THE EFFECT OF TEMPERATURE ON A MOTTLED-EYE[1] STOCK OF DROSOPHILA MELANOGASTER

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Received January 8, 1935

The following paper presents data on the effect of temperature on a mottled-eyed fly, a mutation appearing in a progeny of flies which had been subjected to supersonic radiations in an experiment carried out by HERSH, KARRER and LOOMIS (1930). The original stock was slightly changed by the insertion of sex-linked vermilion by W. P. SPENCER of THE COLLEGE OF WOOSTER. Such was the status of the stock at the beginning of this experiment. Mottled-eyed flies have been reported on rather numerous occasions (MULLER 1928, WEINSTEIN 1928, GLASS 1933, PATTERSON and PAINTER 1931, and GOWEN and GAY 1933). These are apparently quite different genetically.

The accompanying illustrations were prepared by Mr. EMMET MALEY, student at BALDWIN-WALLACE COLLEGE, Berea, Ohio, 1934. They more accurately reveal the nature of the mottled character than could description. These illustrations show samples of the possible variations from nearly complete black pigmentation in figure 2 (A) to a slight shadowing in figure 2 (D). Not only is there variation in the magnitude of the effect but also a considerable variation in the color and compactness of the darkened area. If only a small area is darkened, it is predominantly the dorsal posterior margin of the eye that is affected. This fact may in some way be in accord with the anterior-posterior and dorsal-ventral gradient of development.

The flies used in the experiment were carefully selected for the mottled character from the stock banana-agar bottles at WESTERN RESERVE UNIVERSITY. They had been started at 25°C primarily for increased egg production and then placed at fluctuating room temperature for development. The selected flies were placed in 6×2 bottles on banana media and held at 18°C, 20°C, 22°C, 24°C, 25°C and 29°C. The 24°C and 25°C temperatures were maintained with commercial incubators with a fluctuation of slightly less than a degree. The 18°C, 20°C, 22°C and 29°C temperatures were maintained by laboratory-constructed incubators which were controlled by toluene mercury tube thermostats. These were accurate to .5°C. An underground, unheated chamber was used in the experiment to maintain temperatures below 24°C. During the experiment all temperatures

[1] I wish to express appreciation to Professor A. H. HERSH of the Biology Department of WESTERN RESERVE UNIVERSITY for his constant and helpful advice during the course of the experimental work.

were maintained within the above limits except the 18°C temperature which fluctuated on three occasions approximately two degrees.

The families of flies containing more than fifty progeny, thus produced, were carefully examined. The flies incubated at 25°C and 29°C showed no mottling although some showed a slight suppressed shadowing of the eye as illustrated in figure 2 (D). Such flies with apparent shadowing of the eyes were not considered mottled in this experiment.

The successive increases in temperature decreased the percentage of mottled individuals. This is exemplified by the data in table 1. In addition to determining the percentage of individuals mottled the percentage of the total area of the eye affected was carefully determined. This was done by killing the flies with ether, thus causing them to extend their wings and legs. This treatment facilitated a more constant orientation of the

TABLE 1

The relation of temperature to the percentage of individuals showing the mottled effect in a population.

°C	TOTAL NUMBER EXAMINED	MOTTLED	NON-MOTTLED	PERCENT
18	487	483	4*	99.1
20	346	346	0	100
22	673	335	338	49.9
24	350	144	206	41.1
25	417	1*	416	.23
29	about 200	0	about 200	0

* The data show 4 non-mottled flies at 18° C and 1 mottled fly at 25° C. These are irregularities and may have been due to an error on the part of the author or to impurity in the line as hundreds of flies have since been produced at each of the experimental temperatures without reoccurrence.

flies in balsam. After the flies were thus carefully mounted on their left sides on microscope slides, the right eye was examined with a compound microscope. By the aid of a camera lucida, fifty eyes of fifty flies produced at each of the experimental temperatures were carefully traced on squared paper. This was made possible by the construction of a special adjustible stage lamp for the differentiation of color with the camera. From these data it was possible to determine accurately the percentage of the total

TABLE 2

The relation of temperature to the average area of the eye affected.

°C	NUMBER MEASURED	AREA (PERCENT)
18	50	83.6
20	50	77
22	50	38.1
24	50	18.6

area of the eye affected by the dark pigmentation. The main source of error in thus computing the percentage of area affected is in the orientation of the fly on the slide. Such error is probably small and does not overshadow the observable relationships. The findings are recorded in table 2.

A graphical representation of the relationship of temperature to mottling is illustrated in figure 1.

In the graph (a) represents the relation of the percentage of flies in a population showing the mottled effect to the temperature, while (b) represents the relation of the total area of the eye affected to temperature.

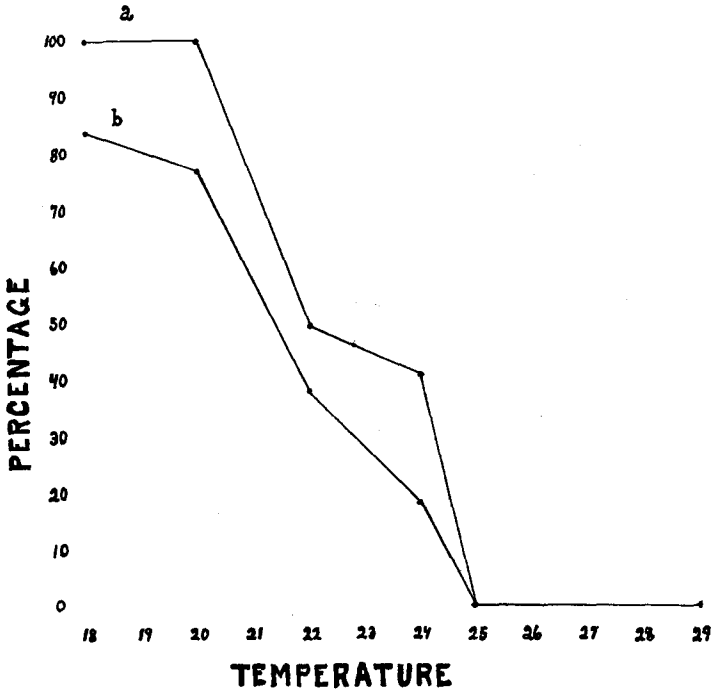

FIGURE 1.—(a). Graphic relation of temperature to the percentage mottled in a population. (b) Graphic relation of temperature to the percentage of the total area of the eye affected.

The four illustrations in figure 2 show typical mottled effects as produced (A) at 18°C, (B) at 22°C, (C) at 24°C, and (D) at 25°C. The mottled effect is distinctly variable for each of the experimental temperatures.

In the course of previous experimentation it seemed apparent that the time during development at which the mottled effect was produced was in the interval from pupation to emergence. This hypothesis was verified by the following procedure. Approximately six large families of mottled flies were started in order to produce large numbers of developing flies simultaneously. The six test-bottles were kept at 20°C. The original adults were removed at the beginning of pupation. The existing pupae

were also removed that day and once every twenty-four hours for the next successive six days. The pupae after being carefully removed from the test bottles were placed in test tubes which contained a wet piece of filter paper and which were plugged with cotton. Approximately fifty pupae were placed in each tube. The first tube of pupae was placed im-

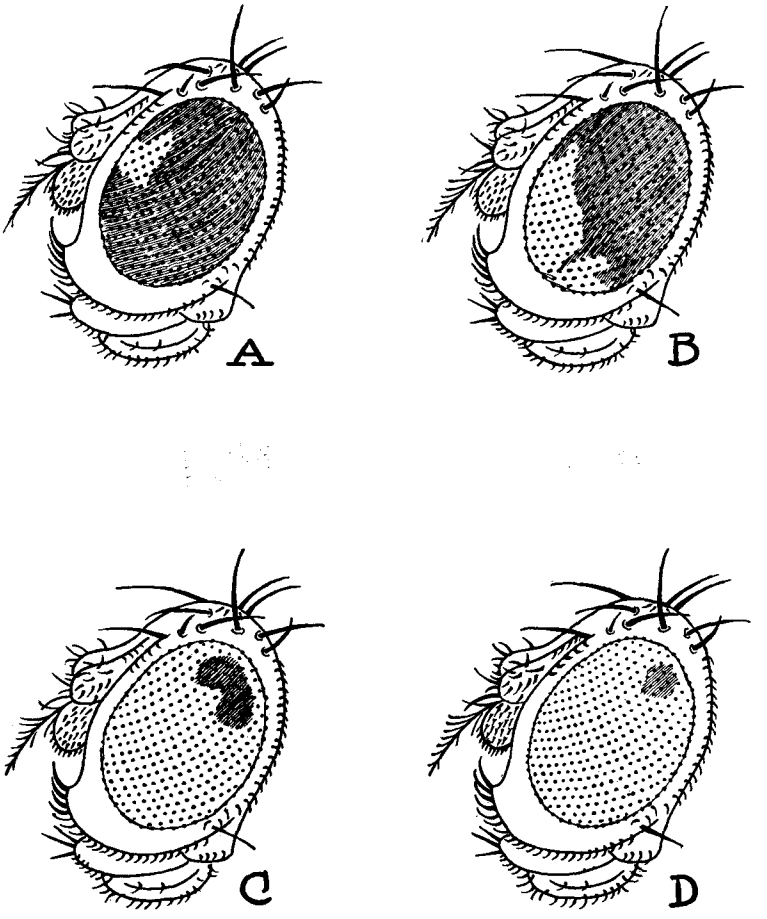

FIGURE 2.—Typically illustrates the variable appearance of the mottled eye at 18°C, 22°C, 24°C and 25°C.

mediately at 27°C and kept there until the flies emerged four days later. Each successive tube of pupae was kept at 20°C one day longer than the preceding tube and placed at 27°C until the flies emerged. As the time was lengthened at the lower temperature, the interval to the time of emergence increased. The number of flies showing the mottled effect increased until all the adult flies were mottled, and the maximum interval from pupation to emergence was ten days at 20°C. Table 3 shows the results of the various tubes of pupae.

TABLE 3

The effect of temperature change during the time interval from pupation to emergence on the mottled character.

TUBE NO.	DAYS AT 20°C	TIME TO EMERGENCE AT 27°C	NUMBER NON-MOTTLED	NUMBER MOTTLED
1	0	4 days	45	0
2	1	5	48	1
3	2	6	31	20
4	3	6	19	22
5	4	7	0	40
6	5	7	0	51
7	6	8	0	39
8	7	8–9	0	56
9	8	8–10	0	48

From table 3 it is evident that if the pupae remain at 27°C until emergence, even though pupation occurred at 20°C, the flies will be non-mottled. Thus the effective period is during the pupa stage. It is also evident from the table that if they remain approximately one-half of the time interval from pupation to emergence at 20°C, they are not affected by later temperature changes.

The previous procedure was then reversed. The larvae were allowed to pupate at 27°C and then carefully removed every twenty-four hours. The first tube of pupae was placed immediately at 20°C and successive tubes kept one, two, three, and four days at 27°C before being removed to 20°C where they were permitted to emerge. Table 4 shows the result of the versed procedure.

TABLE 4

The effect of temperature change during the time interval from pupation to emergence on the mottled character.

TUBE NO.	DAYS AT 27°C	TIME TO EMERGENCE AT 20°C	NUMBER NON-MOTTLED	NUMBER MOTTLED
1	0	8 days	0	51
2	1	6	20	17
3	2	5	57	0
4	3	5	54	0
5	4	4	22	0

Thus if the pupae remain at 20°C until emergence, even though pupation occurred at 27°C, the flies will be mottled. It is again evident that if they remain approximately one-half of the time interval during the pupa stage at 27°C, they are not affected by later temperature changes. It may thus be said that the effective period for mottled-eye is during the first half of the time interval from pupation to emergence.

SUMMARY

1. The mottled-eye character is affected by temperature.

2. The percentage of individuals showing the effect in a population increases with a decrease of temperature over a range of 27°C to 18°C.

3. The area of the eye affected increases with a decrease of temperature over a range of 24°C to 18°C.

4. The mottled effect is erased at incubation temperatures of 24°C or above.

5. The effective period is during the first half of the time interval from pupation to emergence.

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