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STARVATION AND DESICCATION TOLERANCE IN *DROSOPHILA MELANOGASTER* ADULTS: EFFECTS OF ENVIRONMENTAL TEMPERATURE

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Abstract—Adults of a French *Drosophila melanogaster* strain were kept without food in humid and dry conditions and survival time was measured at nine different temperatures ranging from 5 to 31°C. A temperature decrease resulted in an exponential increase of life duration, as would be expected from a slower utilization of reserves. This phenomenon did not extend over the whole temperature range: below a given threshold (around 11–14°C) the exponential increase was not observed and a reduction of life duration occurred at very low temperatures. This result demonstrates some specific, deleterious effects of cold. In the presence of water, starved males always survived longer than starved females. Under desiccating conditions, females survived longer except at very low temperatures, where males survived longer. Survival with water was always much longer than in dry conditions but the ratio of survival time in humid air over survival time in dry air, which is an accurate estimate of tolerance to desiccation, varied according to sex (it was higher in males) and to temperature (it was lowest at middle temperatures). Weight analyses suggested that at medium temperature (25°C), individuals in the presence of water exhausted about 37% of their initial dry body weight before death and died from starvation. Though desiccated flies used only 12% of their reserves before death, the hourly rate of dry weight loss remained the same in both conditions. At 5°C, death occurred in both conditions before the reserves had been thoroughly used.

Key Word Index: *Drosophila*, desiccation, starvation, temperature

INTRODUCTION

A most important factor of the environment is water for any terrestrial animal: numerous structural, functional and behavioral adaptations have been described (Edney, 1977). For example, Arlian and Eckstrand (1975) showed that *D. pseudoobscura* adults could not keep their water content constant unless the relative humidity (R.H.) reached 100%, a condition seldom found in the wild. The water balance is thus maintained by water ingestion and, to a lesser degree, by metabolic water production. Water loss in nature is usually reduced by a behavioral preference for humid environments (Perttunen and Salmi, 1956; Parsons, 1980).

Another much important environmental factor is temperature which is itself linked to the water-balance problem. Since the rate of water loss depends upon the saturation deficit and not upon R.H., the loss for a given R.H. is much higher when temperature increases (Edney, 1977; Parsons, 1980). *Drosophila* species with different ecological niches have a broad range of variability in their tolerances of heat-desiccation stress, as measured by the survival duration of adults in dry conditions (Parsons, 1983). However, the physiological bases of such variation are unknown and many problems remain to be investigated. For example, the relationship between survival and temperature is not known for any species. During starvation in the presence of water, lipidic and non-lipidic reserved are exhausted (David *et al.*, 1975) and are used to produce metabolic water (Arlian and Eckstrand, 1975): some relationship between the amount of reserves used and the survival time under desiccation could be expected.

The present study was undertaken to answer the following questions: what are the thermal response curves of adult survival in humid and dry conditions? Is there a relationship between survival time in desiccating and non-desiccating conditions? What amount of reserves is utilized under these conditions?

MATERIALS AND METHODS

A laboratory strain of *Drosophila melanogaster* originating from Colmar, France, which had been selected for alcohol tolerance, was used. This selection did not decrease the viability and is supposed to have decreased genetic variance, thus providing genetically more homogeneous flies; indeed, the strain is monomorphic for numerous allozyme loci which are polymorphic in French populations. During the selection process, ethanol tolerance increased from 17 to 28% (David and Bocquet, 1977). Then selection was relaxed and ethanol tolerance decreased to its present level of about 20%. Therefore, the tolerance of the Colmar strain used in this study was not very much above that of French natural populations.

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Larvae were grown at 25°C on a killed yeast, high nutrient medium (David and Clavel, 1965) which reduces the effects of crowding. After eclosion, adults were etherized, distributed in groups of ten males or females, and aged 6–7 days at 20°C on maize medium before experimental treatment.

In order to measure survival duration, adult flies were transferred into plastic vials (60 ml) hermetically closed by a plastic cap, without food, For desiccating conditions, two grams of silica gel were put in each vial, under a piece of foam sponge. Since the vials were air-tight, it is assumed that the relative humidity under these conditions was close to zero. As a comparison, other flies were kept in similar vials without silica gel, the piece of foam sponge then being impregnated with 2 ml of water saturated with nipagin, so that the flies could drink. Nipagin was used to prevent any bacterial development. Under these conditions the R.H. was close to 100%. Other experiments (see for instance Van Herrewege and David, 1984) have shown that adults could live in such vial for more than 10 days at 25°C when food was supplied. In our experiments survival time was much shorter so that death occurred from starvation or desiccation but not from anoxia.

Nine temperatures ranging from 5 to 31°C were studied. Above 31°C survival proved to be very short and difficult to measure. Below 5°C, the temperature becomes unfavorable to survival, even for fed flies (Anxolabehere and Périquet, 1970; David, 1988). For each temperature, treatment and sex, three vials of ten adults were tested. The number of dead flies was counted twice a day, at 9 a.m. and 6 p.m., and death was considered to occur at the mid-point between two time measures. For each temperature, at least six independent experiments were carried out at different time intervals, each including (except, in a few cases) both sexes and both (dry/humid) experimental treatments. A three level analysis of variance was done at each temperature: within vial, between vials within experiment, and between experiments.

Dry weight loss was investigated by weighing males after death in dry and humid conditions at 25°C with a torsion balance (precision 0.002 mg). Flies were previously thoroughly desiccated at 70°C in an oven, then compared to a control sample, which had not been experimented upon. Desiccated flies were then washed in ether and weighed again. This procedure allows an accurate estimate of the lipid content (David *et al.*, 1975).

RESULTS

Variability between experiments

As an example, an analysis of variance, made on eight experiments at 25°C, is presented in Table 1. For each treatment the differences between the vials studied in the same experiment are not bigger than expected from the sampling error. In other words, all the flies studied at the same time may be considered as an homogeneous set. On the other hand, differences between successive experiments are always significant. An examination of the mean values failed to show any general tendency over time, as would occur from genetic drift. The most likely interpretation is that the observed variations are due to uncontrolled and unknown fluctuations in the experimental conditions and thus in the physiological stage of the flies. Similar conclusions, not shown, were obtained at other temperatures.

Whatever the origin of these fluctuations, their occurrence leads to a practical result: for statistical analyses, and especially for comparing survival times at different temperatures, the observation unit is the mean value of each experiment, not the value for each fly or the mean value of each vial. This procedure obviously decreases the precision of the overall means but reinforces the conclusion when statistical differences are found. In some cases, and since all the flies studied simultaneously may be considered as an homogeneous set, it will be possible to improve the power of the analyses by comparing associate measures. For example, considering the difference between males and females in each experiment will help to show a consistent variation between sexes.

Variability between individuals and coefficients of variation

Examining the variance between individuals may be a convenient way of appreciating the effects of a given experimental treatment. The overall assumption is that individuals will react more homogeneously when living in optimal conditions while the variance will increase under abnormal, unfavorable conditions (David *et al.*, 1983). Given that in most cases experimental treatments modify the mean value, it is better to consider a relative measure, like the coefficient of variation which is the ratio of the standard deviation to the mean. For each experiment, treatment and sex, the coefficient of variation of survival duration was calculated, generally from the data of 30 flies. These coefficients were then averaged and the values are given in Table 2.

The overall mean is quite high (27.1%). There is no significant difference between experimental (dry or humid) conditions, while a tendency exists for the females to be more variable than males. Considering the effects of temperature, we find that the coefficient of variation is minimum between 8 and 17°C, with an average of 21.7%, and is much higher at high temperatures (between 21 and 31°C, $\bar{m} = 28.8\%$) and also at 5°C.

Table 1. Results of an analysis of variance on adult survival duration in successive experiments at 25°C

	Dry-males			Dry-females			Humid-males			Humid-females		
Source of variation	m.s.	d.f.	*F*	m.s.	d.f.	*F*	m.s.	d.f.	*F*	m.s.	d.f.	*F*
Experiments	157.1	7	11.81*	116.6	7	4.32*	1256.7	7	4.91*	1036.1	6	4.45*
Vials within exp.	6.1	16	0.46	51.8	16	1.92	390.8	15	1.53	283.3	14	1.22
Residual	13.3	196		27.0	204		255.8	194		232.9	183	

m.s.: mean square; d.f.: degrees of freedom; *F*: ratio to residual mean square (significant differences are marked *).

Table 2. Coefficients of variation (in percent) of survival time according to sex, temperature and treatment.

	Dry conditions		Humid conditions		Total	
Temperature	Males	Females	Males	Females	*m*	*n*
5°C	29.8 ± 3.0	43.0 ± 6.7	30.3 ± 5.1	32.8 ± 6.5	34.0 ± 2.8	24
8°C	20.0 ± 2.8	25.2 ± 3.2	20.8 ± 4.3	22.3 ± 3.4	22.1 ± 1.7	24
11°C	17.0 ± 2.3	27.2 ± 2.7	20.2 ± 4.1	25.1 ± 3.4	22.4 ± 1.7	24
14°C	16.5 ± 1.0	18.7 ± 2.6	17.3 ± 2.6	24.5 ± 2.4	19.3 ± 1.2	24
17°C	23.7 ± 2.0	29.8 ± 3.9	31.4 ± 4.5	34.0 ± 3.4	23.0 ± 1.9	26
21°C	20.3 ± 5.1	30.8 ± 3.5	29.8 ± 2.8	29.0 ± 5.0	27.5 ± 2.1	24
25°C	20.5 ± 4.9	30.2 ± 3.7	34.2 ± 2.8	32.0 ± 3.6	29.2 ± 2.1	31
28°C	35.5 ± 8.1	24.5 ± 8.3	25.6 ± 4.1	38.8 ± 7.0	31.1 ± 3.5	24
31°C	26.2 ± 17.2	41.0 ± 18.3	18.8 ± 1.9	22.7 ± 1.6	27.2 ± 6.1	24
mean	23.2 ± 2.2	30.0 ± 2.5	25.8 ± 1.4	29.2 ± 1.5	27.1 ± 1.0	
n	56	56	57	56		225

Survival duration in dry and humid conditions

Experimental results for dry conditions are given in Fig. 1. The shape of the curve suggests an exponential decrease above 11°C. A semi-logarithmic transformation shows a strong linear correlation between 11 and 31°C:

Males: $y = -0.054x + 2.45$;
$r = -0.96$ (d.f. = 43; $P = 0.001$)

residual variance: S2 = 0.011

Females: $y = -0.051x + 2.50$;
$r = -0.95$ (d.f. = 43; $P = 0.001$)

residual variance: S2 = 0.012

(y = log(survival in h); x = temperature in °C)

At lower temperatures a reduction in longevity can be seen. This effect is relatively small in males, but is particularly clear in females.

In humid conditions (Fig. 2), life span is much longer and the curve shows a similar decreasing exponential shape above 8°C. The semi-logarithmic transformation between 8 and 31°C produces the following regressions:

Males: $y = -0.035x + 2.58$;
$r = -0.97$ (d.f. = 50; $P = 0.001$)

residual variance: S2 = 0.0052

Females: $y = -0.034x + 2.51$;
$r = -0.95$ (d.f. = 49; $P = 0.001$)

residual variance: S2 = 0.0069

There is no evidence for an increase in life duration below 8°C in these conditions.

Sex differences

Figures 1 and 2 suggest that the reaction norms of males and females to temperature–starvation and desiccation stresses are not identical. In a dry atmosphere, females live longer than males, except at 5°C (Fig. 3); between 8 and 31°C, the relative difference between survival in females and in males is significantly higher than zero: $t = 4.97$; d.f. = 49; $P < 0.001$.

In the presence of water, however, males survive longer than females, although the difference is not always significant. This is more pronounced at extreme temperatures. Over the whole range of temperatures, the relative difference is significant: $t = -5.47$; d.f. = 55; $P < 0.001$.

Influence of desiccation

Figures 1 and 2 show that survival is always much shorter under desiccating than under humid conditions. Is this difference relatively constant over the whole thermal range? We used the survival ratio in humid and dry conditions as a relative estimate (Fig. 4). The graphs show that the ratio remains low at low and medium temperatures with a slight concave shape of the curve (minimum around 14°C). A clear increase appears at the highest temperatures. Both sexes react in approximately the same way, but there is a major difference with increasing temperature: males become more sensitive than females.

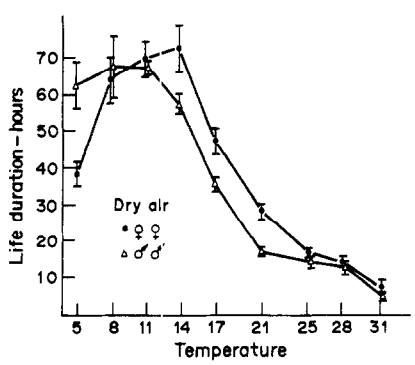

Fig. 1. Influence of temperature on adult survival time under desiccating conditions (relative humidity = 0). Vertical bars show the standard errors.

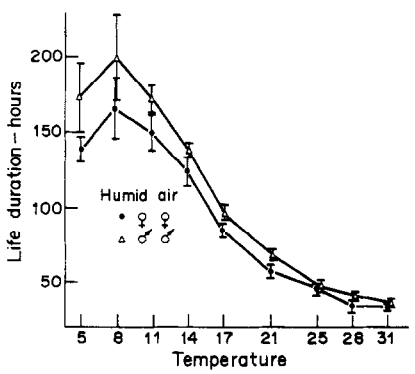

Fig. 2. Influence of temperature on adult survival time in presence of water. Vertical bars show the standard errors.

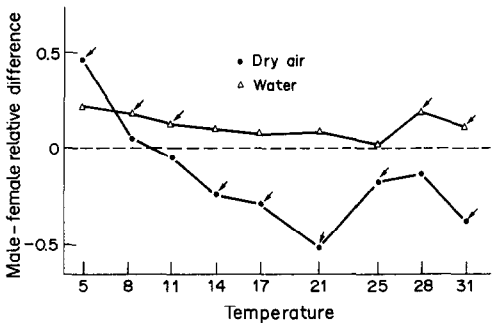

Fig. 3. Analysis of the relative difference between sexes in relation to environmental temperature. △: humid conditions; ●: desiccating conditions; arrows point to values statistically different from zero.

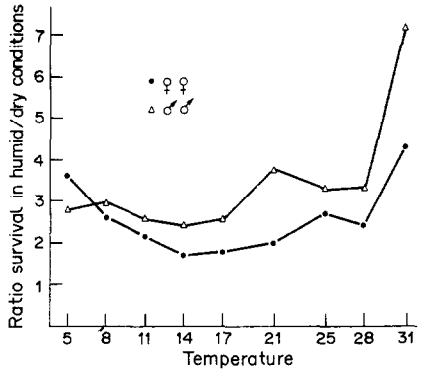

Fig. 4. Influence of temperature on the ratio of survival times in humid and dry conditions.

Dry weight and utilization of reserves

Previous studies have shown that flies, dying of starvation at 25°C in the presence of water, exhibit an important loss of their dry body weight (David *et al.*, 1975). Almost all the lipids of the body are consumed in these conditions and also a large amount of non lipidic reserves.

In order to measure any increase in the consumption of body reserves due to desiccation, males were weighted at 25°C after dying in dry or humid conditions. Similar studies were also carried out at 5°C, in order to investigate the specific, noxious effects of low temperature. The results are presented in Table 3. Males dying from starvation at 25°C (in the presence of water) may be considered as a reference, i.e. they have exhausted all possible reserves before death. Indeed the measures show that almost all the lipids have been used. In dry conditions the flies lost only 12% of their dry body weight, while the control flies lost 37%. One striking result is that the hourly ratio remains the same in both conditions (about 0.8% per h). At 5°C, the hourly ratio is again similar in both dry and humid conditions but it is much lower than at 25°C (0.1% per h). Further, the reserves have not been thoroughly consumed, even in the presence of water (only 25%). These results show the deleterious effect of cold, as previously suggested by the curves shown in Figs 1 and 2. On the other hand, there is no evidence of an increase of the rate of reserve consumption in dry conditions.

DISCUSSION

In a selection of postponed senescence, it has been shown (Service *et al.*, 1985; Service, 1987) that long living strains were more tolerant to various environmental stresses, including starvation, desiccation and ethanol vapors. Since the Colmar strain used in our investigations had been selected for increased ethanol tolerance, we may wonder if this strain is a valid representative of French natural populations. No definite answer can be obtained and further investigations would be needed. We may however point out that, at the time of the experiments, alcohol tolerance in the Colmar strain was not much above that of natural populations (David and Bocquet, 1977). Moreover, tolerance was measured by using an alcoholic solution, while Service *et al.* (1985) found a resistance to alcohol vapors. Finally, an analysis of four different strains selected for increased alcohol tolerance failed to demonstrate any consistent effect upon starvation resistance of these lines (Van Herrewege and David, 1980). So we may suggest that, in our studies, the preliminary selection excerted on the Colmar strain did not alter the physiology of the flies and especially their thermal response.

A first conclusion arising from our observations is that when adults are deprived of food, death does not occur from the same process, whether water is available or not. In the presence of water, death really seems to occur from starvation, at least above 11°C. In this thermal range, we may assume that all possible

Table 3. Analysis of dry weight in controls and in males which died either from starvation (with water) or desiccation

	Total dry weight		Delipidated dry weight		Weight loss		
Treatment	*m*	*n*	*m*	*n*	*m*	*p*	rate
No treatment (controls)	263 ± 3	26	233 ± 3	21			
Starvation, 25°C	166 ± 2	20	163 ± 2	18	97 ± 2	36.9	0.82
Desiccation, 25°C	231 ± 2	29	210 ± 2	23	32 ± 2	12.2	0.86
Starvation, 5°C	212 ± 2	36	—		51 ± 2	19.4	0.11
Desiccation, 5°C	248 ± 3	29	—		15 ± 3	5.7	0.10

m: mean weight in micrograms; *n*: number of flies; *p*: percentage loss as compared to controls; rate of weight loss per h, in percent of controls.

reserves are exhausted before death. Under desiccating conditions, only a part of the reserves disappears and death presumably occurs from water loss. Water loss is known to occur mainly through respiration, and desiccation tolerance seems due to the fact that adult insects control the opening of spiracles (Fairbanks and Burch, 1970; Arlian and Eckstrand, 1975; Edney, 1977). In the present study, we found that in normally-fed adults the water content was 71%, a value in close agreement with previous observations (David *et al.*, 1975). The lethal water content, i.e. the water content when death occurs has not been measured, but we may assume that it is independent of temperature. If this assumption holds, comparing the survival durations in dry and humid conditions gives some idea of the rate of water loss. The dry weight analyses suggest that there is no significant compensation of the water loss by metabolic water. Several authors have found that the rate of water loss was higher in males than in females, as could be expected from the smaller size of males (Kalmus, 1941; Perttunen and Salmi, 1956; Eckstrand and Richardson, 1980; Parsons, 1980). The present study confirms this observation in showing that males are more sensitive to desiccation than females, and also suggests that the rate, which is minimum around 14°C, increases with temperature. This last result would be expected if water loss is due to some evaporation–transpiration process linked to the saturation deficit.

A second major point of this study is the observation of noxious effects of cold below a thermal threshold. In water-fed flies the threshold value is about 11°C, which also corresponds to the developmental zero, i.e. the lowest temperature compatible with development (Cohet *et al.*, 1980). In the presence of food, adult longevity reaches a maximum at about 10°C and sharply decreases at lower temperatures (David, 1988). The nature of these deleterious effects of cold is not known, but we find here that they prevent a convenient use of possible reserves. Under desiccating conditions, these deleterious effects are more pronounced, especially in females: a possible explanation would be that cold impairs the nervous control of spiracles, thus increasing the rate of water loss, but other mechanisms are possible.

A last point is the problem of a thermal optimum. We have two sets of results which relate to homeostasis. First, the minimal relative variability between individuals has been found to lie between 8 and 17°C (Table 2). Second, the minimal rate of water loss, itself related to a low value of the ratio of survival times in humid and dry conditions, was observed to lie between 11 and 17°C (Fig. 4). Although *D. melanogaster* is though to be tropical in origin (Lemeunier *et al.*, 1986), it is now a cosmopolitan species. An adaptation of some physiological features, such as the thermal response, could be expected. The values that we found to be optimal for the French strain used in our experiments correspond to the temperate climate of France. In this respect, a comparison with populations originating in arid or tropical countries would be of great interest.

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