THE SURVIVAL AND CHANGE OF WEIGHT ON SUGAR-WATER MIXTURES OF *DROSOPHILA* MUTANTS AND SPECIES OF DIFFERENT BODY COLOUR

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(With Three Text-figures)

Introduction

It has been shown in a previous paper (Kalmus, 1941 *a*) that the phenotypically yellow offspring of a $\frac{y}{y^+} \times y$ cross in *Drosophila melanogaster*, *simulans*, *subobscura*, and *pseudo-obscura* race A die earlier when starving in dry air than their sibs of wild-type body colour which differ from them in respect of one gene. The yellow flies also lose more weight. In *D. melanogaster* F_2 flies of black or ebony body colour survive longer and lose less weight under these conditions than their wild-type sibs. These differences do not occur in moist air. They are probably due to differences in the water permeability of dark and light cuticles (Fraenkel & Rudall, 1940) resulting in different rates of evaporation.

In a second paper (Kalmus, 1941 *b*) an attempt was made to deduce a number of ecological rules applicable to the life and distribution of insects from these differences, as well as from other physical and chemical differences between light and dark cuticles, the idea being that selection might favour individuals with a dark cuticle in a certain environment, whereas light individuals might thrive equally well or better in other habitats.

Against these deductions one might, however, object that starvation in dry air is a very extreme condition seldom realized for any length of time in the natural life of a species, and therefore a longer survival under these conditions need not necessarily imply any actual selective advantage. The purpose of this paper is to show that a very marked advantage negatively correlated to moisture, of dark mutants and species over their lighter relatives can be demonstrated in experimental conditions having a closer resemblance to natural conditions than starvation, as they provide food and water. Fraenkel (1940) has shown that blowflies which have unlimited access to water and various sorts of sugar offered separately in solid form, may live for many weeks, sometimes much longer than on meat, their natural food. By keeping various mutants and species of *Drosophila* on defined mixtures of sugar and water, differences in survival and change of weight correlated with body colour have been established in the range from pure water to pure sugar. These results are described and discussed below.

Method and Material

The following stocks have been used in the experiments:

D. melanogaster	+ oregon	= wild type
	y^{39e}	= yellow, lighter body colour
	b	= black, darker body colour
	e	= ebony, darker body colour

D. pseudoobscura race A	+	= wild type
	y	= yellow, lighter body colour
D. subobscura	+	= wild type
	y	= yellow, lighter body colour
D. funebris	+	= wild type

D. pseudoobscura and *D. subobscura* are the darkest species, *D. melanogaster* the lightest. *D. funebris* is intermediate in colour, but larger in size.

Drosophila subobscura were mated in vials containing the usual agar-maize-treacle-yeast medium, and transferred to bottles after 1 week. All other species and mutants were cultivated in ½ pt. milk bottles on the same medium. Details of the procedure for obtaining sibs in the F_2 generation differing only in the chromosome which contains the body colour genes are given in an earlier paper (Kalmus, 1941*a*). All flies were collected from bottles at the same time each day, so that the day on which they emerged was known. They were then kept for at least 1 day in bottles containing fresh food.

Two criteria have been used to establish differences in the water metabolism of the flies: (1) the survival on a series of mixtures of cane sugar or other substances with water, and (2) the change, usually the loss, in weight under the same conditions. As mixtures of water and sugar were used, as well as true solutions, all concentrations in this paper are given in weight %.

For the survival experiments batches of 36–40 flies were placed in vials of 35 c.c. capacity stoppered with cotton wool. Each vial contained 3 g. of food-water mixture prepared as follows: Cellucotton was soaked in water or true solution, and some cellucotton was also used in mixtures (cane sugar between 66% and 75%) where a sediment of undissolved solid would have separated from a saturated solution without this precaution. The more concentrated media were prepared by mixing weighed amounts of finely ground powder and water together and pressing the mixture to the bottom of the vials with a glass rod. In the first series an attempt was made to replace the vials at regular intervals with others containing fresh mixtures of the same concentration. However, the loss of flies by the repeated handling appeared more disturbing than the slight inconstancy of conditions due to evaporation and the activity of micro-organisms, which was not very great while the flies were alive. The temperature never fluctuated by more than 2° C. during any experiment. Dead flies were removed at regular intervals and counted according to sex and body colour. For purposes of weighing, fewer flies, usually only four, were taken from vials under the same experimental conditions, to make possible the determination of individual losses in weight. The etherized flies were weighed on a torsion balance showing differences of 0·01 mg.

The survival of y, c and b flies compared with wild-type flies on mixtures of cane sugar and water

Preliminary experiments showed that the relative survival of yellow flies is similar under similar conditions in all three species observed. In *D. melanogaster*, however, it is sometimes difficult to distinguish between wild-type and yellow flies in a dry condition, *D. pseudoobscura* being more suitable for this type of experiment.

Fig. 1 shows the survival times of ten out of twenty wild-type females and ten out of twenty yellow females of this species kept in two vials containing mixtures of water and sugar ranging from 0 to 100%. The survival of starving flies kept in dry air is shown on the right side for comparison. As differences in concentration have a much greater effect on survival at the ends of the scale, more concentrations with high and low sugar content were selected than with medium sugar content. This irregular distribution has been to some extent allowed for in the graph by arranging the distances of the sugar concentrations (c) on the x axis in Fig. 1 according to the formula

$$x = \text{arc sin}\left(\frac{C}{100}\right)^{\frac{1}{2}}.$$

Fig. 1 demonstrates the following facts:

(1) As already shown, the survival time of starving yellow females in dry air is only about $\frac{1}{3}$–$\frac{1}{2}$ of wild-type flies.

(2) The increase of median survival produced by offering starving flies water to drink is considerable for yellow flies and insignificant for wild-type flies.

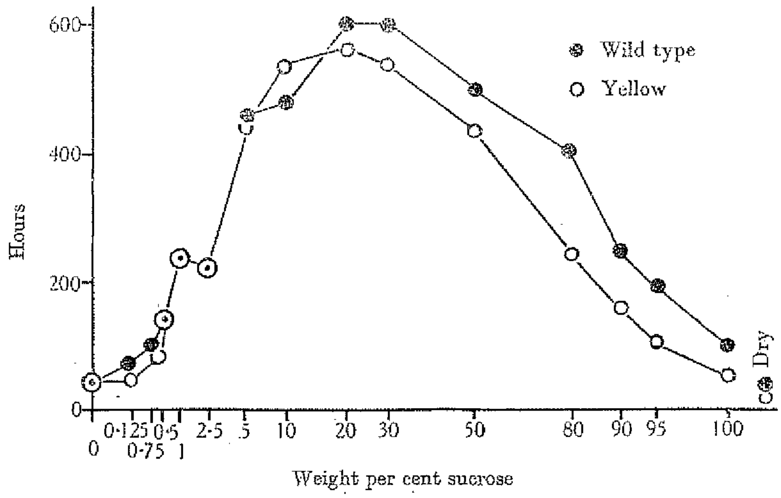

Fig. 1. Survival of ten out of twenty females, offspring from a cross $\frac{y}{y^+} \times y$ of *D. pseudoobscura* race A on mixtures of sucrose and water. Temp. = 18–20° C.

(3) The increase of survival produced by offering flies dry sugar without water is greater than that produced by water alone. It is almost threefold for both wild type and yellow; thus the ratio of survival times is not greatly affected.

(4) The maximum median survival (25 days) occurs at 20–30% sugar in water.

(5) In water-cane sugar solutions containing less than 20% by weight there was no marked difference in the survival of wild-type and yellow flies. At higher concentrations, however, the preponderance of wild-type survivals becomes significant, and increases to nearly three times the value for yellow flies on 100% sugar.

It may be noted that the males in the same experiments showed similar behaviour to the females, except that their survival was somewhat shorter at all concentrations. The longest lived wild-type female in the experiment died after 860 hr. at 10% sugar concentration, the longest lived y female after 834 hr. at the same concentration. Males of both types had a maximum survival of 624 hr. at 30% sugar.

The survival of the phenotypically yellow offspring of crosses $\frac{y}{y^+} \times y$ in *D. melanogaster* and *subobscura* showed differences similar to those of *D. pseudoobscura* at the dry end of the water-sugar mixtures. The phenotypically dark offspring of $\frac{b}{b^+} \times \frac{b}{b^+}$ and $\frac{e}{e^+} \times \frac{e}{e^+}$ crosses in the same species did not, however, show significantly longer survival as compared with the wild type, except in the absence of water and sugar (see Kalmus, 1941*a*). On the contrary, there is a slight preponderance of wild type over ebony at the moist end of the series, although this disappears at the dry end. Clearly the decreased viability of the homozygous *e* or *b* flies does not permit of a clear interpretation of these results. An additional difficulty is that complete separation of the homozygous and heterozygous wild-type flies is impossible in both the mutants, so that there is an unknown amount of increased or decreased viability due to the heterozygous constitution.

The survival of Drosophila melanogaster, funebris *and* pseudoobscura *on sugar-water mixtures*

Differences of survival between mutant and wild-type forms cannot be used directly for a comparison of the adaptation of different species. The greater susceptibility of the light 'yellow' mutants to drought could, for example, be interpreted as being due to a disturbed balance between the gene for yellow and the rest of the genes adapted for the wild-type allelomorph of yellow. If some correlation were established between drought resistance and colour differences of different species of a genus, this would support the hypothesis that light cuticle decreases drought resistance.

6 ♂ and 6 ♀ *D. melanogaster* (or +), *funebris* and *pseudoobscura* (race A) were put together into vials containing mixtures of known water-cane sugar concentration. Two such series of twenty concentrations each were set up containing altogether 1440 flies all of the same age (2–4 days) bred under similar conditions and kept together in fresh bottles for 24 hr. before the experiment started.

For the sake of comparison with previous results, the data for females only are given in Fig. 2, though the males showed essentially similar behaviour. Owing to the lower temperature (15° C.) the absolute maximum of survival of *pseudoobscura* wild type seems to be increased when compared with corresponding values in Fig. 1 (at 18–20° C.); also the maximum survival concentration was slightly shifted from 30% sugar at 18–20° C. to 15% at 15° C. Survival at the dry end of the series, however, appeared to be longer at the high temperature (see p. 202). The longest time that a fly survived was 1128 hr. for a male and female *pseudoobscura* and 1104 hr. for a male and two females in *melanogaster*. The longest survival for a female in *funebris* was also 1104, and for a male 1056. Further, one saw that whereas *pseudoobscura* had the longest of all the median survival times in the whole range of mixtures (at 15 % cane sugar), it was only superior in survival power on mixtures containing more than this percentage of sugar. On mixtures containing more water *pseudoobscura* is significantly shorter lived. The light *melanogaster* has its survival maximum at 5% sugar, where it lives significantly longest; perhaps it can best concentrate the sugar at this level. *D. funebris*, a decidedly larger species, but intermediate in colour, has a lower survival maximum at the same concentration. On mixtures containing less than ½% sugar *funebris* has significantly the longest survival, probably due to its greater stocks of reserve material. The other differences in survival support the thesis that a dark cuticle gives better protection from drought than a light one.

The survival on mixtures of other sugars and water, and on other substances

Some experiments were performed to determine whether there are differences in survival on other sugars and on non-sugars.

In accordance with Fraenkel's (1940) results, glucose proved of somewhat less nutritive value than sucrose. Differences of survival at the dry end of some of the glucose-water mixtures were nevertheless established, the light flies dying first. The same applies to mannitol, a sugar alcohol. The longest survival times at a temperature of 18–20° C. were

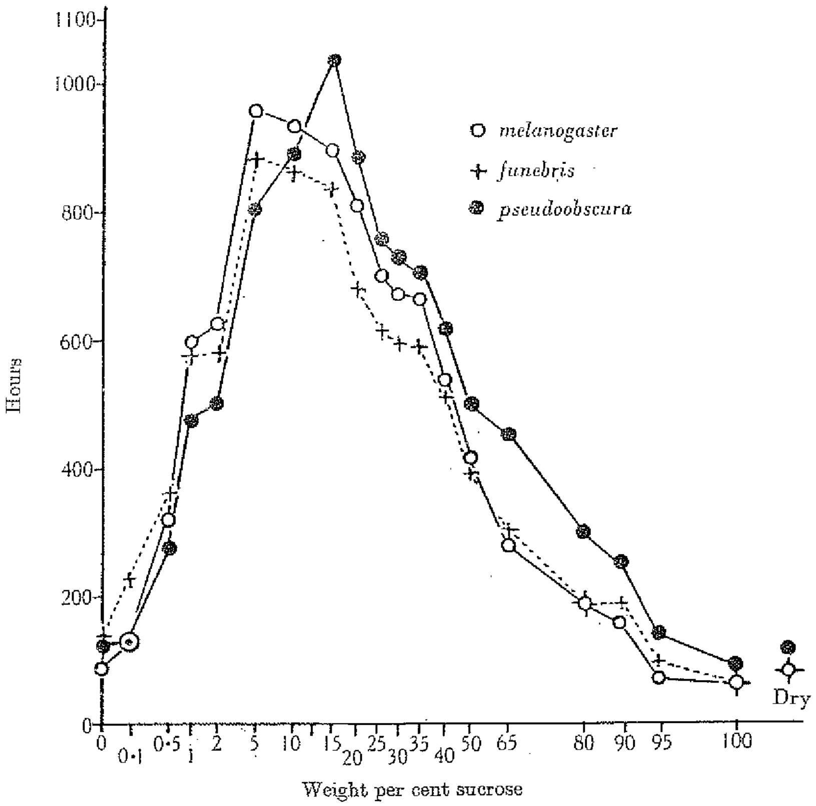

Fig. 2. Survival of six out of twelve females on *D. melanogaster or* +, *D. pseudoobscura* race A and *D. funebris* on mixtures of sucrose and water. Temp. = 15° C.

observed in a series of mixtures of sorbitol, another sugar alcohol, with water. The data for females are given in Fig. 3.

As in the cane-sugar experiments, a difference in drought resistance between the yellow and wild-type forms can be seen at the dry end of the series. The maximum median survival times, however, are longer—820 hr. for +females and 796 hr. for *y* females as compared with 600 and 500 hr. on sucrose at the same temperature. The maximum also appears localized at lower concentrations of sorbitol than of sucrose, in accordance with its greater nutritive value. A closer comparison of results obtained with sorbitol and cane sugar is unprofitable owing to the lower molecular weight of sorbitol. It should be noted that a 10% solution of sorbitol has about the same osmotic pressure as a 20% solution of sucrose.

Attempts were made to discover differences on mixtures of water with salts (cooking salt, Rochelle salt) and urea. However, the flies died very early and were obviously starved and poisoned. On mixtures containing $\frac{3}{4}$ NaCl and $\frac{1}{4}$ cane sugar + 5–20 % water the dark wild-type offspring of crosses from $\frac{y}{y^+} \times y$ in *D. pseudoobscura* survived significantly longer than their yellow sibs.

The change of weight of y and wild-type flies of D. pseudoobscura *race* A *on two mixtures of cane sugar and water*

Table 1 summarizes the results of experiments with flies from a segregating F_2 generation a $\frac{y}{y^+} \times y$ cross kept on mixtures of 20 % cane sugar with 80 % water and 90 % cane

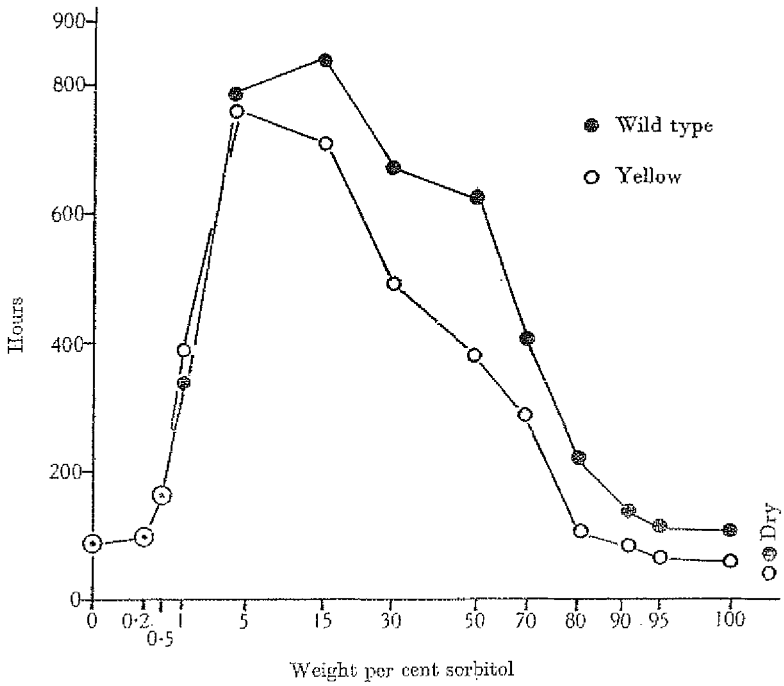

Fig. 3. Survival of ten out of twenty females, offspring from a cross $\frac{y}{y^+} \times y$ of *D. pseudoobscura* race A on mixtures of sorbitol and water. Temp. = 18–20° C.

sugar with 10 % water. The flies were 3–5 days old and had been kept on fresh food for 2 days before the experiment. On the sugar-water mixtures flies were grouped in ten batches (vials), five for each concentration, each vial containing two flies of each sex and body colour (eight to each vial).

Table 1 shows a very gradual loss of weight on the 20 % sugar solution and a much more marked one on the 90 % mixture. Furthermore, there is scarcely any difference between wild-type and yellow flies at the former concentration, but quite a noticeable one at the latter.

Table 1

(All weights in mg.)

Medium	No., sex and phenotype	Av. original* wt.	Av. wt. of survivors after 1 day	% of original wt.	Av. wt. of survivors after 2 days	% of original wt.	Av. wt. of survivors after 3 days	% of original wt.	Av. wt. of survivors after 10 days	% of original wt.
20% weight sugar	10 + ♂	1·32 ± 0·01	—	—	1·15 ± 0·02	87	1·26 ± 0·01	96	1·05 ± 0·03	79
	10*y* ♂	1·29 ± 0·02	—	—	1·10 ± 0·02	85	1·23 ± 0·05	96	1·08 ± 0·01	83
	10 + ♀	1·33 ± 0·02	—	—	1·21 ± 0·03	91	1·28 ± 0·01	96	1·12 ± 0·01	84
	10*y* ♀	1·36 ± 0·01	—	—	1·24 ± 0·01	92	1·33 ± 0·02	98	1·19 ± 0·01	88
90% weight sugar	10 + ♂	1·06 ± 0·01	0·95 ± 0·03	89	—	—	0·84 ± 0·05	79	—	—
	10*y* ♂	1·04 ± 0·01	0·92 ± 0·01	89	—	—	0·63 ± 0·06	62	—	—
	10 + ♀	1·24 ± 0·02	1·08 ± 0·02	87	—	—	0·82 ± 0·06	81	—	—
	10*y* ♀	1·13 ± 0·01	0·94 ± 0·03	85	—	—	0·64 ± 0·05	64	—	—

* Taken 24 hr. after the flies had been put into the vials

The significance of the differences after 4 days between surviving pairs of flies from the same sibs of the same sex but different body colour was calculated as follows:

Table 2. *Differences in loss of weight after 4 days*

On 20% sugar between *y* and + ♂♂ = + 0·025 ± 0·37 mg.
,, ,, ,, ♀♀ = − 0·017 ± 0·036 mg.
On 90% sugar between *y* and + ♂♂ = + 0·168 ± 0·042 mg.
,, ,, ,, ♀♀ = + 0·261 ± 0·055 mg.

Applying the *t* test the first two differences do not appear to be significant, but the second two are slightly so. Similar results were obtained with *melanogaster* and *subobscura.*

Changes in weight of D. melanogaster *and* D. pseudoobscura *on two mixtures of cane sugar and water*

It is not profitable to compare water loss in forms which differ very markedly in size. In Table 3, therefore, *funebris*, a large form, was excluded, and only *melanogaster or* + and *pseudoobscura* race A were used. The latter is somewhat the larger. 3–4-day-old flies had been kept on fresh food for 1 day, the temperature being 20° C. Two males and two females of both species were put in vials containing mixtures of cane sugar and water. Five vials were set up with 20% sugar, five with 85%. The change in weight was determined after 3 days, when seventy-six out of eighty flies were alive. Results for the survivors were as follows:

Table 3

	20% cane sugar mg.	85% cane sugar mg.
D. melanogaster or + ♂♂	Gain 0·109 ± 0·034	Loss 0·184 ± 0·048
D. pseudoobscura race A ♂♂	Gain 0·165 ± 0·058	Gain 0·22 ± 0·078
D. melanogaster or + ♀♀	Gain 0·154 ± 0·047	Loss 0·119 ± 0·043
D. pseudoobscura race A ♀♀	Gain 0·127 ± 0·048	Gain 0·077 ± 0·045

Of the 20% mixtures all flies gained in weight. The results of the *t* test are as follows: *D. pseudoobscura* ♀♀ gained insignificantly less than *D. melanogaster* ♀♀, but there is a slightly higher gain for *pseudoobscura* males. The 85% *melanogaster* flies of both sexes lost in weight, whereas *pseudoobscura* flies gained. The differences appear significant in both sexes.

DISCUSSION

An insect dies from starvation if offered water only. If starving in the absence of water it may die from starvation, desiccation, or their combined action. Previous experiments have shown that in *Drosophila*, yellow mutants die earlier when starving under dry conditions than the wild type (Kalmus, 1941*a*). The presence of water increases the survival of the yellow form in *D. pseudoobscura* to that of the wild type, which is not materially altered under these circumstances. One can therefore infer that when fasting in dry conditions the former die mainly from desiccation, the latter from starvation. Wild-type flies of *D. melanogaster* and *D. funebris* also die mainly from starvation under these conditions.

Before discussing the results of the experiments on survival and change in weight on water-sugar mixtures, the conditions in respect of the water available in the experimental environments must be discussed.

By increasing the sugar percentage in the tubes several factors affecting the water metabolism of the flies are altered simultaneously:

(1) Relative humidity in the air decreases from theoretically 100% in tubes containing water to about 88% in tubes containing mixtures of 63% to almost 100% cane sugar (at 20–25° C.). Saturation deficiency increases in the same range. As the vials were kept in air of about 60–70% relative humidity, the actual humidities were always less, but the differences were about the same. There was a slight decrease in humidity during the experiments.

(2) The proportion of water in the food decreases.

(3) The percentage of sugar from which metabolic water may be produced increases.

(4) Viscosity increases with concentration, and at the higher concentration most of the food is present as crystals. This makes ingestion more difficult.

Factors (1) and (2) act in the same direction, as often happens under natural conditions when proceeding from a wet habitat to a dry one. Factor (3), acting in the opposite direction, can never fully counterbalance (2), as sugar cannot produce its own weight of water. (4) is less important. Fraenkel's (1940) experiments show that flies can ingest enough dry sugar to maintain their energy requirements provided drinking water is offered separately. But they cannot produce enough metabolic water from sugar in the absence of drinking water to compensate their loss of water by excretion, defaecation and evaporation. Consequently in our experiments survival time of dry-kept flies was only moderately increased by offering them dry sugar. Alterations in the length of the survival time depended on the conditions of the flies before the experiment and the constitution of the sugar given. There were no indications that the digestive tract was overloaded. Death at the optimum concentration can be regarded as due to the combined action of starvation and desiccation. If neither were operative we should expect a flat optimal region to our curves, indicating a range over which conditions were quite satisfactory. As this is not found we must suppose that both are operative even at the optimum. The importance of desiccation appears from the fact that with the more nutritive sorbitol it is still active at 15% solutions. Forms where the optimum occurs nearer the dry end of the concentration gradient appear to be better adapted to drought. In accordance with a previously postulated ecological rule, 'Insects exposed to drought are dark' (Kalmus, 1941*b*, rule 12), the wild type of *D. pseudoobscura*, which is black, has its survival maximum at a higher sugar concentration than its lighter 'yellow' mutant or the lighter wild types of *melanogaster* and *funebris*. In the range between this optimum and pure sugar *pseudoobscura* wild type always survives significantly longer than the other forms. On the moist side this behaviour is inverted and there may be a range where the pale forms are at an advantage, perhaps due to their greater power of concentrating sugar; this would be in accordance with rule 13 of the same paper, which states that 'Insects which ingest abundant liquid food are frequently pale'. Rule 9 states that 'long-lived insects are darker than their short-lived relatives', and this seems to be borne out by the fact that the black wild type of *D. pseudoobscura* has the longest survival on its optimal mixture of all the forms observed.

If changes in weight after 3 days are taken as a criterion for resistance to drought, conditions on a mixture of 85% cane sugar with 15% water are such as to enable the dark *D. pseudoobscura* to gain weight, whereas the light *melanogaster* loses weight.

In accordance with Fraenkel's (1940) results, sorbitol was the most efficient of the substances tested in maintaining the life of *D. pseudoobscura*. This accords with the fact that the maximum survival occurs at lower concentrations than on cane-sugar mixtures. The differential behaviour between wild type and yellow therefore becomes manifest over a wider range.

A comparison between the behaviour of *D. pseudoobscura* wild type at 15 and 18–20° C. (Figs. 1, 2) reveals the following relationships. Flies show a maximum median survival of 1040 hr. on 15% sugar at 15° C. and of 600 hr. on mixtures between 20 and 30% sugar at 18–20° C. Survival on mixtures containing more than half the weight of sugar is slightly longer at the higher temperature, but on mixtures containing less sugar the flies live considerably longer at the lower temperature. A possible explanation is as follows: The loss of water by evaporation from the insects is largely controlled by the saturation deficiency (Johnson, 1942) which changes only slightly with temperature in air in contact with a particular sugar solution. On the other hand, the production of metabolic water compensating for this loss is probably considerably increased with temperature. Therefore one may expect that flies would survive longer at higher temperatures in the range of mixtures where the chief cause of death is desiccation. One might further infer that an insect can afford to lose more water in a hot climate than in a dry one. Our results show that light insects are less protected from desiccation than dark ones, and one might see in the longer survival at higher temperatures some proof of rule 11 that 'most non-black insects are found in the tropics. Their related forms become darker as one approaches the poles.' The correlation between colour of drosophilids and latitude in America (Sturtevant, 1921; Dobzhansky, 1937) and in Japan (Kikkawa & Peng, 1938) is in agreement with this rule. In Europe the darker *D. funebris* is reported to live in regions farther north than the lighter *D. melanogaster* (Timoféeff-Ressovsky, 1933).

Summary of principal results

1. Mixtures of sugar and water constitute suitable artificial environments for flies analogous to natural habitats ranging from those with scarce to those with abundant water.

2. Of all the flies investigated *Drosophila pseudoobscura* race A wild type, a black form, showed the longest absolute survival time. The maximum median survival periods of the females in the different experimental series were:

1040 hr. on 15 % cane sugar at 15° C.
600 hr. on 20–30 % cane sugar at 18–20° C.
840 hr. on 15 % sorbitol at 18–20° C.

3. Their mutant yellow sibs survived significantly shorter on mixtures containing more sugar, but had the same survival time, or perhaps even a slightly longer one, on some of the less concentrated mixtures. The maximum median survival periods of the yellow females in this series were as follows:

560 hr. on 20 % cane sugar at 18–20° C.
760 hr. on 5 % sorbitol at 18–20° C.

4. Both forms lost little water in 4 days when kept together on 20 % cane sugar and there was no significant difference in the losses. On 90 % cane sugar, however, the average losses were much greater, the yellow forms losing significantly more weight.

5. Similar differences mentioned in (2)–(4) exist between the wild type and the yellow mutants in *D. melanogaster* and *D. subobscura*. The darker mutants 'black' and 'ebony' in *D. melanogaster* die slightly earlier than the wild type on all mixtures of sugar and water. This difference seems to be diminished in ebony at the dry end of the series.

6. Wild-type flies of the light *melanogaster* and the medium coloured *funebris* survived for a shorter period on the mixtures rich in sugar and longer on the mixtures rich in water. The maximum median survival periods were:

Female *melanogaster* 960 hr. on 5 % cane sugar at 15° C.
Female *funebris* 860 hr. on 5 % cane sugar at 15° C.

7. On mixtures containing 20 % cane sugar *D. melanogaster* and *D. funebris* may lose or gain weight during 3 days and there is little difference between the two. On mixtures of 85 % cane sugar and water, however, the light *melanogaster* loses weight and the dark *pseudoobscura* gains weight in the 3-day period.

8. On mixtures containing more than half the weight of sugar *D. pseudoobscura* survives longer at 18–20° C. than at 15° C., on mixtures containing more water this behaviour is reversed.

9. These results are discussed in conjunction with some previously formulated rules which dealt with the correlation between cuticle colour and some graded ecological factors such as drought, abundance of drinking water, longevity, temperature (geographical latitude).

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