NON-MENDELIAN INHERITANCE OF "HEAT-SENSITIVITY" IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Non-Mendelian inheritance was revealed for the "heat-sensitivity" character of the poikilothermic insect *Drosophila melanogaster*. Genetic analyses were performed on heat-sensitive (S, S_1) strains, derived through indirect selection, and on stocks constructed through extensive chromosomal and cytoplasmic substitutions between strains obtained from two replicate cage populations. The populations were kept for about 7 years under different temperatures (14°–25°) and exhibited different survival. We conclude that the character studied is quantitative, responds to selection pressure and is transmitted through the maternal cytoplasm, while nuclear genes modify its expression.

TEMPERATURE is a common environmental parameter for all organisms and plays a major role in the diversification of life. Ectotherms such as insects are subjected to the direct effect of temperature, a fact that results in various responses of their genetic and biochemical machinery (HOCHACHKA and SOMERO 1973; ALAHIOTIS, MILLER and BERGER 1977; ALAHIOTIS and BERGER 1978; ALAHIOTIS 1979a; ALAHIOTIS 1980; ALEXANDROV 1977). Among those responses increasing attention has been given to changes of enzyme conformation and the transport properties of membranes. If a substantial temperature change takes place, the ability of an ectotherm to survive is dependent on its genetic capacity to compensate for the temperature change. Hence, appropriate selection might increase or decrease the heat resistance or heat sensitivity. By applying such selection to individuals of an insect population, one should be able to construct strains having high resistance to temperature shock and others having high sensitivity. If such strains are analyzed genetically, the mode of inheritance of heat sensitivity, in particular, whether it behaves as a simple Mendelian factor or whether it is a quantitative character with a considerable additive genetic component, can be determined. The elucidation of the situation could contribute to a new understanding of temperature compensation. To test this experimentally we used *Drosophila melanogaster*. Genetic analysis of heat-sensitive and heat-resistant lines that we selected revealed that the survival rate is chiefly determined by cytoplasmic inheritance but also depends to some extent on the nucleus.

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MATERIALS AND METHODS

Fifty isofemale lines, maintained in mass culture under standard conditions (25°, 43 ± 4% mean relative humidity and in the cornmeal sugar-agar food medium; ALAHIOTIS 1976) for about 2 years in our laboratory, were tested for their ability to survive when subjected to heat shock (40° for 25 min). The original inseminated females were captured from a natural population in Gavros-Achaïa, Greece. From these lines two were chosen: one with the highest and another with the lowest tolerance to the heat shock treatment. Afterward, indirect selection was performed for several generations as follows: from each of the two lines approximately ten sublines were derived; each subline was generated from a single pair (1♀ × 1♂). The parents of each subline were transferred to a new food vial three times in order to increase the number of the progeny obtained; from each subline at least 60 progeny were tested. Three-day-old progeny were anesthetized with ether and placed in empty glass vials (10♀ and 10♂ per vial) for 2 hr before treatment. The vials contained no food, and moistened cotton plugs were forced into a position well below the surface of a Grant water bath into which the vials were immersed. After the heat shock, the flies were placed under standard conditions (25°) for 20 hr, and the percentage of the flies that remained alive was calculated. The untreated siblings of the most sensitive subline of the sensitive line or the most resistant subline of the resistant line were used to generate the next generation.

Ten additional strains of *D. melanogaster* were also used in this investigation; the genetic constitution of these strains is described in the RESULTS and DISCUSSION section (Table 4). The stocks CCC_{cC} and DDD_{cD} originated from two replicate cage populations maintained for approximately 7 years at 14° and 25°, respectively. Extensive chromosomal and cytoplasmic substitution between these strains was carried out. The common parents of these populations had been caught in Cephalonia, Greece. A detailed description of the populations is given elsewhere (KILIAS, ALAHIOTIS and PELECANOS 1980). The construction of the stocks referred to in Table 4 has been achieved using the balance stock *M-5; Cy/Pm; Ubx/Sb* (LINDSLEY and GRELL 1968), and the crossing schemes are presented elsewhere (KILIAS and ALAHIOTIS 1982).

RESULTS

Indirect selection experiments: From the indirect selection experiments for heat sensitivity (Figure 1) two strains have been derived, one "resistant" (*R*) and one "sensitive" (*S*). These strains exhibit a ~20-fold difference in survival rate when subjected to heat shock. We seem to have succeeded in increasing heat resistance in the *R* strain, an achievement that has already been reported (MORRISON and MILKMAN 1978). After about ten generations of selection the sublines of each *R* or *S* line exhibited considerable homogeneity with respect to their survival, which was not true in the early generation (Table 1). This indicates that the selection experiment not only increased the heat resistance (in the *R* strain) but also contributed to stabilizing the genotypes of the *S* and *R* strain making them more appropriate for genetic analysis. The coefficients of variation (V) in the 16th generation of selection for the *S* and *R* strains are much lower than in the third generation: $V_{S(3)} = 106$; $V_{S(16)} = 64.82$; $V_{R(3)} = 66.6$; $V_{R(16)} = 7.89$. Since there was heterogeneity in the survival values among the sublines of each original line in the early generations, we may conclude that the original 50 isofemale lines maintained in the laboratory for about 2 years had not reached isogenicity.

Genetic analysis of the heat sensitivity character: Virgin *S* or *R* females, 3 days old, were mated with *R* and *S* males, respectively. Progeny from each reciprocal cross were collected and subjected to temperature shock, when they were 3 days old. Figure 1 shows that the survival values obtained from such heterozygotes are similar to those found for their mothers, in seven sequential

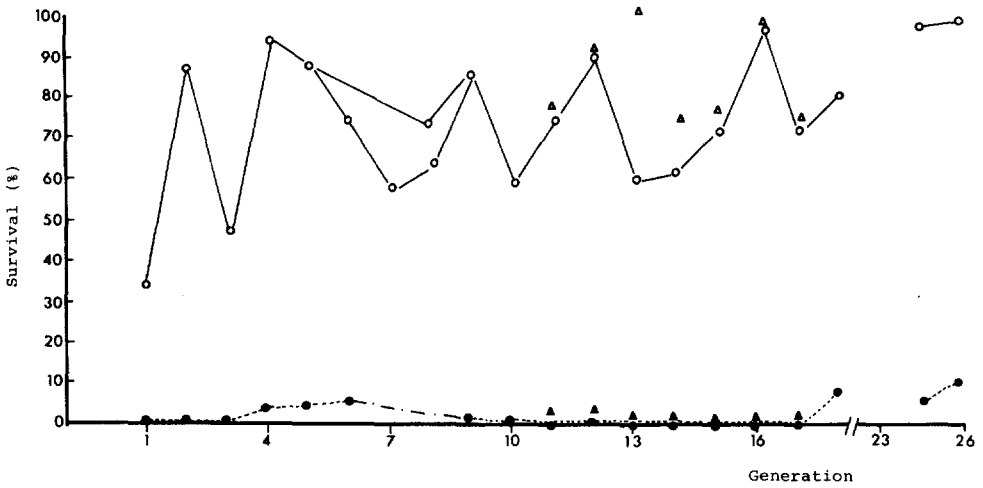


FIGURE 1.—Indirect selection for resistance and sensitivity to heat shock. O——O, selected resistant; ●- - -●, selected sensitive; △, hybrids from resistant females and sensitive males; ▲, hybrids from sensitive females and resistant males.

TABLE 1

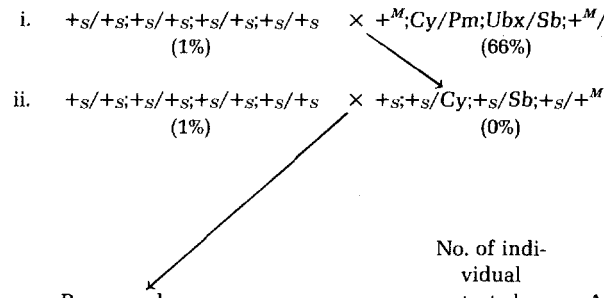
Survival (after 25 min at 40°) of the sublines of S *and* R *strains in the 3rd and 16th generation of selection*

Generation	Stocks	
	S	*R*
	0	18.46
	12.57	45.76
	4.00	3.03
3	3.03	6.75
	5.00	23.07
	0	30.35
	1.85	13.95
		46.66
		20.27
		8.69
	3.38	91.30
	3.03	87.50
16	2.70	92.13
	6.25	95.00
	0	

For each subline at least 60 progeny were tested.

generations of selection. This difference between reciprocal crosses suggests that the inheritance of the heat-sensitivity character depends on the origin of the cytoplasm of the egg. This leaves open the question whether the particular maternal chromosomes that are inherited with the proper cytoplasm influence the survival values.

The contribution of individual chromosomes to the genetic basis of the heat



Progeny classes	No. of individual tested	Alive	Survival (% alive)
(1) +S/+S (or →);+S/Cy;+S/Sb;+S/+S (or +M)	1068	90	8.42
(2) +S/+S (or →);+S/Cy;+S/+S;+S/+S (or +M)	886	102	11.51
(3) +S/+S (or →);+S/+S;+S/Sb;+S/+S (or +M)	803	45	5.09
(4) +S/+S (or →);+S/+S;+S/+S;+S/+S (or +M)	625	48	7.68

FIGURE 2.—Crossing scheme and table of responses of each backcross progeny class to heat shock (40° for 25 min). Unmarked chromosomes from the marker stock are designated. $+^{M}$.

sensitivity trait was analyzed by the cross illustrated in Figure 2. Chromosomal analysis was perfromed with the aid of a balanced marker stock (*Cy/Pm, Ubx/Sb*) for the 2nd and 3rd chromosomes. Females from the sensitive strain were mated with males from the balanced stock (cross i, Figure 2). For the next generation, sensitive females were mated with heterozygous males (cross ii, Figure 2). Such heterozygotes were found to exhibit survival values similar to those detected for their mothers: no intermediate values were observed. (The balanced stock was found to exhibit 66% survival and can be considered as resistant.)

The survival of the progeny from cross ii (test cross: Figure 2) was generally of the same order of magnitude in each genotypic class and resembles that of the *S* strain. This observation differs from the report of MORRISON and MILKMAN (1978) (for strains isolated from flies collected in the United States) that the major factor(s) for heat sensitivity are on the 2nd chromosome. According to our data, some response can be attributed to the 2nd chromosome (heterozygotes for *Cy* exhibit higher survival than the sensitive homozygotes; contingency $\chi^2_{4-2} = 6.2$, $P < 0.05$). Moreover, heterozygotes for *Sb* exhibit lower survival ($\chi^2_{1-2} = 5.2$, $P < 0.05$) contrary to findings of MORRISON and MILKMAN (1978) that the 3rd chromosome makes a small additional contribution. Furthermore, homozygotes for the II and III wild-type-sensitive chromosomes exhibit slightly higher survival than the *S* strain (Figure 2). Thus, it appears that the survival rate does not depend, or depends to a quite minor extent, on the nucleus. All of these observations support the view that the inheritance of the heat sensitivity character under investigation in our strains is not monofactorial but is controlled by additive effects. The information obtained from Figures 1 and 2 strongly suggests that a cytoplasmic factor might be determining the heat sensitivity response. The increase in heat tolerance upon selection was not considered great enough to justify similar analysis for the time being.

Reciprocal F_1 hybrids between another pair of S_1 and R_1 strains, established through indirect selection (for ten generations) from two of the initial 50 isofemale lines, were found to exhibit genetic behavior (Table 2) analogous with

TABLE 2

Survival (after 25 min at 40°) of the S_1 and R_1 strains and their reciprocal hybrids

Stock	No. of individuals tested	Alive	Survival (% alive)
S_1	322	21	6.50
R_1	350	267	76.24
S_1R_1	240	76	31.67
R_1S_1	342	207	60.53

that of the strains already described (Figures 1 and 2), *that is*, the heat shock sensitivity is inherited cytoplasmically. The fact that the two reciprocal hybrids (S_1R_1 and R_1S_1) presented in Table 2 do not show the extreme survival values of their mothers could also be attributed to a hypothetical scheme in which a single X-linked locus determines the S_1 or R_1 phenotype. If S_1 and R_1 are co-dominant, such that the S_1/R_1 heterozygote shows the average survival of S_1 and R_1 alone, then the results for R_1S_1 and S_1R_1 would be very close to the observed values. To rule out this hypothetical explanation we examined the survival of males *vs.* females and found no difference in their survival.

The genetic information obtained so far regarding the inheritance of the trait under investigation in two independently derived pairs of strains (R, S, R_1, S_1) favors the view that survival appears to be determined mainly by a specific cytoplasmic state. To obtain stronger evidence regarding the possibility of heat sensitivity being controlled by a dominant allele or being a sex-linked character, substitution for the X, second or third chromosome pair of the S_1 strain was carried out. The crossing scheme followed to isolate the strains listed in the Table 3 is given in Figure 3. Virgin females from the S_1 original strain were crossed with *M-5; Cy/Pm; Ubx/Sb* males (balanced stock). Heterozygotes (females and males) from this cross were used to generate the second generation. Selected progeny from that generation resulted in the isolation of two substitution lines for the second and third chromosomes. For the constuction of the strain that bears the X chromosome from the balanced strain and the remainder from the S_1 stock, an additional cross (generation 3) is required. Thus, *M-5*/+s_1;+s_1/+s_1;+s_1/+s_1 virgin females were mated with *M-5*;+s_1/+s_1;+s_1/s_1 males, and the appropriate progeny were selected (Figure 3). Finally, to isolate a strain carring the nucleus from the balanced stock in the S_1 cytoplasm, *M-5*/+s_1;*Cy*/+s_1;*Ubx*/+s_1 females were mated with *M-5;Cy/Pm;Ubx/Sb* males, and the appropriate progeny were selcted (Figure 3).

The original balance stock used to perform the chromosomal and cytoplasmic substitutions exhibits high survival values and has been considered as resistant. Stock 1 (Table 3) carries a nucleus from S_1 flies in the S_1 cytoplasm like the original S_1 stock. However, we have used the +s_1/+(s_1 or –);+s_1/+s_1;+s_1/+s_1 stock, instead of the original S_1, as a control. This stock had been constructed through a crossing scheme similar to that followed for the isolation of the chromosomal substitution lines.

Table 3 gives the results of the tests made on strains in which both homologues of each chromosome pair have been substituted. The control stock (Table 3,

TABLE 3

Survival values of the strains derived by chromosomal and cytoplasmic substitution as shown in Figure 3[a]

Strain	Cytoplasmic background	No. of individual tested			Alive			Survival (% alive)		
		G_6[b]	G_7	G_8	G_6	G_7	G_8	G_6	G_7	G_8
(1) $+s_1/+s_1$ (or →);$+s_1/+s_1$;$+s_1/+s_1$	S_1	390	63	402	22	1	34	5.64	1.59	8.45
(2) *M-5/M-5* (or →);$+s_1/+s_1$;$+s_1/+s_1$	S_1	92	80	150	0	1	6	0.00	1.25	4.00
(3) $+s_1/+s_1$ (or →);*Cy/Pm*;$+s_1/+s_1$	S_1	98	202	182	39	77	44	39.79	38.11	24.17
(4) $+s_1/+s_1$ (or →);$+s_1/+s_1$;*Ubx/Sb*	S_1	691	106	311	37	8	25	5.35	7.54	8.03
(5) *M-5/M-5* (or →);*Cy/Pm*;*Ubx/Sb*	S_1	219	57	243	57	19	58	26.76	33.30	23.87
(6) *M-5/M-5* (or →);*Cy/Pm*;*Ubx/Sb*	M[c]	231		65	143		35	61.90		53.84

[a] Heat treatment was performed as described in the MATERIALS AND METHODS section.

[b] G denotes generations after the first cross (Figure 3) performed for the constructions of these strains.

[c] M denotes that the cytoplasm carried by stock 6 is that of the original marker stock used for the substitutions performed.

stock 1) which carries the three major chromosomes and the cytoplasm of the S_1 behaves as sensitive. Since about half of the individuals of that strain must carry, in heterozygous state, a chromosome IV coming from the resistant balanced strain, recessive genes on this chromosome do not appear to be involved in the control of the survival. Second, the survival values obtained for strains bearing the X or the third chromosomes from the resistant balanced stock are of the same order of magnitude as those of the sensitive strain(s). This observation rules out the possibility of the character being sex linked or controlled by a dominant allele located on the third chromosome (Tables 2 and 3). Third, in terms of the second chromosome we see that this chromosome increases the survival rate (as previously has been noticed, Figure 2) in comparison to the control values ($\chi^2_{1-3} = 83.53$, $P < 0.001$ for G_6; $\chi^2_{1-3} = 30.85$, $P < 0.001$ for G_7; and $\chi^2_{1-3} = 26.75$, $P < 0.001$ for G_8). This response to the 2nd chromosome is higher in the case in which both homologues (Table 3) instead of one (Figure 2) have been substituted. It must be emphasized here that the survival rate of this strain (Table 3, stock 3) is much lower than that of the resistant balanced stock ($\chi^2_{3-6} = 136.7$, $P < 0.001$ for G_6; $\chi^2_{3-6} = 19.38$, $P < 0.001$ for G_8). This fact does not support the view that the major contributing factor is located on the 2nd chromosome, and the possibility of heat sensitivity being controlled by a dominant allele (on that chromosome) does not hold true. However, some response can be attributed to that chromosome that appears to act in a quantitative sense.

Fourth, as regards the cytoplasmic effect on the survival rate, we see that the constructed strain that exhibits complete exchange (stock 5) displays low survival (Table 3). This strain carries a resistant balanced stock nucleus in sensitive cytoplasm (S_1). The survival rate observed for that strain is not as low as that of the control stock, possibly due to the additive contribution of the second chromosome. In terms of the stock under investigation, the action of the

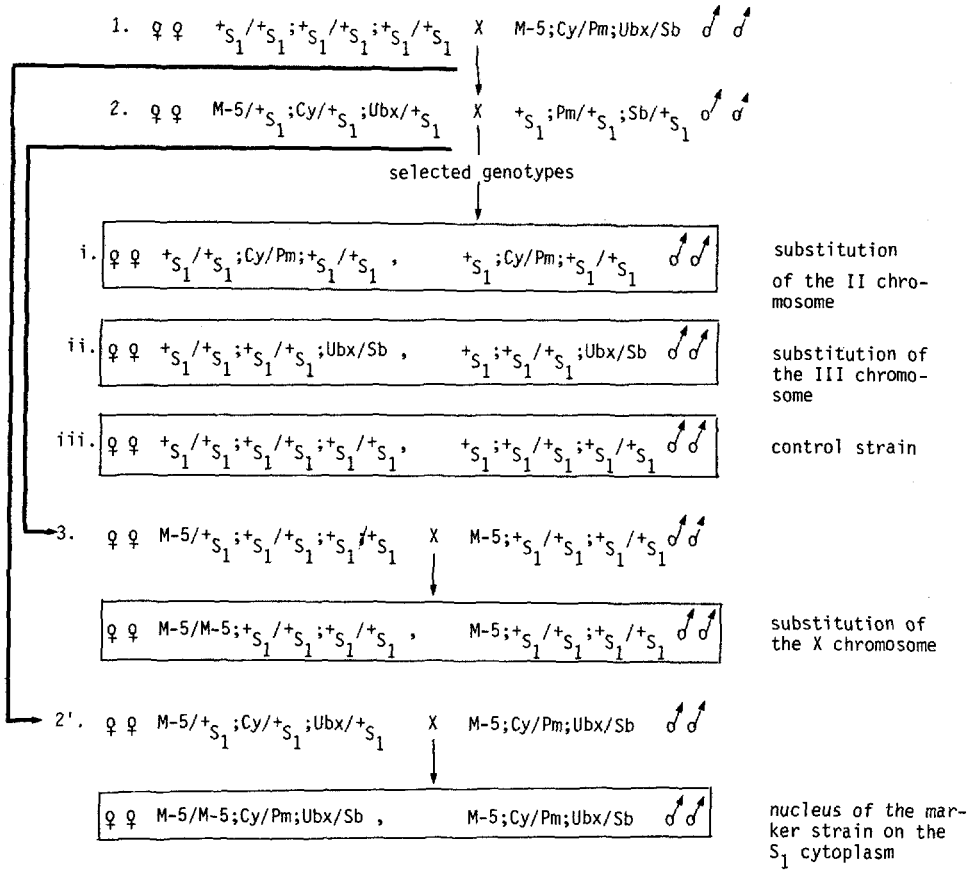


FIGURE 3.—Crossing scheme followed to isolate strains having the X, *II* and *III* chromosome substituted. A strain with complete exchange as well as a control strain has been constructed through the crossing scheme presented here. In all strains the cytoplasm is that of the sensitive (S_1) strain.

three major chromosomes is not additive, a fact that rules out, once again, any contribution of the X and third chromosome.

The genetic analysis of the heat sensitivity performed in two different sensitive strains (S, S_1) leads to the same conclusion, that is, the survival rate is mainly controlled by cytoplasmic factor(s), although the nucleus also intervenes in a minor way. This mode of inheritance could be further supported if we exclude the possibility that maternal effects are responsible for our observations. One way to rule out such effects is to do test crosses over several generations between individuals from the sensitive and resistant strains. However, this approach could result in an uncontrolled amount of genetic heterogeneity in the following generations. We think that a more appropriate way to examine the possible maternal effects is to construct a composite strain that associates the homozygous genotype of the resistant strain with maternal cytoplasm from a sensitive one; the survival rate of such a strain will indicate

how the chromosomes interact with the cytoplasm in the long run. Stock 5 in Table 3, which exhibits complete exchange, has the characteristics required for this test. Although one-fourth of the individuals of that strain must carry, in the heterozygous state, a chromosome IV coming from a sensitive mother, recessive genes on this chromosome have already been shown not to control survival. Since the survival rate of this stock has remained low several generations after the synthesis, the effect of the chromosomes does not appear to accumulate through the generations. In the long run, this observation argues against maternal influence. The possibility of some maternal effect being involved in heat sensitivity is clearly quite small and restricted only to dominant allele(s) located on chromosome IV. Furthermore, no delayed effect in relation to the long-term interaction between nucleus and cytoplasm was observed in the case in which strains with complete exchange as regards the *C* and *D* nucleus and cytoplasm (Table 4) were tested about 15 generations after their derivation.

Survival experiments using flies from long-term cage populations maintained under different temperatures: Additional experiments were carried out using strains obtained through extensive chromosomal and cytoplasmic substitution between the DDD_{cD} and CCC_{cC} strains, which are of different origin than the strains previously described. The aims of these additional experiments were: (1) to test whether an association exists between heat sensitivity and the temperature at which the populations were maintained for about 7 years and (2) if this were the case, to get more information on the genetic nature of the character in order to reinforce or reject the conclusions reached.

Table 4 shows that flies from the C population (14°, stock 2; CCC_{cC}) are more sensitive to heat shock than flies obtained from population D (25°, stock 1, DDD_{cD}; contingency $\chi^2_{1-2} = 175.34$, $P < 0.001$). Since DDD_{cD} and CCC_{cC} flies and their chromosomal and cytoplasmic substitution lines were not taken directly from the cage populations but were reared for one (DDD_{cD}, CCC_{cC}) or many (substitution lines) generations in common conditions (25°) before the heat shock, the differential survival of the *C* and *D* flies was not due to acclimation. Hence, we may assume that the differences regarding the survival rate between the two cage populations, which were maintained for nearly 7 years under different temperatures (14°-25°), reflect a selective effect of the temperature with regard to the factor(s) responsible for the ability of *D. melanogaster* to survive under extremely high temperatures. Genetic analysis revealed again that the trait is under maternal influence. Stocks 3 and 4 (Table 4) contain the X, *II* and *III* chromosomes in foreign cytoplasms. That is, stock 3 has the *C* chromosomes in the *D* cytoplasm and the opposite situation is true for the stock 4. The survival of the CCC_{cD} resembles that of the DDD_{cD} (Table 4); these strains share common cytoplasm but different nuclei. The same situation is true for the DDD_{cC} and CCC_{cC} strains (Table 4). The strain CCC_{cD} exhibits higher tolerance than the strain DDD_{cC} ($\chi^2_{3-4} = 115.52$, $P < 0.001$). No intermediate values were obtained for these strains that exhibit complete exchange. Here, again, the contribution of the cytoplasm in determining survival is clear.

Table 4 also shows the genetic constitution of the chromosomal substitution lines, as well as the contribution of each of the two autosomal chromosomes (*II*

TABLE 4

Genetic and cytoplasmic constitution of the strains derived by chromosome and cytoplasmic substitutions and the responses of each strain to heat shock (40° for 25 min)

Stock	Genetic constitution with regards to chromosomes			Cytoplasmic background	No. of individuals tested	Alive	Survival (% alive)
	X	*II*	*III*				
1. DDD_{cD}	D	D	D	D	1396	796	57.02
2. CCC_{cC}	C	C	C	C	1141	350	30.67
3. CCC_{cD}	C	C	C	D	219	119	54.33
4. DDD_{cC}	D	D	D	C	732	131	17.89
5. DCC_{cC}	D	C	C	C	273	143	52.38
6. DCD_{cC}	D	C	D	C	419	53	12.64
7. DDC_{cC}	D	D	C	C	253	78	30.83
8. CDD_{cD}	C	D	D	D	419	24	5.72
9. CDC_{cD}	C	D	C	D	380	50	13.15
10. CCD_{cD}	C	C	D	D	469	84	17.91

and *III*) and the X chromosome to the incidence of survival. From the same table we see two interesting findings. (1) The (*D*) X chromosome introduced into *C* genetic background and cytoplasm increases the heat tolerance of the fly (compare stocks 2 and 5; Table 4; $\chi^2_{2-5} = 45.70$, $P < 0.001$). The same is true for the opposite situation in which the first (*C*) X chromosome decreases the heat tolerance of the fly carrying the rest of the genome *D* and its cytoplasm (compare stocks 8 and 1, Table 4; $\chi^2_{8-1} = 342.32$, $P < 0.001$). Thus, the X chromosome appears to play a role in determining the survival of the fly. (2) When the X and *III* or the X and *II C* chromosomes are in the *D* genetic background and cytoplasm (stocks 9 and 10; Table 4) the heat tolerance of the flies is decreased as compared with that of the control DDD_{cD} strain ($\chi^2_{9-1} = 230.37$, $P < 0.001$; $\chi^2_{10-1} = 215.46$, $P < 0.001$). However, these values are much lower even than those of the CCC_{cC} strain (Table 4; $\chi^2_{9-2} = 45.13$; $P < 0.001$; $\chi^2_{10-2} = 27.50$, $P < 0.001$), a fact which does not favor location of the factor(s) responsible for the survival of the flies only in the nucleus. This view is also supported by the fact that there is no additivity of the chromosomal effect on survival.

A more quantitative sense of the contribution of each component (cytoplasm and chromosomes X, *II* and *III*) to survival can be obtained by comparing members of pairs of stock that differ from each other in a single component and calculating an average value for the shift of heat tolerance in a particular direction for all stock pairs that differ only in that component. Thus, by comparing stocks 1 with 8 and 2 with 5 (Table 4) we see that on the average (weighted according to the number of individuals tested in each pair) the *C* chromosome X decreased survival by 38.34%. Similar comparisons of stock 4 with 6, 3 with 9, 5 with 7 and 10 with 8 (Table 4) reveal that on the average the *C* chromosome *II* increased survival by 12.9%. For chromosome *III*, comparisons of stocks 3 with 10, 7 with 4, 5 with 6, and 9 with 8 show that the *C* chromosome *III* increased survival by an average of 22.5%. Finally, when stocks 1 and 4 and

3 and 2 are compared, the *C* cytoplasm decreased survival by an average of 33.1%. From this sort of analysis it appears that the *C* chromosome X has as strong an effect as the *C* cytoplasm in decreasing the survival of the heat-shocked flies.

When we take into consideration the reciprocal chromosomal transfers (Table 4, strains 6 and 7 as compared with strains 9 and 10) we see again that the X, *II* and *III* chromosomes alone do not control the inheritance of the character under study. That is, strains 6 and 7 do not show high tolerance as one would expect if the *D* chromosomes alone contributed to the incidence of the survival. The X and *II* or the X and *III D* chromosomes, when they are introduced into the *C* cytoplasm, decrease survival ($\chi^2_{6-1} = 254.85$, $P < 0.001$; $\chi^2_{7-1} = 58.97$, $P < 0.001$) instead of increasing it. This is not true for the reciprocal transfers (Table 4, strains 9 and 10). This obvious asymmetry, in combination with the other data of Table 4 discussed before, could be explained if we assume that the major factor(s) controlling the heat sensitivity character is cytoplasmically inherited but that the trait also depends to some extent on the nucleus. That is, the *C* or *D* cytoplasm acts in combination with the nuclear genetic background; if this background has been disrupted, the cytoplasmic effect is also modified.

Selection at either the strain or the population level possibly results in a complex combination of nuclear and cytoplasmic elements which together determine the incidence of the heat sensitivity trait. This view is supported by the data in Figure 4 in which the frequency distribution for survival approaches that of a continuous distribution.

The nature of the survival phenomenon is largely unknown. One can ask

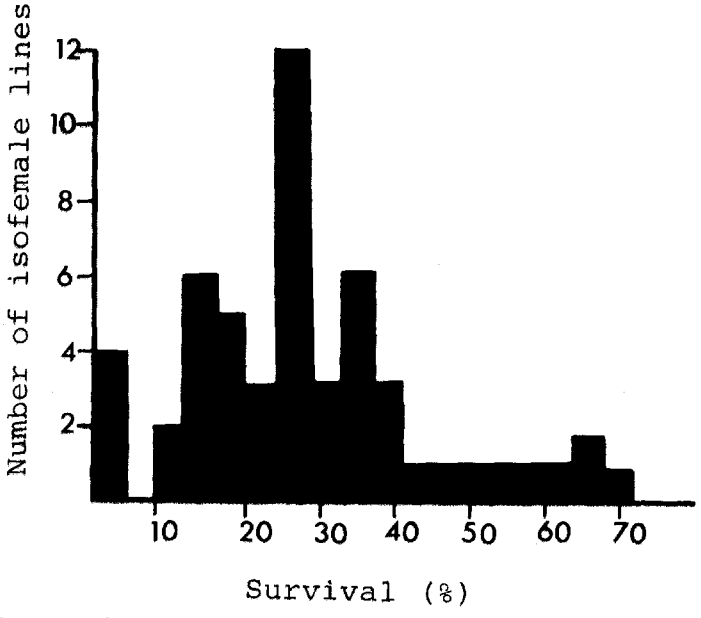


FIGURE 4.—Frequency distribution of survival of 50 isofemale lines (see MATERIALS AND METHODS).

whether or not the differences between the S_1 and R_1 lines are due to the mechanism of stress recognition. If this is true, then the two strains might exhibit different low (thresholds) and upper limits of heat shock response. Figure 5 shows that no difference exists in terms of the thresholds or the upper limits of heat shock of the S_1 and R_1 strains. Their difference in survival is expressed at 40°. These observations indicate that the differential response of the S_1 and R_1 lines to heat shock is not due to the mechanism of stress recognition.

DISCUSSION

The mode of inheritance of the heat sensitivity trait was investigated by utilizing two sensitive strains (S and S_1) derived independently through short-term indirect selection. Moreover, two additional strains, one sensitive (C) and one resistant (D), which were derived by long-term direct selection were used.

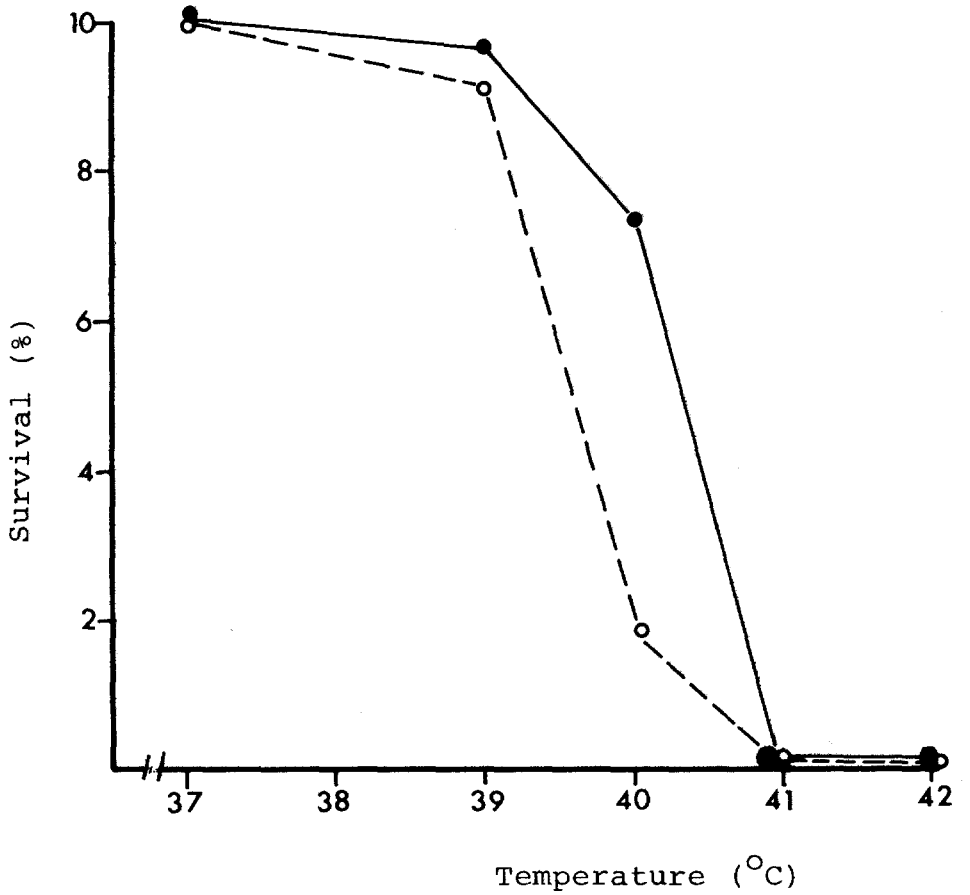


FIGURE 5.—Survival of the S_1 and R_1 strain in different temperatures for 25 min (○- - -○ S_1 strain; ●——● R_1 strain).

Several conclusions can be drawn from our experimental results that have been presented. (1) The character investigated is quantitative and responds to selection (indirect or direct); it also appears to be under complex genetic control. (2) The incidence of the survival rate is chiefly determined by cytoplasmic inheritance but also depends to some extent on the nucleus. (3) The influence of the nucleus at least in the S and S_1 strains, is associated mainly with the second chromosome, which acts in a quantitative manner. However, the contribution of the 2nd chromosome is not clear in another experiment using the strains CCC_{cC} and DDD_{cD}, obtained by direct long-term selection, whose survival rates depend on both the egg cytoplasm and the nucleus. This observation indicates that some variability may exist among the strains investigated in terms of the inheritance of the character under study. In any case, the maternal influence through nucleocytoplasmic regulatory interactions seems to be a major contributory factor for the heat sensitivity (or heat resistance) trait. Hence, we have described here a case of inheritance of heat-sensitivity that differs from that reported by MORRISON and MILKMAN (1978) for strains isolated from flies collected in the United States where the major factor(s) for heat sensitivity is on the 2nd chromosome. With respect to the findings of MORRISON and MILKMAN (1978), we hypothesize that selection may have occurred for a heat-sensitive mutant located on the 2nd chromosome in their study but not in ours.

Furthermore, the nuclear effect does not accumulate through successive generations and thereby does not diminish any initial extrachromosomal maternal inheritance. On one hand, maternal effect(s) could be attributed to the action of an intracellular population of extrachromosomal elements, which would tend to be regularly transmitted through mitosis but would be liable to undergo quantitative or qualitative variations through the action of nuclear genes. If this is the case then one would expect to observe delayed effects of the genotypic substitution. On the other hand, maternal effects, at the molecular level, may be based on the stability of proteins stored during oogenesis or on the existence of a long-lived mRNA that the embryo receives with the egg cytoplasm (GERASIMOVA and SMIRNOVA 1979). Since in our experiments heat sensitivity is maintained at a low level for many generations in flies that have undergone complete chromosome exchange (no delayed effects were observed), we conclude that maternal effect does not account for our observations. This situation strongly supports the view already presented that the differences in heat sensitivity we detected in *D. melanogaster* are to a large extent determined by cytoplasmic factor(s), although this is modified to varying extents by nuclear influences. In conclusion we suggest that the survival rate is determined by a complex combination of cytoplasmic and nuclear elements; if the nuclear background has been disrupted, the cytoplasmic effect can also be modified.

As has been already mentioned, the differential response of the sensitive and resistant stocks is not due to the mechanism of stress recognition. Another mechanism(s) has to be involved, such as the thermolability of protein synthesis. Recent experiments have shown that a relationship exists between the survival of the flies and the pattern of heat shock proteins synthesized (ALAHIOTIS and STEPHANOU 1982; G. STEPHANOU, S. N. ALAHIOTIS, C. CHRISTODOULOU and V. MARMARAS, unpublished data), a fact that may represent a physiological argu-

ment for the adaptive significance of the heat shock proteins. This convergence between survival and the cellular level of heat shock proteins also holds true for other species (e.g., for *Ceratitis capitata*, STEPHANOU *et al.* 1982; for yeasts, MCALISTER and FINKELSTEIN 1980; and for sea urchins, ROCCHERI, DI BERNARDO and GIUDICE 1981). The existence of heat shock protein polymorphisms in natural populations (PETERSEN, MOLLER and MITCHELL 1979) strengthens the significance of these proteins as a contributing factor for the temperature adaptation of Drosophila.

On the other hand, the heat shock phenomenon seems to be related to viral effect(s) such that an integrated or unintegrated viral genome stimulates synthesis of certain proteins when the cell is incapacitated by increased temperature (SCOTT, FOSTEL and PARDUE 1980). Taking into consideration this situation, we could also attribute the differential ability of the flies to survive heat shock to a mechanism that involves a differential viral incorporation and action.

The effect of the cytoplasm in controlling heat sensitivity is apparent in the present study in three independently derived sensitive strains. Many factors could cause such effects. Of particular importance is organelle (mitochondria) inheritance. One avenue of communication between the organelle and the rest of the cell may lie in the regulation of organelle protein synthesis. Another avenue may lie in signals of organelle origin that feed back to the nucleus and regulate the synthesis of particular proteins or even modulate the cell cycle itself (SAGER 1977; BIRKY 1978; TZAGOLOFF, MACINO and SEBALD 1979). Thus, the differential nucleocytoplasmic regulatory interactions which seem to be the major contributing factor for the heat sensitivity and heat resistance could be also attributed to a diversification of the mitochondrial genes. The existence of polymorphic mitochondrial proteins has been shown recently (JEFFREYS and GRAIG 1976).

Taking into consideration the genetic basis for the ability of flies to survive under temperature stress, we can now explain why experiments by us (G. STEPHANOU and S. N. ALAHIOTIS, unpublished data) and others (e.g., SCHENFELD and MCKEHNIE 1979) failed to correlate the survival of *D. melanogaster* (in extreme temperature) with genotypes of enzymic loci. Moreover, we may suggest that the temperature-dependent catalytic differences revealed for the enzymic products of many polymorphic loci (ALAHIOTIS, MILLER and BERGER 1977; ALAHIOTIS and BERGER 1978; ALAHIOTIS 1979a, b; MILLER, PEARCY and BERGER 1975; ALAHIOTIS 1982) can be considered as additive components in determining the incidence of survival. From this point of view, temperature stress appears to be an important component of natural selection in maintaining protein polymorphism.

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