit percent mortality) and their error for males (♂♂) and females (♀♀) of C lines of experiments E1 and E2 in G0, G4, and G8. The time of maintenance in laboratory is also indicated (T, months)

		C		T
		♂♂	♀♀	
E1				
	G0	7.60±1.30	7.66±0.84	1
	G4	12.56±2.80	12.48±3.33	6
	G8	11.94±1.96	14.73±1.70	11
E2				
	G0	9.36±0.87	8.09±0.79	4
	G4	9.48±2.03	10.07±1.78	9
	G8	15.23±1.80	15.27±1.11	14

selection (exception: males of S line in E1 with 2.3% decrease). The results obtained after four generations of selection for the R lines are similar (with the exception of males of E1 with 2.4% increase), but the fiducial limits of R and C lines still overlap. Comparing R and S lines in two out of four cases, one finds is no overlapping. For the S lines in the fourth generation, a higher decrease in females is obtained (22.5% in E1, 21.2% in E2), and in males the fiducial limits of S and C lines still overlap. Thus, after eight generations of selection, R and S lines are differentiated from their respective controls and the selection response is similar in R and S lines.

LD50 values for the progeny of reciprocal crosses between R and S lines after the eight generations of selection are shown in Tables 1 and 2. Although most of these values are within the fiducial limits of the LD50 values of the control lines, in seven out of eight possible cases they are more similar to those found for their mothers. This tendency could be an expression of a biological fact.

Discussion

The selection responses were similar in both "resistant" and "sensitive" lines after eight generations of selection. This observation differs from Morrison and Milkman (1978), who found a much greater selection response of the sensitive line. But these authors do not describe the evolution of control lines during selection, and it is not possible to interpret their graph of survival with the information given in their paper. Stephanou and Alahiotis (1983) also obtained two strains that exhibit differences in survival rate when subjected to heat shock, but the isofemale lines that they used has been maintained in the laboratory for about 2 years before the selection experiment and they did not carry out a control, so their results cannot be compared with those obtained in the present study.

After the fourth generation there is no clear response to selection, which could be explained if the number of genes controlling heat shock resistance were relatively low. Parsons (1986) states that "the variability existent in natural populations for ecological phenotypes, detected by discrete differences among isofemale strains, and the analysis of many directional selection experiments are only interpretable assuming relatively few genes of relatively large effect. Data consistent with this conclusion come from traits ranging from morphological to physiological and, in addition, for ecological and ecobehavioral traits important in determining distribution and abundance of a species". The ability to withstand a high temperature shock may be considered one of these traits. This hypothetical genetic architecture would permit a rapid genetic change in response to stress, in this case, heat stress.

Morrison and Milkman (1978) consider that the major factor(s) for heat sensitivity are on the second chromosome for strains isolated from *Drosophila melanogaster* collected in the United States. Stephanou and Alahiotis (1983), according to the results of genetic analysis of their heat-sensitive lines, conclude that the heat sensitivity character of *Drosophila melanogaster* presents a non-Mendelian inheritance and is transmitted through the maternal cytoplasm, while nuclear genes modify its expression. With respect to the findings of Morrison and Milkman (1978), Stephanou and Alahiotis hypothesize that selection may have occurred for a heat-sensitive mutant located on the second chromosome.

In our study, LD50 values for the progeny of reciprocal crosses between resistant and sensitive lines after eight generations of selection are more similar to those found for their mothers' line, and their fiducial limits do not overlap with the fiducial limits of their fathers' line in seven out of eight possible cases. This suggests that the inheritance of the heat shock resistance character may depend in part on the origin of the cytoplasm of the egg, but with our data we cannot discriminate between true cytoplasmic inheritance or maternal effect. Neither can we establish the possible significance of heat-shock genes studied by several authors [see review by Lindquist (1986)] for the results obtained up to now.

The decrease of heat shock resistance observed in laboratory culture conditions is in agreement with other observations that support the existence of a general capacity for rapid genetic response to environmental conditions. This capacity is detected in different traits (morphological, behavioral, physiological) both in laboratory (Powell 1974; Anderson 1973; Cavicchi et al. 1985; Pascual et al. 1990) and natural (Grant 1986; Gibbs and Grant 1987; Pimm 1988; Prevosti et al. 1988, 1990) populations.

The increase in the homogeneity of response to heat shock, as shown by the 95% fiducial limits of LD50

values of C lines, and the increase in the slope of regression lines between log.dose and mortality percentage probit seem to suggest that the change occurring in the laboratory consists in a decrease of initial population variability.

We have not found any references to this behavior of control lines, which suggests that the reduction of variability consists in the loss of those combinations of genes that confer high resistance. These combinations are possibly more important in natural populations, which can be subjected to high temperatures or other stresses, than in laboratory populations, for which the environmental conditions are more constant. Natural selection could be responsible for the maintenance of these combinations in nature.

The reduction in variability observed in the laboratory agrees with a general observation by Parsons (1986) that both phenotype and genotype variability tend to be higher at conditions of severe stress imposed by physical and biological environments, especially for quantitative traits of importance in determining survival.

Note that the LD50 values of males and females of the initial generation of experiment one (the progeny of wild, inseminated females) are only exceeded in one case: females of resistant line in the fourth generation of experiment one. This result is explained by the loss of variability in laboratory conditions mentioned above.

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