EXPERIMENTS ON TOLERATION OF TEMPERATURE BY DROSOPHILA.

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In connection with experiments by one of us on the effect of high temperature on the amount of crossing over in *Drosophila* it became obvious that differences exist among various cultures of this fly in the ability to withstand temperatures above the normal range and in the ability to breed at these temperatures. A series of comparative tests was therefore undertaken in order to gain more accurate information along these lines.

Experiments on a single culture of *Drosophila melanogaster*, grown on sterile yeast medium, were reported by Northrop[1] in 1920. He wished to find out whether the offspring of cultures kept at high temperatures acquired the ability to tolerate temperatures still higher. He found that no such adaptation occurred, and that flies could be carried at temperatures above 29°C. for one generation only. These flies would however breed normally according to his findings if returned to normal temperatures for a period of 24 hours.

Since Northrop's work was upon a single stock of *Drosophila* only, it seemed of interest to us to determine whether there was not a difference in the upper temperature limit among different cultures of *Drosophila*, which might be genetically determined.

Our work indicates that with optimum food conditions it is only the exceptional stock of *Drosophila melanogaster* which will not breed at 31°C. Furthermore our work suggests the basis for the failure to breed at high temperatures as observed by Northrop and by ourselves.

[1] Northrop, J. H., *J. Gen. Physiol.*, 1919–20, ii, 313.

Our tests were carried on in electrically controlled bacteriological incubators. While by no means as constant as the apparatus used by Northrop, these were sufficiently so for all practical purposes. Checks were run on certain of the experiments in a water bath used in the Department of Chemistry which varied less than 0.1°C., with identical results in each case. The control incubator was carried at approximately 24°C. Most of the high temperature tests were made at 31°C. Approximate records of the limits of variation of these incubators were kept with Taylor maximum and minimum thermometers, and these showed variations of less than 1° on either side. In the case of the high temperature tests, Northrop[1] and Plough[2] have indicated that 29°C. was the critical temperature, which our experiment confirms, and our present material was constantly above this point in the high temperature work.

The medium used for the cultures was the usual banana agar, made up in fairly large quantities, placed in bottles, and kept in a refrigerator until used. A pinch of powdered magic yeast was dropped in each bottle before the paper and the flies were introduced. This medium, if carefully made and used within 2 or 3 days, gives far larger numbers of flies than the sterile solution of Northrop. The cultures are practically uniform and the conditions closer to those in the wild state.

EXPERIMENTAL.

Our first tests were made with a number of different cultures of *Drosophila melanogaster* collected from various widely separated regions. Following this series we tested a number of mutant stocks and wild stocks of several other species of *Drosophila*. Table I gives the results of these tests and also the source of the stocks tested. In general these tests were made with two bottles of each stock containing about three pairs of flies apiece. Whenever a test culture failed to develop, however, it was repeated at least once, so that each negative result represents at least four bottles tried.

Geographically Different Races of Drosophila melanogaster.—The data given in the table show in general that wild stocks of *Drosophila*

[2] Plough, H. H., *J. Exp. Zool.*, 1917–18, xxiv, 147.

melanogaster can be bred for indefinite periods at a temperature of 31°C. on banana agar. The stocks from Amherst, Georgia, and Sweden were carried for eight, ten, and eleven generations before they were lost and it is very probable that more careful methods, with a larger number of separate cultures would make it possible to carry them indefinitely. At 31°C. the flies begin to hatch within 8 days. They are smaller than under optimum conditions and somewhat less viable. All of these conditions appear to be due to the hastening of development with the result that the larvæ take in less food. Up to 31°C. there is no evidence that the stocks cited show any effects which make breeding beyond the second generation impossible as Northrop states. In the case of the Devil's Lake stock no difficulty was experienced in carrying the flies for fourteen generations and the stock was only discontinued at that point because we moved to Woods Hole where incubators were not available.

An interesting point in connection with these stocks has to do with the geographical origins. It was thought possible that stocks from southern or tropical climates might show greater resistance to heat than those from farther north. That this idea has no basis in fact is obvious. Stocks from Sweden and North Dakota go as well at 31°C. as those from Georgia and Florida.

In the case of the stock collected at Attleboro, the results follow those found by Northrop with his stock. Three distinct trials failed to give any offspring beyond the first generation. In addition the stock has been tested at least as many times more in another series of tests with identical results. This stock is as viable as any of the others at normal temperatures. It differs only in that it has been kept in culture since the summer of 1919 and has therefore been subjected to a considerably higher degree of inbreeding. This has apparently resulted in the isolation of a type genetically different from the wild stocks commonly collected.

Mutant Stocks.—With respect to the various mutant stocks the tests show a uniform failure to tolerate a temperature of 31°C. for more than one generation. This is more or less expected in view of the fact that most of the *Drosophila* mutants appear to be less viable than the wild stocks. This is very striking in the case of the so called II–III stock (a combination of three second chromosome

TABLE I.

Summary of Tests of Drosophila Stocks at 31° C.

Stock, source, and year collected.	No. of tests.	Result in F_1.	Result in F_2.	No. of generations carried at 31°C.
D. melanogaster				
Normal wild stocks from:				
Amherst, Mass. (H. H. Plough, 1922).	1	Normal.	Same.	8 (died).
Georgia (J. Krafka, 1922).	1	"	"	10 (").
Sweden (A.H. Sturtevant, 1922).	2	"	"	(a) 11 ("). (b) 4 (discontinued).
Florida (M. Grossman, 1922).	1	"	"	3 (").
Devil's Lake, N. D. (C. Thompson, 1920).	3	"	"	(a) 3 (died). (b) 7 ("). (c) 14 (discontinued).
Attleboro, Mass. (H. H. Plough, 1919).	3	"	(a) No offspring. (b) ± 75 " (c) ±100 "	1 (died). 2 ("). 2 (").
Mutant Stocks:				
eosin-miniature.	2	Normal.	(a) 10–15 flies. (b) No offspring.	2 (died). 1 (").
black-purple-curved.	2	(a) ±20 flies. (b) Normal.	" " " "	1 ("). 1 (").
II–III stock (black-purple-curved-hairy-scarlet-spineless).	2	(a) No offspring. (b) ±50 flies.	" "	Died F_1. 1 (died).
xple stock (scute-echinus-cut-vermilion-garnet-forked).	2	(a) ±50 flies. (b) ±20 "	" " " "	1 ("). 1 (").

vermilion-garnet-forked.	10	Normal.	No offspring	1 (died).
alternated III $\left(\frac{\text{ru D pe e}^s}{\text{h st ss}} \times \text{h st ss}\right)$.	2	"	(a) " " (b) ±10 "	1 ("). 2 (").
III-ple (ru h D st pe ss e^s).	1	±30 flies.	No "	1 (").
D. simulans				
Woods Hole, Mass. (A. H. Sturtevant, 1922).	1	Normal.	±10 offspring.	2 (died).
Alabama (A. H. Sturtevant, 1922).	1	± 40 flies.	No "	1 (").
Scarlet eye (C. W. Metz, 1921).	2	No offspring.		Died F_1.
D. virilis (C. W. Metz, 1921).	2	Normal.	(a) Normal. (b) ±15 flies.	3 (discontinued). 2 (died).
D. funebris Amherst, Mass. (H. H. Plough, 1921).	2	(a) ±10 flies. (b) No offspring.	No offspring.	1 ("). Died F_1.
Norway (A. H. Sturtevant, 1919).	1	" "		" "
D. immigrans (A. H. Sturtevant, 1921).	2	" "		" "
Chymomyza procnemis (C. W. Metz, 1921).	2	Normal.	(a) ±25 flies. (b) ±50 "	2 (discontinued). 4 (").

mutant genes with three in the third). This stock is very weak and rather difficult to keep going at normal temperatures. It is the only mutant stock from which an F_2 generation could not be secured at 31°C. Two of the stocks gave a few offspring in the F_2 generation but these flies would not breed. In the case of the mutant types the failure to breed seems to be simply a result of the weakened condition. The physiological difference is not specific but it is nevertheless genetic in the sense that it is one of the effects of most of the mutant genes. This point is emphasized further by the results of crossing these stocks as described below.

Different Species.—The tests of wild stocks of certain other species indicate that some are able to breed continuously at 31°C., while some, like the *D. melanogaster* mutants, will not tolerate this temperature. It is interesting to compare the geographical range of these species with the results of these tests. *D. funebris* and *D. immigrans,* according to Sturtevant,[3] are northern forms and not commonly found in tropical climates. These species show the most marked inability to breed at 31°C., usually giving no offspring even in the F_1 generation. On the other hand, *D. virilis* and *Chymomyza procnemis,* which are found in the tropics, can apparently be bred at 31°C. as well as the stocks of *D. melanogaster.* It is curious that *D. simulans,* which Sturtevant has shown to be so similar to *melanogaster,* and to parallel its range, cannot be bred at 31°C., at least as far as the available stocks are concerned. These data indicate that species differ genetically in their ability to tolerate high temperature, and that this is of some importance in determining the normal range of the species.

Experiments at Higher and Lower Temperatures, Indefinitely and for Short Periods.—A number of tests indicate that 31°C. is the highest temperature at which any of the *Drosophila* cultures can be bred. Most of the stocks which will go at 31°C. were tried at 32 and 33°C., and while a few gave offspring for one generation, these could never be carried further. At these temperatures the bacterial growth in the food is very active, the agar usually becomes partly liquified, and the bottle goes bad. However, the fact that flies do, now and then,

[3] Sturtevant, A. H., North American species of *Drosophila, Carnegie Inst. Washington, Pub. 301,* 1921.

come through, indicates that this is not the cause of the failure of the culture.

We have confirmed Northrop's finding that there is no evidence of hereditary adaptation to high temperature. Stocks which will breed indefinitely at 31°C. cannot be bred at 33°C. even after six to eight generations of continuous exposure to the lower temperature.

At a temperature of 29.5°C. most of the cultures will breed with difficulty. In several tests the black-purple-curved stock and the eosin-miniature stock failed after the first generation. On the other hand the vermilion-garnet-forked stock was carried for four generations at this point without difficulty, before being discontinued. The wild Attleboro stock was also carried at this temperature for the same period, but the number of offspring was small and difficulty was experienced in keeping it going. The wild *D. funebris* and *D. immigrans* stocks which showed marked inability to tolerate 31°C. gave larvæ and pupæ at 29.5°C., but no imagos hatched out. This temperature therefore appears to be close to the critical temperature for stocks which will not go at the higher point.

Northrop reported that his stock of *D. melanogaster* could be carried indefinitely at 30°C. if the cultures were brought back to the optimum temperature for a period of 24 hours or more during each generation. We find that the stocks which will not go at 31°C. will recover from the effects of exposure to it for one generation, but the period at lower temperature necessary for recovery is longer. Most of the mutant stocks will breed again at 31°C. if returned to 24°C. for about 4 or 5 days. The vermilion-garnet-forked stock gave a small number of offspring in the second generation at 31°C. when the F_1 flies were returned to 24°C. for 72 hours. On the basis of such observations Northrop concluded that the eggs of the flies kept at high temperatures were injured, but that normal eggs would be produced if the parents were returned to normal temperatures and these normal eggs would develop at high temperature. No proof of this idea was given and our crosses of flies exposed to high temperatures with control flies shows it is by no means an adequate explanation.

Extraction and Crosses.—Since the inability to breed at 31°C. seems to be an inherited character, a number of crosses were made between stocks which would go at 31°C. and those which would not, in order to determine the behavior of the gene or genes which are responsible for it. Two such cases where mutant characters are involved may be cited. The Sweden wild stock was crossed to the black-purple-curved, and the Georgia stock to the sex multiple. In both cases the normal heterozygous stock would breed indefinitely at 31°C. like the wild parent, but extracted flies, showing any of the mutant characters, failed to breed at this point although they went perfectly well at the control temperature of 24°C. Some twenty-five tests of this sort, with three pairs in each bottle were made in an attempt to discover whether the reaction to temperature was due to a separate gene which would segregate from the mutant genes. The negative result indicated that the inability to withstand high temperature is not separable from the mutant genes and is simply one of the physiological effects of the presence of the mutant genes themselves. As suggested above, these characters all produce a weakening, which, if for no other reason, would probably prevent their survival in a tropical climate.

With regard to crosses of the single wild *D. melanogaster* stock which refused to breed at 31°C., the Attleboro stock, our evidence is still inconclusive. This stock crossed to Amherst wild stock gave normal offspring at 24°C. and the hybrid stock will breed indefinitely. The same is true of crosses made to any of the mutant types at 24°C. When the Attleboro by Amherst cross is made at 31°C., normal F_1 flies hatch. Six bottles of these F_1 flies with two pairs each failed to give any offspring at 31°C. although many larvæ appeared in the bottles. The same cross at 24°C. went without difficulty. While further confirmatory tests are necessary, this result suggests that a dominant gene or genes in the Attleboro stock produce failure to tolerate a temperature of 31°C.

Evidence of the Effect of High Temperature on the Germ Cells from Crosses.—The nature of the effect of the high temperature on those flies which will not breed is an interesting question. Normal flies are to be expected for one generation since the germ cells which form the F_1 are matured at the normal temperature before the exposure. In cultures of the F_1 flies hatched at 31°C. and continued at that temperature, copulation takes place and a large number of eggs are laid. In all cultures at high temperatures more eggs are laid on the glass above the food than in cultures at normal temperatures. In spite of their normal appearance, practically none of these eggs give rise to larvæ. The few which do give rise to larvæ and imagos, whether of the Attleboro or any mutant stock, can never be carried any further.

We have examined sections of the gonads of flies newly hatched at 31°C., and except for their small size, they are similar to the gonads of normal flies of the same age. Both mature eggs and sperm are present.

We then tried crossing males and females from susceptible stocks kept at 31°C. for one generation to females and males hatched at 24°C., placing the bottles at the former temperature. If the germ cells of either were injured then no offspring could be expected, just as in the case where the F_1 flies were mated in mass cultures. The results are shown in the following table. The mutants being sex-linked, all classes hatched out in the F_2 generation; the heterozygous flies could be bred for an F_3 generation but those having mutant characters would not go.

TABLE II.

Crosses Made at 31°C.

F_1 flies hatched at 31°C.		F_1 flies hatched at 24°C.	Total No. of bottles started.	No. from which F_2 hatched.
vermilion-garnet-forked ♀	×	Amherst wild ♂	7	7
vgf ♂	×	" " ♀	7	5
" ♀	×	Attleboro wild ♂	1	1
" ♂	×	" " ♀	1	1
" ♀	×	vgf ♂	5	3
" ♂	×	" ♀	3	0
		F_1 flies hatched at 31°C.		
		(From non-susceptible stock.)		
vgf ♀	×	Devil's Lake wild ♂	2	2
		(From susceptible stock.)		
" ♀ and ♂	×	vgf ♂ and ♀	23	0

These data show clearly that while susceptible stocks will not go for more than one generation at 31°C. when inbred, both males and females will produce normal offspring at this temperature when mated to other flies which have hatched at a lower temperature or to flies which breed normally at 31°C. Clearly, then, neither the eggs nor the sperm are injured by the exposure to 31°C. since they perform their function normally enough in crosses. The reason for the failure to produce offspring after the first generation seems to lie either in a failure of the sperm to reach the eggs—that is unsuccessful copulation—or in the failure of the fertilization process itself. This problem is now under investigation and we cannot as yet answer the question. As noted above F_1 flies hatched from these heat-sensitive stocks copulate normally as far as outward appearances indicate at 31°C., so that it would appear that fertilization was not successful. It has not been determined whether sperm are actually present, however, in the seminal receptacles of the females.

SUMMARY.

1. Most wild stocks of *Drosophila melanogaster* can be bred indefinitely on banana agar at a temperature of 31°C. There is no relation between the geographical origin of these stocks and their ability to tolerate this temperature.

2. A single wild stock has been found which will breed for only one generation at temperatures above 29°C. The offspring hatched at 31°C. will breed normally at 24°C. This difference from other wild stocks is apparently genetic, but its genetic basis has not yet been worked out.

3. The mutant stocks of *D. melanogaster* tested by us will breed for only one generation at 31°C. and their offspring at this temperature are also fertile at 24°C. This condition is apparently a physiological effect of the presence of any of the mutant genes in a homozygous condition.

4. Similar tests indicate that wild stocks of *D. virilis* and *Chymomyza procnemis* will breed at 31°C., while *D. simulans*, *D. immigrans*, and *D. funebris* will not. The last two species are northern forms not commonly found in the tropics.

5. Both male and female flies from mutant stocks hatched at 31°C. produce offspring at this temperature if mated to flies hatched at 24°C. Their germ cells are therefore capable of development, and the cause of their failure to develop at 31°C. when inbred must lie either in the failure of the germ cells to reach each other or in the fertilization process itself.