Inherited Behavior in Drosophila

J. P. SCOTT, Wabash College

Introduction

It is a scientific truism that simple phenomena yield to analysis more readily than complex ones, and since the day of Fabre it has been a common impression that the behavior of insects, while sometimes forming an intricate pattern, is little affected by variations of the environment. Therefore, the observation of Dr. H. H. Strandskov that the stock of brown-eyed *Drosophila melanogaster* at the University of Chicago reacted more vigorously to light than did the wild appeared to offer fruitful ground for genetic experiment.

Materials and Methods

Three methods of measuring the response of an animal to a given stimulus suggest themselves: (1) the threshold value of the stimulus required to produce a reaction, (2) the strength of an opposing stimulus sufficient to overcome the effects of the stimulus studied, and (3) the speed of reaction to the given stimulus. The first method was abandoned because of the difficulty of exactly controlling the secondary stimulus (vibration) involved in the reaction of Drosophila to light. The second method would make the situation even more complicated, and the third has a disadvantage in that it is practically impossible, in a small, active animal like the fruit fly, to measure separately reaction time (time between the stimulus and reaction) and the speed of the reaction (time required to move a certain distance). However, the last method, including both reaction time and speed of reaction in a single measurement, was worked out to a point where consistent results were obtained and was adopted.

The apparatus designed (Fig. 1) was essentially a closed glass tube cut into three lengths of 6.1 cm., 18.3 cm., and 6.1 cm. The ends were made of two pieces of the same microscope slide, and the cut edges ground smooth. The different sections of the tube could be cut off from each other by an opaque barrier composed of two cover-slips cemented

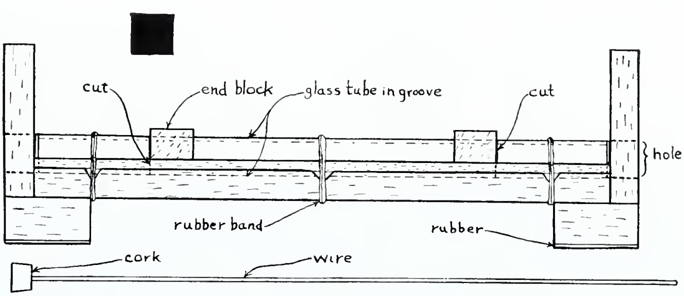

Fig. 1. Barrier, wooden rack carrying glass tube, and plunger. x ¼. The end blocks allow the short sections of the tube to be rolled out of line without loss of flies. The entire wood surface is painted black. The rack is one-third as wide as it is long.

to thin black paper. The tube was fastened horizontally upon a rack, and, since it had only 1.6 cm. internal diameter, little room was present in which the flies could react to gravity. A beam of light from an enclosed 40-watt Mazda electric bulb was shone directly down the tube, the illumination at the joints being approximately 6 and 16 candle power. Examination showed that the light was reflected evenly from the inner surface of the tube with little interference from the joints. All tests were made in a dark room, so that very little light entered the sides of the tube.

McEwen (1917) found that the reactions of females were interfered with by the number of eggs carried, and that the maximum phototropic response was developed at 3-5 days. Therefore, males only were used, raised at a temperature of 24-25°C. on cornmeal-agar-molasses medium and removed daily at 4 p. m. from a fresh stock bottle. These were fed on dilute medium which had been allowed to go to vinegar and which keeps indefinitely. On the fourth day at 4 p. m. the flies were shaken out into the testing tube and 19 out of the 25 or 30 present were selected at random and pushed with a plunger into one of the small sections of the tube. This was tightly stoppered with cotton and placed in the dark for three hours.

During the test an attempt was made to avoid all jarring involved in handling the rack and lifting the opaque barrier which replaced the cotton plug. The tube containing the flies was placed on the lighted end of the rack and the whole left in that position for two minutes. The rack was then reversed and entirely covered for three minutes. The cover was removed, the barrier lifted, and the time at which the tenth fly reached the second cut in the tube recorded with a stop watch. All flies were then pushed into the lighted end and the procedure repeated for a total of 10 trials. An air temperature of 24-25°C. was maintained throughout the test.

All of the stocks used had been inbred over a period of years by reason of the small number used to start each new stock bottle.

The wild stock, which is normal, shows the same pigment in the eyes throughout adult life. These flies emerge from the pupa case with grey bodies, the black pigment being at first concentrated in a spot in the abdomen. The body becomes progressively darker with age, finally becoming yellow with black bands.

On the other hand, brown flies emerge from the pupa case with light brown eyes, which rapidly darken to a cherry red. The body pigment at emergence is darker than that of the wild, and the black markings are always more intense. Brown flies are smaller in size and more constantly active under stock-bottle conditions and, in this particular stock, at least, appear to lay their eggs somewhat later after mating, with the result that there are more sterile matings than in the wild stock.

White is epistatic over brown, and, with an eye to future genetic experiments, the white stock was also tested. This type entirely lacks red pigment in the eyes. As in the wild, yellow body pigment is absent at emergence but appears gradually with age in both eyes and body. It probably does not reach the full depth of color seen in the wild stock. Development is slower than with the wild, but the stock lays its eggs promptly after mating so that sterile matings are practically unknown. Under favorable conditions size may exceed that of the wild, but under unfavorable conditions it may be more greatly reduced. White flies are decidedly less active than the wild. Both brown and white soon disappear in competition with wild in stock bottles.

Analysis of the Response

When highly stimulated by vibration the flies respond to the light by almost immediately starting toward it in a body, crawling in straight lines. When less highly stimulated the flies wait some time after exposure to the light before crawling toward it and may crawl only a short distance. The effects of a vibratory stimulus last for several minutes.

Tests of the white, brown, and wild stocks were made, with the results shown in Table I. The brown stock differs from both the wild and white in mean and standard deviation. Wild and white differ in the mean, but not significantly in standard deviation. The brown stock is therefore faster and less variable than the other two.

Table I. Time of Phototropic Response of Flies Subjected to Minimum Vibration

Stock	No. Trials	Mean	Standard Deviation
Wild	30	17.89±0.59	3.25±0.42
Brown	39	14.49±0.23	1.46±0.17
White	30	21.23±0.76	4.18±0.54

As Carpenter (1905) originally showed, the phototropic response of Drosophila takes place only when the flies are mechanically agitated. Under the conditions of the test, the flies are agitated at the begining by the placing of the tube upon the rack. While in the lighted end the flies constantly collide with one another, and they are jarred slightly by the reversal of the rack and friction of the barrier with the cut edges of the tube. All other sources of vibration were eliminated so far as possible. From the variable behavior of the wild and white stocks it appeared that this amount of vibration was somewhere near the minimal amount necessary to obtain a response in these groups. The brown stock, on the other hand, appeared to be highly stimulated at all times.

It was then supposed that the essential difference between the brown stock and the others was a difference in susceptibility to vibration. A small amount of additional vibration was produced by vibrating a ruler attached to the table on which the rack was resting, just before the barrier was lifted. The results are shown in Table II. With the exception of one lot of brown flies which was lost, the same flies as in Table I were used.

The mean time of the brown stock is significantly reduced by this amount of vibration, but the others are little affected if at all. It can therefore be concluded that the brown stock is more susceptible to vibration than the wild or white, since the latter two stocks may be made to respond as rapidly as the brown by sufficient shaking. It is obvious that the difference is hereditary, but it has not been demonstrated that the difference is actually produced by the *bw* gene.

TABLE II. TIME OF PHOTOTROPIC RESPONSE OF FLIES SUBJECTED TO REGULAR JARRING

Stock	No. Trials	Mean	Standard Deviation
Wild	30	18.23 ± 0.58	3.18 ± 0.41
Brown	30	12.59 ± 0.24	1.34 ± 0.17
White	30	20.09 ± 0.64	3.53 ± 0.46

On the other hand, there is no indication of difference in susceptibility to vibration between the white and wild. The foundation for the difference appears to be simply the more sluggish movements of the white flies. Cole (1922) was unable to get any consistent response of white flies to changes in intensity of light, but this factor is not involved in the present experiment.

The susceptibility of Drosophila to small amounts of vibration is amazing. Passing trucks on the street and a small power saw in the lower floor of the building in which the first experiments were made were sufficient to produce unrepeatable results. The flies were found to be responsive to small bits of lint left in the tube, which had to be kept perfectly clean, and to be markedly disturbed by variations in temperature of as little as half a degree. Below 22° C. the brown stock would sometimes react more slowly than the wild. Freshly fermented food fed the adults produced sluggish behavior, and the conditions under which the larvae were reared also produced variation, probably through alteration in size. In order to get repeatable results environmental conditions must be rigorously controlled.

McEwen (1917), although keeping only a very rough control over environmental and accessory hereditary factors, concluded that age, sex, the size of the wings, and specific genes affect the response to light. Cole (1922) reported that the photic response (in the vertical direction) was made faster by an increase in illumination. Summarizing the available evidence, it may be said that the "phototropic" response of Drosophila is affected by several different hereditary (internal) factors and by the following important environmental factors: light, vibration, temperature, and food.

Discussion

The amount of variation in the data given is accounted for by accidental variation of the environmental factors mentioned above and by errors of measurement introduced by the operator. Payne (1911) used a technique which approaches fairly closely the one described here. Adjusted for different length of tube, his figures show an average time of about 13.5 seconds for wild flies. The faster time can probably be accounted for by the increased jarring involved in his technique. In any case, the test described in this paper gives the result expected under the mechanistic theory of behavior, a reaction predictable in terms of probability under a controlled set of conditions.

However, the idea that environmental factors play only a small part in determining the behavior of insects is obviously incorrect in this case. Under natural conditions, with all the controlling factors varying and largely independent of each other, an enormous variety of reactions to light can be observed.

This being so, the use of the term "tropism" becomes questionable. Loeb's (1918) idea that the energy derived from a stimulus helps to move an animal toward or away from that stimulus (producing a "forced movement" or tropism) was discredited on the grounds of oversimplicity by Jennings (1923), who replaced it with the theory that with regard to injurious and beneficial stimuli an animal tends to come to rest in the most favorable position, though not necessarily responding directly to the stimuli. That is, tropisms are adaptive behavior. The adaptive value of the "phototropic" reaction of Drosophila has been pointed out by Mast (1911) to be a means of escaping engulfment in rotten fruit. Jennings further concludes that all behavior is more or less adaptive, and that the term "tropism" is therefore meaningless.

Still, superficial observation will discover many cases of behavior in the lower organisms in which an animal apparently responds positively or negatively to a single stimulus. Given the proper conditions, a fruit fly will hasten directly toward a source of light, paying no attention to other stimuli. On analysis, this behavior is found in a situation where one stimulus, that of vibration, is so intense as to overcome the effects of all other stimuli.

This sort of behavior is not confined to the lowly organized animals. A man might be said to exhibit negative thermotropism in the face of a forest fire, or to be negatively phototropic at a temperature of 100°F. Even if tropism were redefined to mean unusual susceptibility to a particular stimulus toward which a positive or negative reaction was given, the term would become entirely relative and so retain practically no descriptive power. The analysis of behavior in Drosophila, at any rate, leads inevitably to Jennings' conclusion that "tropism" should be abandoned as a specific scientific term.

Summary and Conclusions

1. An attempt was made to measure the response to light of inbred wild, brown, and white stocks of Drosophila.

2. The technique used was the measurement of the average time required to traverse a distance of 18.3 cm. toward an electric light under standard conditions.

3. The mean times for the three stocks differ significantly, white being the slowest and brown the fastest. The brown stock gave less variable results than the other two.

4. The difference between brown and wild was found to be produced by differential susceptibility to vibration, whereas the difference between white and wild appears to be caused by the slow walking speed of the former.

5. These hereditary differences in behavior do not appear to be caused directly by differences in eye pigmentation.

6. The following major environmental factors also affect the response to light: vibration, light, temperature, and food.

7. The assumption that insect behavior patterns are controlled almost exclusively by heredity does not apply to Drosophila.

8. The data suggest that the meaning of the word "tropism" is entirely relative and that the term should therefore be abandoned.

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