CRITICAL FREQUENCY OF FLICKER AS A FUNCTION OF INTENSITY OF ILLUMINATION FOR THE EYE OF THE BEE

By ERNST WOLF

(*From the Laboratory of General Physiology, Harvard University, Cambridge*)

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I

The use of the reaction of the honey bee to a moving stripe system for testing visual acuity and intensity discrimination has been described in detail in previous papers (Hecht and Wolf, 1928–29; Wolf, 1932–33 *a*, *b*; Wolf and Crozier, 1932–33). The motion of the striped pattern under the experimental conditions was always such that a sudden but not too rapid lateral displacement was made, to which the bee reacted by moving from its straight course of progression with a sharp turn against the direction in which the pattern was moved. For different visual acuities, threshold intensities were determined for this response, and in the case of intensity discrimination the minimal difference in brightness of alternating stripes was found at which the bee reacts by the characteristic reflex.

In previous experimental tests the speed of translocation of the pattern in front of the bee's eye was kept so small that no fusion of the alternate stimuli could take place. Experiments concerning the flicker phenomenon in the facetted eye of an arthropod have thus far been made only with larvae of the dragon-fly (*Aeschna cyanea*; Sälzle, 1932). As index of this animal's reaction to flicker there was used a reflex, the throwing forward of the labium toward a moving object, which occurs as long as the speed of repeated flashes of light is below the critical frequency for fusion of the sensory effects. Having thus only one source of information about the number of single impressions which can be perceived separately by an insect's eye, as a function of illumination, the critical fusion frequency of intermittent stimulation by light was studied in the honey bee. Using the bee's reaction to moving patterns we have a means of more direct approach for test as compared with the "catch" reaction in the dragon-fly, and a certain body of precise information is already available concerning the relationship between visual acuity and illumination for this eye.

Bees are positively phototropic and negatively geotropic; they tend to creep upward upon a transparent inclined surface which is illuminated from underneath. If a visible pattern be moved below the creeping plane, we obtain a typical response to the displacement of the pattern, as described. The bee's response to a moving stripe system, however, can be obtained if the system is not shifted while the bee begins its journey up the inclined surface, and even while the pattern is in continuous motion in one direction of constant speed. The bee's path then involves a continuous creeping against the moving stripes, from the lower edge of the field to one of the sides of the compartment, at a fairly low angle of slope. In case the bee enters the field from the side against which the stripes are moving, it runs straight across the field to the other side of the compartment without showing any tendency to creep upward; if entering from the opposite side, *i.e.* moving in the direction of the moving pattern, it takes a sharp turn of almost 180° and crawls quickly out of the field, up the walls of the chamber, continuing to walk hanging from the cover of the compartment until it again enters the field from below or from the other side, then showing the same reaction once more. At very high speeds of motion of the pattern, however, it quite often happens that the bee, apparently unable to move against the stripes, "swims" so to speak with the stream with excessively rapid movements of the legs. In spite of a certain variation in the behavior of different individuals, the critical intensity of light and frequency of flicker at which the bee shows the first definite reaction to the moving pattern under varying experimental conditions can be determined rather accurately.

This reaction of the bee was employed in the following way. For pattern plate a round mud-ground glass plate, 50 cm. in diameter, was used. On the ground surface, which is the upper side of the disc, 20 sectors of opaque black paper are glued, in such a way that there is made a sector wheel with 20 black and 20 translucent sectors of the same size. The breadth of each sector on the central side of the disc is so chosen that the visual angle sustained by it is great enough so that the sectors passing underneath the creeping plane can be reacted to at the lowest intensities used during the test (Fig. 1). With a sector wheel, for which the angular speed is the same at any distance along the radius, the flicker frequency for the bee is the same regardless of its position nearer to the center of the disc or to the periphery. The peripheral part of the ground glass disc which is in the field of vision of the bee is illuminated by light reflected from a mirror underneath,

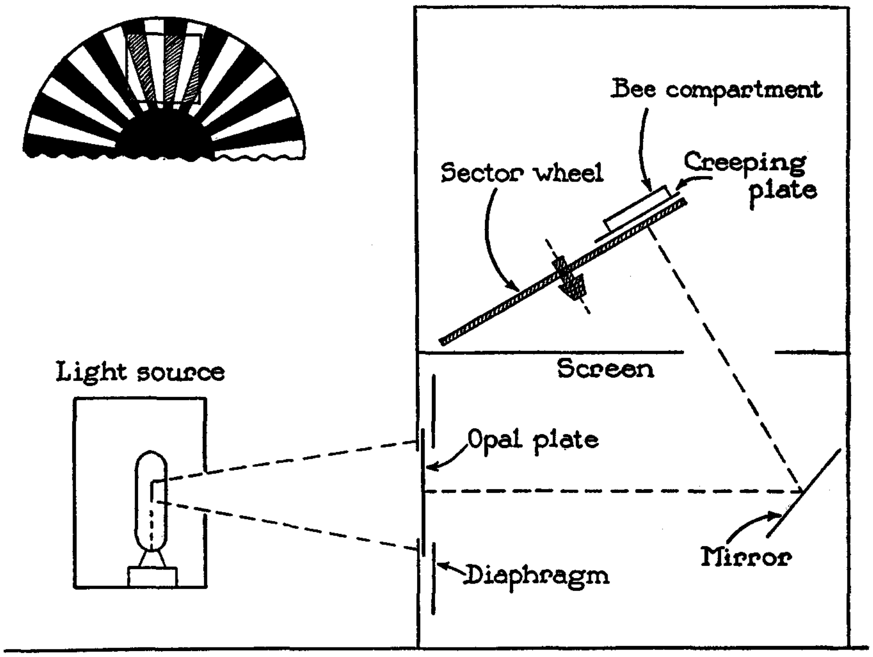

FIG. 1. Diagram of apparatus for measuring critical frequencies of flicker at different light intensities.

which gives an even illumination of the bee's visual field (Fig. 1). The source of light is a 1000 watt concentrated filament lamp which can be placed in three different positions on an optical bench. The positions are 15 cm., 55 cm., and 175 cm. from a diffusing screen in the wall of the dark room containing the apparatus. The amount of light admitted into the dark room is controlled by an accurately calibrated diaphragm for each position of the source. The intensities available on the upper surface of the ground glass wheel are measured by means of a Macbeth illuminometer. The intensity values obtained for the different settings of the diaphragm and positions of the light source are plotted against the scale readings of the diaphragm. Thus three smooth calibration curves are obtained. From these there could be read with sufficient accuracy the threshold intensities at which the bees just give the first noticeable response to the motion of the stripes at given velocities of rotation of the disc.

The ground glass plate is mounted on an axle running in ball bearings and rotated smoothly by a D. C. motor of which the speed is controlled by a rheostat and transmitted by a system of reduction gears to the disc. By using pulleys of different diameters for transmission, and by adjustment of the rheostat, the velocity of rotation could be so varied that almost any speed was obtained, providing flicker frequencies between 2 and 70 per second. This range proved to be wide enough for study of the critical frequency of flicker over a range of intensities of 4 logarithmic units.

During test a bee, after the wings have been clipped, is put into the compartment, above the rotating disc. The speed of rotation is adjusted for a certain value, measured with a stop-watch, and kept constant. The variation in speed is negligible, as repeated stop-watch readings before, during, and after the test showed. The bee then creeps over the illuminated field and reacts in the typical manner to the sectors passing by, provided the light intensity is high enough. By opening or closing the diaphragm the intensity is found at which the bee just begins to show a reaction to the motion of the visual field. For this threshold response at a given flicker frequency the associated threshold intensity is measured. This test is repeated 10 times for each given flicker frequency, with 10 different bees, to give a mean value represented as one point on the curve illustrating the relation between flicker frequency and illumination (Fig. 2). It has been shown previously that on account of the uniformity of the members of a colony of bees it is justifiable to take bees for single tests only once, instead of repeatedly (Wolf, 1932–33 *a*, *b*).

II

Flicker frequencies were chosen from 2.4 to 52.6 flickers per second, 18 selected frequencies covering this range. Some tests were made at higher frequencies, up to 68 per second, but responses of the bees could not be obtained. At a flicker frequency of 55.3 per second 3 bees gave uncertain reactions at the highest intensity of light available. Many other bees which were tested did not react at all. It seems therefore fairly certain that the minimum interval for intermittent stimulation, such that the stimuli can be reacted to separately, is at a frequency of about 55 per second. This value compares nicely with

TABLE I

Critical Intensity of Illumination for Threshold Response in the Bee as Function of Frequency of Flicker

Frequency of flicker per sec.	I	P.E.$_I$
	millilamberts	
2.4	0.011	±0.0018
3.1	0.019	0.0019
4.0	0.033	0.0018
6.0	0.064	0.0040
8.0	0.111	0.0056
10.6	0.171	0.0043
13.3	0.256	0.0161
17.2	0.546	0.0419
21.7	0.706	0.0306
23.8	0.761	0.0902
27.0	0.915	0.0634
31.7	1.270	0.0699
35.7	1.281	0.0837
38.4	1.633	0.0600
42.5	2.956	0.0633
47.6	7.474	0.587
50.0	11.85	0.711
52.6	57.66	3.682

the findings for the dragon-fly (Sälzle, 1932); the maximum value found there was 59.7 per second.

The data for critical flicker frequency at different intensities between 1/100 and 100 millilamberts are presented in Table I. The values for threshold intensities are *mean* values for the number of bees tested in each case ($n = 10$), with the probable errors of the threshold intensities.

The data show that at low flicker frequency the intensity for the threshold response is small. As the flicker frequency is increased, the intensity has to be increased, at first more rapidly and then only

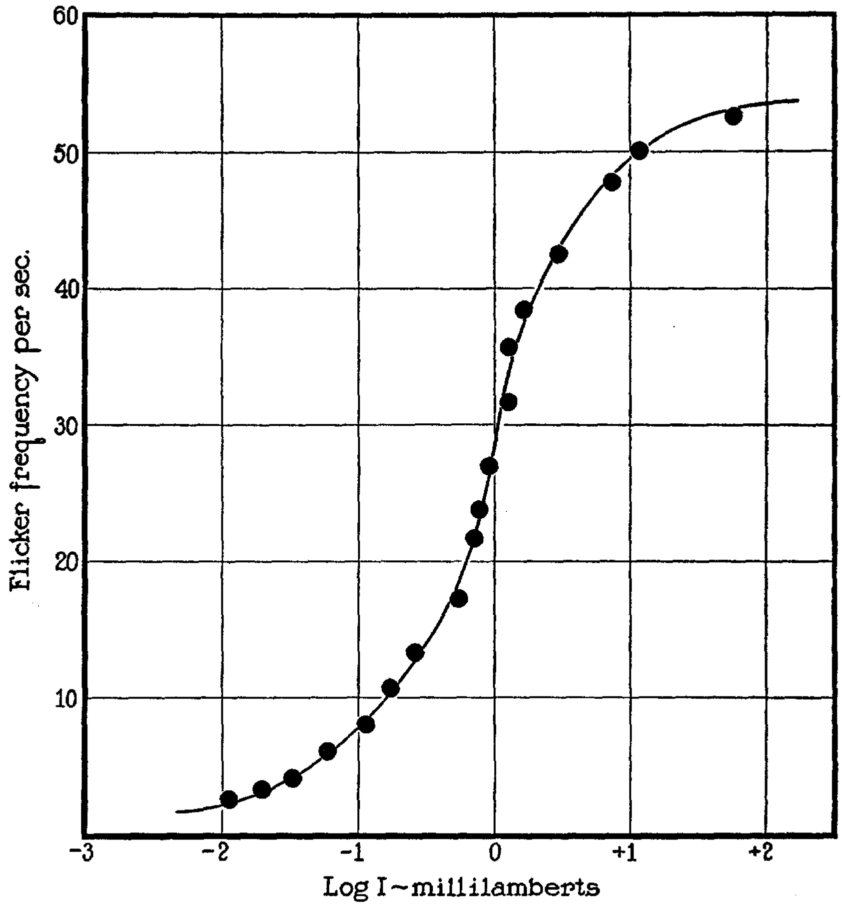

FIG. 2. Relation between critical flicker frequency and illumination. Single readings from 180 bees at 18 different flicker frequencies. The points represent averages of 10 tests.

slightly, with relatively great increase of frequency, until finally at very high flicker frequencies the increase in intensity is again greater.

The data are plotted in Fig. 2, where the flicker frequency is plotted against the logarithm of intensity. Fig. 2 shows that the points representing the mean values fall on a smooth S-shaped curve. This curve has great similarity to the ones given by Sälzle (1932) for the dragon-fly, and to the visual acuity curve for the bee (Hecht and Wolf, 1928–29; Wolf, 1932–33 *b*).

In comparing the flicker curve for the bee with the visual acuity curve it is significant that the threshold for visibility in the visual acuity tests and the lowest intensity at which the bee reacts to a very low flicker frequency are identical. By using still lower frequencies for flicker, and decreasing the intensity correspondingly, no reaction of the bee is obtained. The bee starts to react as soon as the intensity is above 0.007 millilamberts with flash frequency above 2.4 per second. At a speed of less than 2.4 sectors per second passing in front of the bee's eye, no reaction could be obtained even at higher intensities. While the bee is moving freely in the compartment the slow motion of the sectors is negligible compared with the bee's own velocity of progression, so that no reaction takes place. We thus have to assume that at least two changes from light to dark have to take place to cause the reaction of a bee which is permitted to move freely.

With higher flicker frequencies the threshold intensity for response increases. In comparing the flicker curve with the visual acuity curve it can be shown that the increase is in the two cases identical. The inflection point of the flicker curve occurs at an intensity of about 1 millilambert, which is found also for the visual acuity curve (Hecht and Wolf, 1928–29; Wolf, 1932–33 *b*). At higher flicker frequencies, above the inflection point, the intensity has to be increased more rapidly per unit increase in flicker frequency to cause the bee to react, until at a frequency of about 54 flickers per second the curve reaches a maximum level and flattens out, the intensity for the response being very high. This intensity corresponds fairly well to the highest intensity for visual acuity tests at which the bee's eye reaches the maximal resolving power.

For further comparison of the flicker curve with the visual acuity curve the following considerations are of importance. It has been assumed for the human eye (Hecht, 1927–28) and the bee's eye (Hecht and Wolf, 1928–29) that the mosaic of retinal elements, or in case of the facetted eye the ommatidia, have different thresholds. The increase in resolving power of an eye at higher intensities can thus be explained by the assumption that with increasing intensity more and more elements are actively functioning. The elements with different thresholds being distributed over the eye at random, with higher intensities it is to be assumed that elements with the same threshold lie closer together and provide thereby a higher visual acuity.

In studying the relations between threshold intensities and critical flicker frequencies the same threshold relations for the different ommatidia must play a part during response. At low intensities, while only few elements are functional and thus very much wider apart from each other than at higher intensities when others come into play, bees can react only to a slow flicker frequency, giving the reacting elements far apart time enough to be stimulated over a certain period to cause a photochemical effect during stimulation and to come back to the original threshold condition during the period of darkness. If at a low intensity the flicker frequency is made greater, the time of exposure to light for the elements with a low threshold and the corresponding period in dark would be smaller than necessary for causing adequate photochemical effect in the retinal element or for providing enough time to build up in darkness enough new photosensitive material necessary for reaction to the next flash of light. This means that the elements come to a stationary condition where light and dark reactions come to an equilibrium without setting off any impulses during illumination (Hecht, 1922–23, 1931; Hecht and Wolf, 1931–32; Sälzle, 1932).

As the intensity is gradually increased the bee is able to react to higher and higher flicker frequencies. With higher intensities the thresholds of more and more elements of the ommatidial mosaic are reached. As their distribution at random calls for the assumption that the distance between functional elements becomes smaller and smaller, faster transitions from the unexciting to the exciting state of the ommatidial surface can be reacted to. At an illumination such that the thresholds of all elements are exceeded, the maximum capacity of perceiving flicker singly is arrived at. As with increasing intensity more elements with higher thresholds come into play, the amount of light impinging upon such elements is greater, causing a photochemical effect during which the photosensitive material present is decomposed. During the period in darkness, which with higher flicker frequency is shorter, the photosensitive material is replenished probably by a reaction of second order following the mass law. Thus for elements of higher and higher threshold the period in the dark needs to be shorter to bring them back to their original condition of excitability. Consequently the bee can react to higher frequencies of flicker at higher intensities. If, however, the flicker frequency is increased still further, which gives each element again shorter times of exposure and shorter periods for recovery, we again find the elements in a stationary state during which light and dark reactions come to an equilibrium not affected by any one flash of light because the photosensitive material is below threshold concentration, nor replenishing during the dark period any considerable amount of photosensitive material, because the light reaction does not really get started, and consequently does not cause any reaction in the opposite sense. Under these conditions the flickering visual field for the bee has the effect of a stationary one uniformly illuminated, and no reaction to the rapidly moving sectors is obtained.

III

The variability of the determination of the threshold intensities at which the bee begins to react to different flicker frequencies is of particular interest. In previous tests on intensity discrimination of the bee in relation to visual acuity (Wolf, 1932–33 *a*, *b*; Wolf and Crozier, 1932–33) it was shown that the *variation* of the increase in light intensity is a function of the width of the stripes and of the illumination. The analysis of the data indicated that the amount of variation depends in part upon the frequency of alternate stimulation of the ommatidia of the bee's eye.

In testing the bee's reaction to different flicker frequencies at different illuminations, a study of the variation of threshold intensity necessary to give threshold response gives further support to the assumption that the variation depends on the frequency of transition of the retinal elements from one state of excitation to the other.

For the threshold intensities which just elicit the first response of the bee, the probable errors were computed according to Peter's formula (Table I). With increasing flicker frequency from 2.4 to 52.5 per second, the probable error increases over a thousand times.

The logarithm of P.E.$_I$ is plotted against flicker frequency in Fig. 3. The points for P.E.$_I$ at different flicker frequencies fall on an inverse S-shaped curve, compared with the flicker curve given in Fig. 2. Log P.E.$_I$ for threshold intensity increases smoothly for flicker frequencies between 2.4 and 25 per second. For frequencies between 25 and 42 per second P.E.$_I$ keeps more or less on an even level and rises more rapidly than for the lower part of the curve for frequencies above 45. Comparing the respective points for different flicker frequencies, in

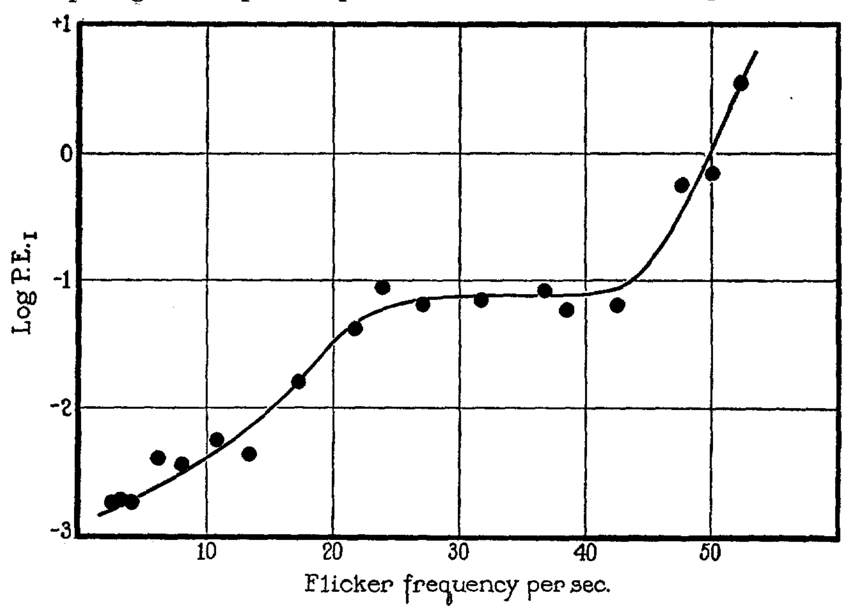

FIG. 3. Relation between the logarithm of the probable error of threshold intensity I and flicker frequency.

Figs. 2 and 3, it is apparent that in the lower part of the curve in Fig. 2 for a relatively small increase in flicker frequency the increase in intensity has to be rather great for causing threshold response; in Fig. 3 over the same range of flicker frequencies P.E.$_I$ increases smoothly. The middle part of the flicker curve (Fig. 2) is steep, which means that over a relatively wide range of increasing flicker frequency the increase in intensity needs to be only slight. For the corresponding flicker frequencies, in Fig. 3, log P.E.$_I$ increases only very little which corresponds to the small increase in intensity over the steep part in Fig. 2. The flicker curve above the inflection point tends to flatten out rather rapidly, with increasing flicker frequency, which means in terms of intensity that its increase has to be rather great for a small increase in flicker frequency to cause the bee to react. The corresponding part for flicker frequencies in Fig. 3 shows that $P.E._I$ increases steeply up to the highest frequency at which the bee was found to give a definite reaction.

This variation of threshold intensity in relation to flicker frequency can be interpreted with the help of our previous findings (Wolf, 1932–33 *a*; Wolf and Crozier, 1932–33). At low intensities and the corresponding low flicker frequencies, where only few elements are concerned —for the others the intensity is below threshold—the occurrence of alternate stimulation of the functional elements is small. As the intensity increases and more elements come into play the critical flicker frequency increases and with it the occurrence of alternate stimulation of elements, for which reason we have to expect an increase in the variation of the threshold intensity (*cf.* Wolf and Crozier, 1932–33), up to the point where for relatively small increase in flicker frequency the intensity increase had to be great. For the range over which increase in flicker frequency is high but increase in intensity small—which means that only for few additional elements the threshold is reached and consequently the increase in alternate stimulation is only slight—$P.E._I$ stays almost at an even level. For any further increase in flicker frequency the intensity has to be raised more rapidly again. New series of elements come into function by which the frequency for alternated stimulation for neighbor elements is growing and with it the probable error for measured intensity for threshold response. This agrees in a very striking way with the analysis previously given (Wolf and Crozier, 1932–33) upon the basis that the variation of the measured intensity for threshold response depends upon the intensity of the excitation induced, and that in this intensity there are two distinct elements; namely, the intensity of illumination and the frequency of exposure.

In Fig. 4 the data on variation are presented in still another way. The graph shows that the P.E. for threshold intensity is a power function of the intensity (*cf.* Wolf, 1932–33 *a*). This relationship will be understood readily by remembering that any increase in illumination (ΔI) calls for addition of elements to the set of functioning ones already concerned at that particular intensity. As more elements are involved, the chance of alternate stimulation of neighbor elements increases, and with that $P.E._I$ has to increase according to our previous considerations. Fig. 4 thus gives an illustration of the dependence of

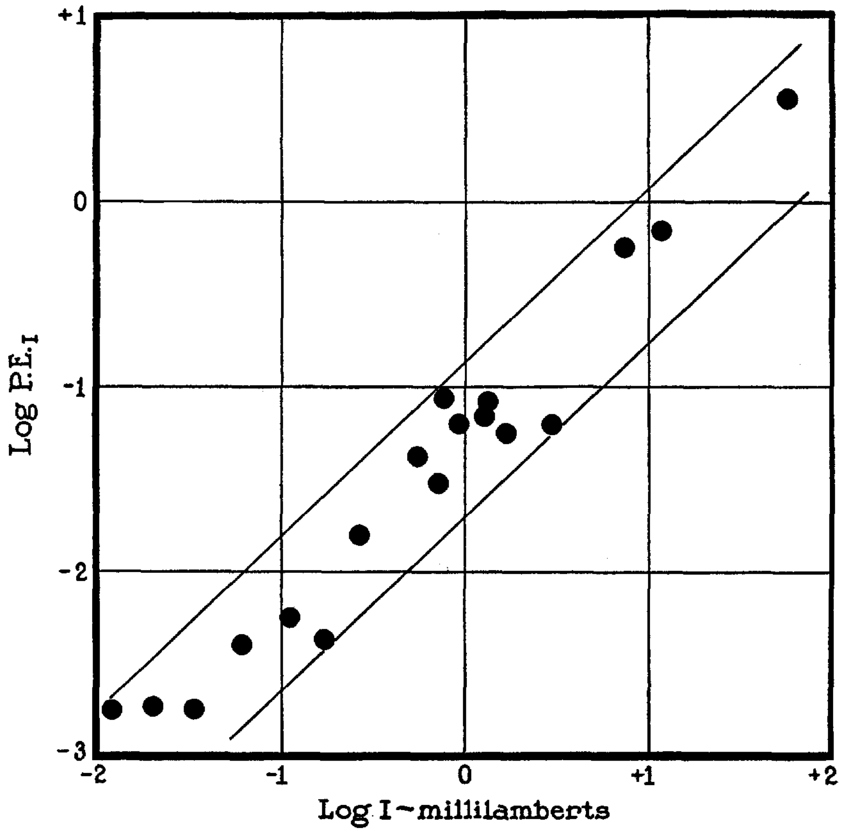

FIG. 4. Relation between the logarithm of the probable error of threshold intensity I and light intensity at which a response of the bee is obtained.

the variation of the threshold intensity upon the flicker frequency from a different point of view than is concerned in Fig. 3.

SUMMARY

The bee's characteristic response to a movement of its visual field is used for the study of the relation between critical frequency of flicker

and illumination. The critical flicker frequency varies with illumination in such a way that with increasing flicker frequency the intensity of illumination must be increased to produce a threshold response in the bee.

The illuminations required to give a response in a bee at different flicker frequencies closely correspond to the intensities for threshold response in visual acuity tests. This is due to the different thresholds of excitability of the elements of the ommatidial mosaic.

An analysis of the variation of the values for threshold intensities at the several flicker frequencies shows that the variation depends upon flicker frequency and upon the number of elements functioning at different intensities.

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