

THE DARK ADAPTATION OF THE EYE OF THE HONEY BEE

BY ERNST WOLF AND GERTRUD ZERRAHN-WOLF

(From the Biological Laboratories, Harvard University, Cambridge)

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I

The increase of sensibility to light of organisms which are kept in darkness is well known and quantitative descriptions of dark adaptation have been made for vertebrates and invertebrates. Data are plentiful for the dark adaptation of the human eye and have been treated analytically (Aubert, 1865; Best, 1910; Hecht, 1919–20, 1921–22, 1922–23, 1926–27, 1934*a*; Kohlrausch, 1922, 1931; Lasareff, 1914, 1926; Piper, 1903; and Pütter, 1918, 1920). For lower vertebrates we find data for the eye of the chick (Honigman, 1921) and the tadpole (Obreshkove, 1921). Similar studies are those on the tunicate *Ciona intestinalis* and the lamellibranchs *Pholas dactylus* and *Mya arenaria* (Hecht, 1926–27). In all cases the course of dark adaptation could be followed and conclusions drawn as to the velocity and range of the photosensory process.

For invertebrates other than those just mentioned only few data are available. By means of the change of phototropic response, light and dark adaptation were followed in the gastropod *Agriolimax* and quantitative data for the change of sensibility with time were obtained (Wolf and Crozier, 1927–28; Crozier and Wolf, 1928–29). For arthropods, data are only available for the eye of *Limulus* (Hartline, 1929–30), for which the electrical response in the optic nerve was taken as a measure of the change in sensitivity and for a variety of insects where tropistic changes were taken as a measure of adaptation (Dolley, 1929; von Buddenbrock and Schulz, 1933). Much information has been accumulated concerning the pigment migration in the compound eyes of arthropods during dark adaptation (for reference see: Parker, 1932), but no quantitative relations between pigment migration and the photochemical changes within the eye were established.

For the study of dark adaptation of an insect eye the honey bee was chosen. A body of precise information is already available for the visual capacity of the bee's eye (Hecht and Wolf, 1928-29; Wolf, 1932-33 *a, b*, 1933-34).

II

The reaction of the honey bee to moving stripes in its visual field has been applied successfully to studies on visual acuity, intensity discrimination, and critical flicker frequency (Hecht and Wolf, 1928-29; Wolf, 1932-33 *a, b*, 1933-34). The method permits the determination of threshold values for light intensities at which the bee just gives a noticeable response. Originally the same method was applied to study of the course of dark adaptation. It was found, however, that the speed with which determinations of threshold values for light intensities could be made was not fast enough to give an adequate determination of the course of dark adaptation. A more certain way of testing had to be developed. If a bee is tied down so that it can make only head movements, the bee shows a very definite response to the slightest motion of a stripe system in front of the eye by a co-ordinated movement of the antennae *against* the direction in which the stripes are moved. The antennae might be quiet or moving, but as soon as the stripes are shifted to the right the antennae are seen at once to take a definite almost rigid position. The left antenna points at an angle of 90° to the axis of the bee's body, while the right antenna is extended straight forward, the two thus including an angle of 90°. As soon as the motion of the pattern is reversed, the antennae take a reverse position; *i.e.*, the right is extended at 90° to the body axis and the left points straight forward. This reaction occurs with great certainty and each time with the reversal of the motion of the pattern. This index response has great advantages over the one previously used for visual tests with the honey bee, because threshold determinations can be made rather rapidly; this is important for the study of dark adaptation.

The bees used for experimentation were taken from our own colonies and brought into the laboratory. The wings are clipped under slight anesthesia. After the animals were fully recovered they were fitted into glass tubes about 5 cm. long with their heads sticking through a thin rubber membrane. In this position they can move their heads freely. The tube is held in position in front of a striped pattern (Fig. 1). In the tubes the bees will live for days. They are fed several times a day and take food readily.

Before dark adaptation tests are made the animals have to be fully light adapted. On account of the fact that the ommatidia of each eye include a very great area of the head and point in different directions, it is important that each element obtains a sufficient exposure to light. In case not all elements are equally well adapted to light it might easily happen that one set of elements which was well adapted would not arouse a response at a given testing intensity while some others which had not the necessary exposure to light might cause the reaction. For

adaptation, therefore, a box of opal glass was constructed $30 \times 30 \times 20$ cm. with a 150 watt bulb outside of each surface. The illumination at the center of the box was 142 millilamberts, and was sufficiently uniform to secure an equal exposure of all the elements of the eye to light. On account of the strong illumination outside the box precautions have to be taken that the temperature within the box is kept constant, because any rise in temperature would doubtless influence the velocity of the process of dark adaptation. By ventilating the adapting box properly the temperature was kept between 27 and 28°C. This temperature corresponded within about 0.5°C. to the temperature of the dark room in which the bees were

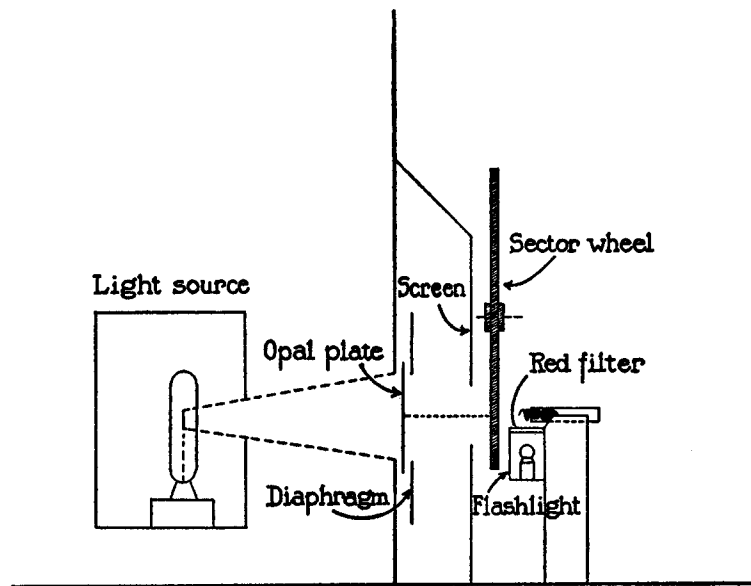


FIG. 1. Diagram of apparatus for measuring threshold intensities for excitation at different periods of dark adaptation in the bee.

tested. For all tests we always made it a point to keep the temperature during exposure and during test at the same level. The bees were exposed to the light after they were tied into the glass tubes. About 10 bees were light adapted simultaneously by mounting them in their tubes on a celluloid stand so that no shadows could interfere with an appropriate adaptation.

For dark adaptation test the bees were taken into a small dark room where they were exposed to a moving pattern at different intervals of dark adaptation (Fig. 1). Thus determinations were made of threshold intensities at which the bees gave the first noticeable response to the moving pattern. The pattern system consists of a ground glass plate 50 cm. in diameter on which 20 opaque black sectors are

pasted, leaving equally wide translucent spaces in between. The sector wheel has advantages over a system of parallel stripes because its motion can be reversed more smoothly and the bee's reaction easily followed when turning the wheel slowly around its axis to the right or to the left. Only a square area of the sector wheel, 7×7 cm., close to its periphery, is used as a test field. This area is illuminated from behind by a 200 watt concentrated-filament lamp. The amount of light coming from the source is controlled by an accurately calibrated diaphragm. With help of this diaphragm and different diffusing screens placed in front of the source, the light intensities can be varied over 4 logarithmic units.

The bee in its glass tube is placed on a stand close to the sector disc. The motion of the antennae can be seen without difficulty at high light intensities. At low intensities the antennae have to be viewed from above against a low red illumination. For this illumination a concealed flashlight bulb is used, in front of which there is a dark red filter (Wratten Filter No. 88 A) which permits only wavelengths above 6930 Å.u. to pass through. The longest wave-length which can be perceived by the bee's eye is about 6800 Å.u. (Bertholf, 1931). It therefore is perfectly safe to observe the bee's reaction to the light passing in between the sectors under these conditions. If the same filter is placed in front of the light source and the reactions of the bees studied, no response can be obtained even at the highest light intensities.

For dark adaptation tests 10 bees are adapted to light simultaneously for 15 to 20 minutes. The lights are then turned off and the bees brought into the small dark room and kept in a black box. After a certain interval the first bee is placed on the stand in front of the sector wheel, and while opening the diaphragm as quickly as possible the wheel is moved right and left until the first response of the bee can be observed. The interval of time since the animal was brought into darkness and the diaphragm reading are noted. Then the diaphragm is closed again and the next bee is tested in the same manner, and so on, until all bees which were previously light adapted have been tested. In no case was the same bee tested twice without having been fully light adapted before the new test. This was done to avoid any effect of the light to which the bee was exposed during the first test on the succeeding reading.

The first set of experiments was done during the fall of 1933 and a curve describing the course of dark adaptation was obtained. It was evident, however, that the method of exposure to light to secure light adaptation before test did not involve adaptation of a sufficiently large number of ommatidia to give an adequate description of the process of dark adaptation. New series of experiments were carried on in the spring of 1934 which gave better results, particularly because we knew by that time what the approximate range of intensities for the bee's reaction would be, so that the apparatus could be adjusted accordingly. Furthermore, it was evident from the first series of experiments that the accuracy of the decision as to the bee's reaction to the moving pattern at low intensities depends largely upon the state of dark adaptation of the observer. Thus the experiments had to be done by two people. While one was in darkness testing the bees the other took

down the times for dark adaptation and the diaphragm readings which were later translated into intensity values with the help of calibration curves.

III

The data for the course of the bee's dark adaptation are presented graphically in Fig. 2, where the logarithms of the threshold intensities

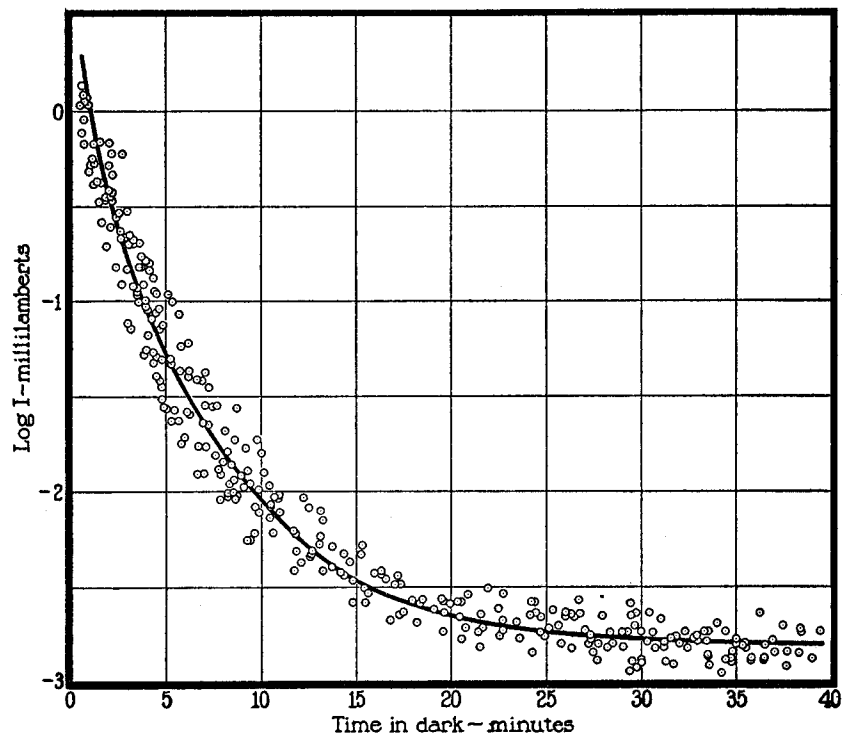


FIG. 2. Relation between threshold intensities and time of dark adaptation for the eye of the honey bee.

for response are plotted against the times of dark adaptation. The points fall on a smooth curve which shows that during the first few minutes in darkness the sensitivity of the bee's eye increases rapidly, then more slowly, until after about 25 to 30 minutes of dark adaptation the increase of sensitivity is minimal, so that one can assume that after 30 minutes' stay in darkness the process of adaptation is complete. The 311 points which are plotted in Fig. 2 are taken from

a great number of individuals, some of them having been used only once for test, some of them used repeatedly. In case animals were used more than once they were always fully light adapted before each new exposure to the testing intensity. Since all individuals were taken from the same colony it seemed justifiable to treat the data *en masse* (*cf.* also Wolf, 1932–33 *a, b*; 1933–34).

The curve given in Fig. 2 shows that the dark adaptation of the eye of the bee follows a regular course. The sensitivity increases over 1000 times within about half an hour. The points plotted lie in a ribbon the width of which is apparently proportional to mean I . This indicates that the relative variation of intensity required to give the index response is constant at all levels of sensitivity during dark adaptation (*cf.* Wolf, 1932–33 *a, b*; Wolf and Crozier, 1932–33).

While from a mass plot, as given in Fig. 2, it is not apparent where the points determined on a single individual would lie on the curve, tests were made with a group of individuals which were numbered and used repeatedly for experimentation, so that each individual might be treated singly to see whether the curve fitted to the points would be identical with the one given in Fig. 2.

Two series of experiments were carried on. In the first case 11 animals were used and tested so many times, always with light adaptation between tests, that for each individual a sufficient number of points was obtained to fit a curve. For the second series 7 bees were used. For each series of experiments the points were plotted separately. In Fig. 3 the data obtained for the 11 animals of our first series are represented by different symbols to indicate the amount of individual variation. The curve drawn through the points is the same as that given in Fig. 2. The fit of this curve seems to be adequate for every single animal. The data for the second series of repeated tests gave the same results. Since observations were made at intervals of dark adaptation previously used, the points fall on top of the readings of the first series; to avoid confusion the data of this series are omitted in Fig. 3.

It is of interest to compare the data obtained for the dark adaptation of the eye of the honey bee with the velocity and the range of adaptation in other organisms. Among the few quantitative data available the best are those for the human eye (Hecht, 1919–20; 1921–22;

1926–27). The sensitivity of the human eye increases about 10,000 times within half an hour during dark adaptation, after which further increase in sensitivity is hardly noticeable. The same relation between time and completion of dark adaptation is found for the eye of the bee. If we compare the adaptive capacity of the human eye

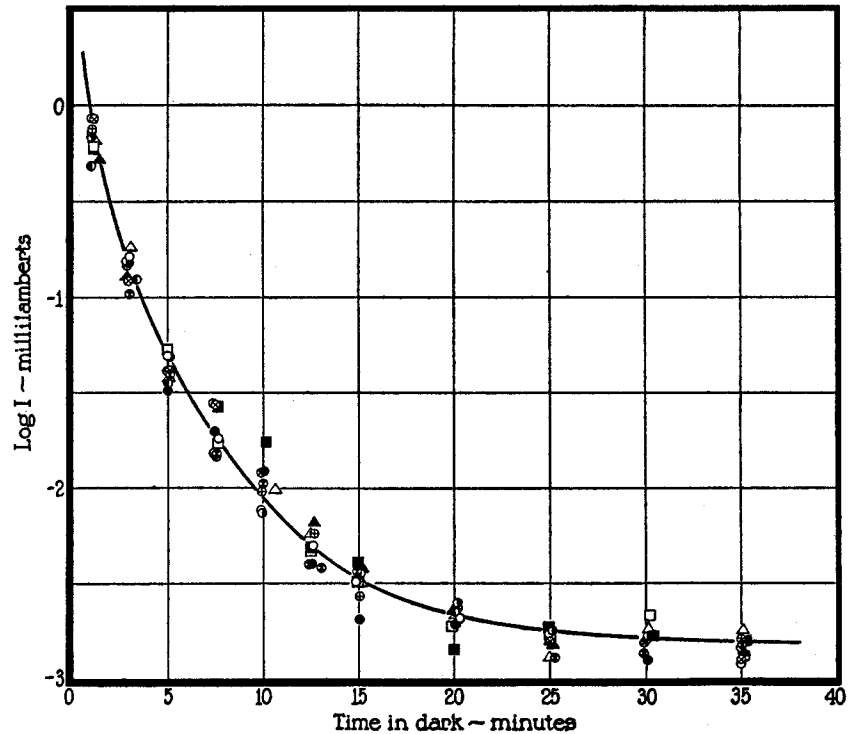


FIG. 3. Relation between threshold intensities and time of dark adaptation for 11 individuals which were tested repeatedly. The threshold values for each individual are represented by different symbols.

with that of the bee we have to keep in mind that the range of dark adaptation in the human eye depends upon the number of functioning elements. The range of adaptation increases in proportion to the number of retinal elements concerned in vision. Since we cannot limit the number of elements in the bee's eye by an artificial pupil a comparison can only be made if we take the human retina as a

whole. Under such circumstances we find that the range of adaptation of the human eye is 10 times greater than for the bee.

If we recall that a dark adaptation curve for the human eye shows distinctly two portions, namely one for the adaptation of the cones and the other for the rods, it is noteworthy that the intensity range covered by the ommatidia of the bee's eye is pretty much the same as that which we find for the rods of the human retina.

If we attempt to analyze our curves in terms of the usual equation for the chemical reactions going on during dark adaptation, we meet a good deal of difficulty. From the measurements of intensity discrimination by the bee (Wolf, 1932-33 *a, b*) Hecht computed (1934 *b*) that the dark process in the bee's eye is bimolecular. The curve determined in Fig. 2 is definitely not second order. Dark adaptation measurements should in some way reflect the fundamental nature of the dark reaction. No complete theoretical relationship is at present available for the necessary connection between these two (for an approximate one, see Hecht, 1934 *a*). In this particular case the data are probably complicated by the migration of retinal pigments during dark adaptation, which would probably make the early course of the dark adaptation as measured here appear more rapid than without this complication. Relevant data on the time course of pigment movement are not available. At the same time, this effect, if it is involved, might also appear in the measurements of intensity discrimination and visual acuity. The curve in Fig. 2 is quite accurately rectilinear when $\log \log \left(\frac{I_t}{I_f} \right)$ is plotted as a function of time in dark, where I_t is the threshold intensity at time t , and I_f is the intensity for threshold response at complete dark adaptation; in fact, this is the curve drawn in Fig. 2. No simple theory as to why this relationship is obtained has yet been forthcoming, but clearly when a complete theory becomes available it must satisfy the quantitative conditions expressed by this equation.

SUMMARY

Bees which are held in a fixed position so that only head movements can be made, respond to a moving stripe system in their visual field by a characteristic motion of the antennae. This reflex can be

used to measure the bee's state of photic adaptation. A curve describing the course of dark adaptation is obtained, which shows that the sensitivity of the light adapted bee's eye increases rapidly during the first few minutes in darkness, then more slowly until it reaches a maximum level after 25 to 30 minutes. The total increase in sensitivity is about 1000 fold.

The adaptive range of the human eye is about 10 times greater than for the bee's eye. The range covered by the bee's eye corresponds closely to the adapting range which is covered by the rods of the human eye.

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