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

1. Breeding temperature and crowding

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Summary. Survival time following a high temperature shock of *Drosophila subobscura* adults in dry air has shown great variability. This experimental condition involved desiccation as the first cause of death. Here survival is studied under saturation humidity, so that the mortality may be imputed only to thermic stress. We analyze the influence of culture temperature and crowding on resistance for different sex and age of the adults. The results show strong influences of these environmental factors on heat shock resistance and show interactions with the age and sex of the adults. We suggest that these facts could be due to acclimatization and/or to adaptation. The acclimatization would occur during development and would affect physiological processes related to aging of the flies. The adaptation would take place for selection, acting through differential mortality before the heat shock. Of course, other processes could be significant. Whatever the causal explanation, it will be necessary in any future research related with heat shock resistance to take these factors into account.

Key words: Heat shock resistance – Breeding temperature – Crowding – *Drosophila subobscura*

Introduction

Temperature and humidity are the most important factors in the environment which influence the physiology (Wigglesworth 1972) and the distribution area (Parsons 1983) of insects. Their effects are constantly interacting and they should always be considered together.

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. In the absence of direct evidence from nature, laboratory studies may provide useful information on the relationship between survival and time of exposure to a high temperature. Parsons (1980, 1983) points out that periods of short-term, high- and low-temperature stress are the most effective in defining climatic races of *Drosophila melanogaster*, and that some concordance has been detected between the stress levels needed to produce a detectable effect, both within and among species, and the biogeographical level (Nix 1981; Parsons 1986).

Early studies on the ability to withstand a high temperature shock in *Drosophila subobscura* adults showed great variability in survival times and a strong influence on these times by different extrinsic and intrinsic factors: culture temperature (Maynard-Smith 1956) and adult age (Hollingsworth and Bowler 1966). The lethal temperature chosen was 33.5° – 34 °C, and the shock was carried out in dry air; in this condition, desiccation becomes the first cause of death. In this paper we observe the ability to withstand a high temperature shock (33° ± 0.5 °C) in *Drosophila subobscura* adults of different sex and age under saturation humidity, so that the mortality may be imputed only to thermic stress. It is evident that the first step is to determine which environmental factors influence the resistance of a population to heat stress. We study the effect of two environmental factors, culture temperature and larval density. It is necessary to discover their influence and try to understand their action if we want to interpret future studies correctly: comparison of population resistance, experiments on selection for resistance or sensitivity to a heat shock, etc.

Materials and methods

The flies used in this study were derived from a population from Tibidabo, Barcelona. This region is characterized by hot summers. The progenies of 12 wild females captured in October, 1980, were used to found a population box with 110 males and 115 females. This was kept at 17° ± 1.5 °C for 6 months prior to the extraction of samples for high temperature treatments and maintained at this temperature throughout the experiment. To obtain flies for experimental purposes, eggs were obtained by introducing a fresh petri dish of ethanol-acetic acid agar medium seeded with live yeast into the population box; the eggs were then transferred to culture bottles which contained 25 cc cornmeal sugar-agar food medium, and were subsequently kept either at 17° ± 1.5 °C or 25° ± 1.5 °C.

Three breeding conditions were studied: (I) 17° ± 1.5 °C and 100 eggs/bottle; (II) 17° ± 1.5 °C and 300 eggs/bottle; (III) 25° ± 1.5 °C and 100 eggs/bottle. Temperature and density conditions (I) can be considered as optimal for the laboratory culture of *Drosophila subobscura* (Nogués 1976). Adults that emerged during the period of greatest emergence were transferred to fresh food bottles on the day of emergence, in groups of 25–30 males or females, and their age was noted. These adults were kept at their developmental temperature until the shock. Their ability to withstand a high temperature shock (33° ± 0.5 °C) at 100% relative humidity was measured as the median lethal dose, LD50 (Finney 1971). The heat shock was performed by introducing the adults into a climatic chamber (50 × 40 × 40 cm) that regulated temperature and humidity at the values required during treatment. Moreover, temperature and relative humidity during treatment were checked by means of a hygro-thermograph.

A sample of 300 adults of each age, sex and condition was tested, using five different exposure times (doses) and two replicates/dose. Depending on age, sex and breeding condition, the differences in resistance were very important and the same doses gave considerable mortality differences. Consequently, a dose of 4 h gave 20% mortality in 2-day-old males but 100% mortality in 24-day-old males at breeding condition I. Thus, doses were changed depending on age, sex and breeding condition from 2 to 6 h at intervals of 30 min. Tested flies were transferred to empty tubes immediately before the shock and were again transferred to a new fresh food bottle after testing and placed in standard conditions (17° ± 1.5 °C) for 24 h; then the percentage of flies that died was calculated. Controls were used to estimate death caused by handling.

The median lethal dose, LD50, has been estimated by calculating the probit regression line, using the maximum likelihood estimation by the iterative method (Finney 1971). Another parameter used is the slope of this line as an estimator of $1/\sigma$ (Finney 1971), where σ is the resistance variability in the population, that is, the variability in the population of the maximum dose that an individual can survive.

ANOVAs have been carried out using the statistical packet "BMDP Statistical Software" (Dixon et al. 1983), in particular 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 and common doses. The correlation coefficients, their significance and the age (days) – resistance (log. LD50) regression lines have been calculated using the P6D program ["Bivariate (Scatter) Plots", Steve Chansen] of this statistical packet. The comparison of slopes of these regression lines has been carried out using a parallelism test (Cuadras 1982) and the comparison of the mean of the weighted percentages, using a test based on arcsine transformation (Sokal and Rohlf 1981).

Results

Table 1 shows the mean of the weighted egg-to-adult viability and the percentage of emerged males for the three conditions studied. With increased crowding (condition II), differences in egg-adult viability are detected, but the sex ratio does not vary. With increased breeding temperature (condition III), the egg-adult viability is very much lower and the frequency of males is significantly lower.

Figure 1 and Table 2 show the LD50 values for males and females raised in each of the three conditions.

Individuals kept at optimal breeding conditions (condition I) show a rapid decline in their ability to withstand this lethal temperature when their age increases ($P < 0.001$). This decline is stronger in males (Table 3, b, I). Males were more resistant than females from 2 to 8 days of adult age ($0.001 < P < 0.01$), for 10 and 12 days there

Table 1. Means of weighted egg-adult viability ($\overline{\%V}$) and emerged males ($\overline{\%♂♂E}$) for the three conditions. N = number of replicates for each condition. NH = total number of eggs at each condition. t = t values for the conditions compared

Condition	N	NH	$\overline{\%V}$	$\overline{\%♂♂E}$
I	13	20,400	77.32	48.74
II	5	15,000	62.85	49.35
III	9	18,700	44.12	46.01

$\overline{\%V}$	Conditions	t
	I–II	24.156***
	I–III	51.384***
$\overline{\%♂♂E}$	Conditions	t
	I–II	0.352
	I–III	4.308***

*** $P < 0.001$

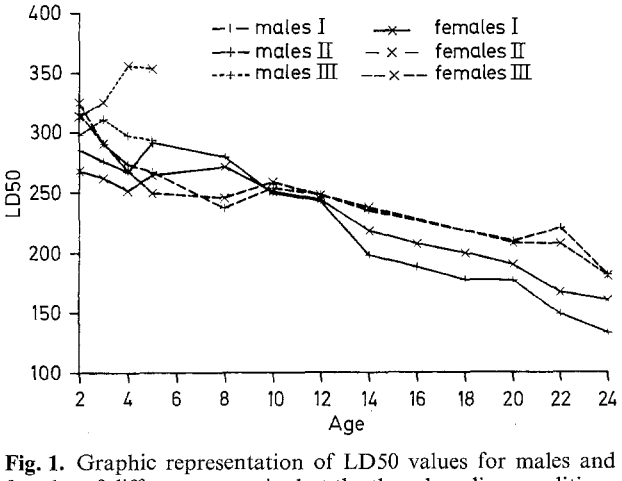

Fig. 1. Graphic representation of LD50 values for males and females of different ages raised at the three breeding conditions (I, II, III)

Table 2. LD50 (in min) for males and females raised at conditions I, II and III. Slope of regression lines (log. dose – probit % mortality) and their error (S±e) are also given

Age (days)	I		II		III	
	LD50	S±e	LD50	S±e	LD50	S±e
♂♂ 2	285.37	13.92±1.60	315.94	10.05±1.81	297.77	7.53±2.25
3	275.93	18.03±1.94	290.64	9.75±1.72	311.08	12.19±1.46
4	267.34	13.50±1.65	273.72	17.41±2.03	297.57	9.85±1.78
5	291.81	10.34±1.43	267.03	12.71±2.71	293.67	7.19±1.27
8	279.85	15.96±1.73	237.64	14.76±2.64		
10	249.79	11.83±3.44	254.22	12.73±1.53		
12	243.08	10.58±2.10	248.45	12.89±1.56		
14	198.23	7.83±1.79	235.32	21.85±2.14		
16	188.34	8.78±2.19				
18	177.61	6.88±1.33				
20	177.29	6.74±1.51	210.59	12.04±2.53		
22	148.55	3.32±1.06	221.48	14.60±2.42		
24	133.02	4.82±0.69	182.38	6.14±1.11		
♀♀ 2	267.83	9.24±1.43	324.70	12.14±3.28	313.39	4.44±1.22
3	262.01	13.24±1.68	290.72	13.39±1.85	325.16	12.60±1.55
4	251.70	11.81±1.67	268.58	19.19±3.95	356.65	8.34±1.97
5	264.71	10.38±1.48	249.67	14.63±1.63	353.93	7.82±1.43
8	271.67	10.29±1.44	246.03	16.04±1.67		
10	251.19	8.96±2.04	259.00	12.55±1.55		
12	244.40	8.07±1.14	248.10	12.27±1.50		
14	218.54	9.62±1.99	238.09	17.75±1.79		
16	207.85	10.99±1.81				
18	200.24	10.51±2.12				
20	190.65	10.18±1.88	208.98	8.34±1.92		
22	167.21	10.38±1.05	208.24	8.79±2.22		
24	160.49	8.83±0.96	181.69	5.69±1.06		

are no significant differences between sexes ($P=0.770$) and after 14 days of age, females survive longer than males ($0.01<P<0.05$).

With increased crowding (condition II), sex differences are reduced or absent for all ages tested ($P=0.245$ from 2 to 14 days and $P=0.940$ from 20 to 24 days) and the heat shock resistance declines less strongly with age (Table 3, b, I–II). After 14 days of age, adults raised at the highest density are more resistant than those raised at the optimal density ($P<0.001$).

Individuals kept at 25°±1.5°C (condition III) as larvae and adults are more resistant to the heat shock than those raised at 17°±1.5°C ($P<0.001$). Up to 5 days of age, females are more resistant than males ($P<0.001$). It was not possible to continue the study for older adults in this condition because they died before the shock.

Slope values of the regression lines showing the relation between log. dose-mortality percentage probit (Table 2) seem to show random variation in females raised at condition I. At this condition, a lower slope is observed for males at older ages (from 14 to 24 days). However, this slope appears greater than that for females at younger ages (from 2 to 12 days). In breeding condition II, slopes show erratic behavior, although the lowest value corresponds to the oldest age in both sexes. The

Table 3. (**a**) Correlation coefficients age (days)-resistance (log.LD50) for males and females raised in breeding conditions I and II (R). P=signification of correlation coefficients. RL=regression lines. N=number of data. (**b**) Comparison of slope of these regression lines

	N	R	P	RL
(a) I				
♂♂	13	−0.964	<0.001	Y=2.5178-0.01485X
♀♀	13	−0.947	<0.001	Y=2.4718-0.00996X
II				
♂♂	11	−0.933	<0.001	Y=0.4807-0.00793X
♀♀	11	−0.933	<0.001	Y=2.4826-0.00834X

(b) I ♂♂–♀♀
Homogeneity of variances: $F=$ 1.47 (11, 11g1)
Parallelism test: $t=$21.26*** (22g1)

II ♂♂–♀♀
Homogeneity of variances: $F=$ 0.90 (9, 9g1)
Parallelism test: $t=$ 2.37* (18g1)

I–II ♂♂–♂♂
Homogeneity of variances: $F=$ 1.58 (11, 9g1)
Parallelism test: $t=$31.06*** (20g1)

♀♀–♀♀
Homogeneity of variances: $F=$ 0.97 (11, 9g1)
Parallelism test: $t=$ 8.33*** (20g1)

* $P<0.05$
*** $P<0.001$

comparison of slope values for conditions I and II reveals that slopes are similar or greater for condition II (exceptions: males of 2 and 3 days and females of 24 days). In breeding condition III, slopes are lower in both sexes and in all the ages than the values obtained in the other two conditions (exception: males of 3 days for conditions II–III).

Discussion

The relationships between resistance, age and sex can be interpreted within the framework of the survival mechanisms of the species when faced by heat stress.

In this species, both sexes are capable of early reproduction about 48 h after emergence. According to Hollingsworth and Bowler (1966), this fact would agree with the decline observed in both sexes with age in the ability to withstand a heat shock of 34 °C in dry air. In this sense, our results for the variation of heat shock resistance (33 °C) with age under saturation humidity do not disagree with their observations. However, our results also show that this decline is not similar in both sexes at optimal breeding conditions (condition I). Male resistance declines more strongly than female resistance so that, although males are more resistant than females for the younger ages, females survive longer than males for the older ages tested (Fig. 1). At the optimal breeding conditions used, it is known that *Drosophila subobscura* females show higher longevity than males (Nogués 1976). It is probable that the greater resistance of the older females is related to their greater longevity. From an adaptive point of view, it is clear that it will be advantageous for the survival of the species that the females keep an important capacity to withstand adverse conditions like high temperature until advanced age. The lower resistance of males of advanced ages is probably due to the more rapid aging that the males of this species suffer. From an adaptive point of view, it is clear that survival of males in advanced age is not essential for the survival of the species. It is more difficult to interpret the greater resistance of young males in comparison with young females.

The difference in resistance between the flies bred in conditions I and II may be due to differential mortality before the shock, due to crowding, or to physiological processes occurring during their development. We have detected differences in egg-adult viability (Table 1) that would support the first possibility. Adults raised at the greater larval density (condition II) show a greater resistance for nearly all the ages tested; this points to the possible importance of the observed differences in the egg-adult viability. However, the crowding in this breeding condition is possibly not severe. Parsons (1986) indicates that phenotypic variability and the manifestation of genotypic variability tend to be high under conditions of severe stress imposed by physical and biological environments, especially for quantitative traits important in determining survival. The greater slope of log. dose-probit % mortality regression lines (Table 2) points to a lower variability for the resistance at condition II which would indicate, according to Parsons, that this breeding condition does not suppose a higher stress. This is also indicated by the lack of variation of sex ratio, although egg-adult viability is significantly lower. On the other hand, the increase in larval density produces a marked decrease in body weight (Sokoloff 1955; Miller 1964). Also (Miller and Thomas 1958) adult longevity may increase when body size decreases due to larval crowding, although perhaps longevity would decrease with severe crowding.

In accordance with these results, adult longevity could tend to become greater in both sexes when larval density increases. This would agree with the greater resistance found for both sexes in adults raised at condition II, which is especially clear over 14 days of age. And it would also explain that the heat shock resistance declines less strongly with age at breeding condition II (Table 3, b, I–II). It is more difficult to explain the equal resistance of both sexes for all ages. It is very likely that both processes indicated and possibly others have some influence, and that their relative importance depends on the larval density condition.

Maynard-Smith (1956) studied the capacity of *Drosophila subobscura* flies to survive at high temperature shock (33.5 °C in dry air) and studied the changes in this capacity according to the temperature at which they had previously been kept (15° or 25 °C). Individuals which had been kept at 25 °C either a larvae or as adults survived for much longer than those kept at 15 °C. We have also found striking changes in the capacity of adults of *Drosophila subobscura* to withstand high temperature shock under saturation humidity, depending on the temperature at which they were raised and kept before the shock (Fig. 1).

These changes may be regarded as acclimatization, in the sense that individuals which have been reared at 25 °C are better able to preserve their life and function when exposed to a high temperature than those reared at 17 °C. However, it is possible that these differences were due to adaptation. Considering the markedly lower egg-adult viability observed (Table 1), the variation of sex ratio (Table 1), and the high mortality of adults kept at 25 °C before the shock, we cannot exclude a selective process leading to adaptive changes. The lower slopes normally obtained at breeding condition III (Table 2), in comparison with the slopes obtained at breeding condition I for the same age, indicate a greater variability for the resistance which, according to Parsons (1986), could be a consequence of the strong stress suffered by the individuals reared at 25 °C. At this temperature, *Drosophila subob-*

scura cannot accomplish its biological cycle and, on the other hand, the significantly lower egg-adult viability confirms the negative effect of high developmental temperature especially in males, as indicated by sex ratio. If the differences observed in the flies reared at 25 °C were only due to selection, we would expect a greater resistance in males than females, which does not occur. It is known that the increase of breeding temperature accelerates developmental time and, consequently, it is likely that it affects aging. This observation could explain the effect observed in the males which have a lower longevity than the females in *Drosophila subobscura*. Of course other processes could be significant.

Whatever the causal explanation, our results show strong interactions of crowding and breeding temperature with age and sex in heat shock resistance. Therefore, these factors should be taken into account in any future research related to heat shock resistance.

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