CRITICAL ILLUMINATION AND CRITICAL FREQUENCY FOR RESPONSE TO FLICKERED LIGHT, IN DRAGONFLY LARVAE

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I

The interpretation and the theoretical implications of measurements of reaction to flickered light have been given preliminary consideration in the case of data obtained with the honey bee and with the sunfish *Lepomis* (Crozier (1935–36); Crozier, Wolf, and Zerrahn-Wolf (1936–37)). For a variety of reasons it was desirable to extend the observational foundation for the proposed development. Curves expressing critical illumination as a function of flicker frequency (Wolf (1933–34); Wolf and Zerrahn-Wolf (1935–36 *b*)) have been obtained from homogeneous data for *Apis* and for *Lepomis*, and for critical frequency as a function of illumination with *Lepomis* (Crozier, Wolf, and Zerrahn-Wolf (1936–37)). The reciprocal curves for the same organism, *Lepomis*, are not identical; their differences have been in certain essential respects predicted (*cf.* Crozier (1935–36); Crozier, Wolf, and Zerrahn-Wolf (1936–37)). The meaning of these differences is of prime significance for the attempt to utilize such measurements for a theory of the mechanism of excitation; one important aspect of their meaning concerns the propriety of utilizing *averages* of measurements of reactiveness, in the way which has become almost axiomatic but which seems to be incorrect. We have proposed to test further the reality of these differences, and the capacity to predict their interrelations, by means of additional experiments with another animal. For this purpose we chose the larvae of a dragonfly, *Anax junius*. In the present paper we describe the results secured when the relationship between flicker frequency and illumination for threshold response is concurrently measured in each of the

two ways conveniently and necessarily open to examination, namely by (1) determining mean critical flicker frequency (F_m) as a function of illumination (I); and (2) mean critical illumination (I_m) as a function of frequency of flicker (F). The two curves differ in predictable ways, entirely consistent with the results we have already analyzed in the case of *Lepomis*; moreover, they are also concordant with the interpretation to which the data on *Apis* have been submitted. It has seemed to us important to carry through this experiment with two organisms (*Apis*, *Anax*) in which the eyes are of the same general sort, and which presumably possess but a single type of visual cell; the duplex composition of the typical vertebrate retina presents a special problem, since the respective contributions of rods and cones are to be separated in the analysis of the result when the whole eye is open to excitation. A following paper deals with the use of these findings in devising an interpretive theory of response to visual excitation as it involves the discrimination of intensities.

The predictive requirements of the situation are derived from the view that threshold response to flickered light is a matter of intensity discrimination (Crozier (1935–36)), and involves the statistical comparison of groups of effects. (1) The curve for F_m as a function of I should lie above that for I_m as a function of F; (2) the shape of the two curves should not be the same, and the discrepancy between them (measured by $F_m - F$) should pass through a maximum as I increases, at the inflection of the $F_m - \log I$ curve; (3) the variation of I ($=\sigma_I$) should be directly proportional to I_m, when the critical illuminations are measured at fixed flicker frequencies; (4) the variation of F, when F_m is determined at fixed illuminations, should pass through a distinct maximum near the inflection point of the $F - \log I$ curve.

We have already considered some of the evidence proving that the variation of the measured quantities in such experiments is, under suitable conditions, a property of the system of which the performance is under examination as an index of excitability (Crozier (1935); (1935–36); Crozier, Wolf, and Zerrahn-Wolf (1936–37)). This being the case, serious obstacles confront any attempt to pass from a series of homogeneous averages, as of intensity to produce a certain effect, to a mechanism for the basis of the effect. One conclusion emerging

from this is, that the mean increment of intensity ΔI just recognizable as greater than a given intensity (I_1) is primarily a statistical quantity, a property of a parameter of a frequency distribution, which possesses certain novel characteristics. In a succeeding paper it is shown that the practical recognition of these characteristics apparently brings about a unifying interpretation of all available relevant data pertaining to tests in which phenomena of intensity discrimination are the basis of the measurements.

The investigation of these matters is confronted by certain intrinsic difficulties, which it is believed can be circumvented by a procedure of the general sort which we have developed. It is obvious that in any quantitative investigation of the reactive capacity of a given organism one is concerned with sources of variation arising in (1) the nature and use of the instrumental aids to measurement, their ultimate precision and structural inadequacies, and (2) the intrinsic variability of the reacting organism. The procedural scheme we have adopted, based largely upon experience (*cf.* Crozier (1929); (1935)), is in outline this: to obtain at each of a number of magnitudes of an independent variable the same number of estimations (of the same or as nearly as possible the same inherent precision, so far as the adjunctive measuring devices are concerned) of the reactive capacities of the same individuals. Means of these measurements for these individuals are then averaged, thus minimizing the "instrumental errors," and the dispersion of the individual means gives an estimate of the individual variability; when this index of dispersion is considered as a function of the independent variable a measure is obtained of the organism's capacity to vary its performance as a function of the fixed conditions (Crozier (1929); (1935)). The adequacy of this is attested chiefly by (1) the consistent account thus empirically secured of the capacity to vary performance—and the incidentally demonstrated absurdity of the idea that organic variability is complex and "indeterminate"—and (2) by the demonstration, which we shall illustrate, that a given group of individuals of one type enables one to sample the capacity for variance which one single individual of these can exhibit. The experimental difficulty here is, that to obtain adequate data for *direct* proof of (2) would involve tests over a length of time which is in general prohibitive and impracticable, partly because the single tested individual might be expected to change in a significant way during the tests. The proof is thus necessarily in part statistical, but appears to be reasonable and adequate. We do not give any extensive account of the application to these data of the method of "analysis of variance" (Fisher and Mackenzie (1923)), largely because the consistent use of the *same* individuals introduces an unknown restriction upon degrees of freedom in comparing the different tables of data; the limitation here is properly termed "organic," and is not of the sort contemplated in routine statistical practice (*cf.* Crozier and Pincus (1935–36)). It should be said, however, that the general cor-

relation method (variance analysis) has been applied to these data, without yielding any additional information or guidance beyond that given by the methods here used.

II

The 12 individuals employed for these tests were selected from a lot secured in November, 1935; they were numbered and kept in separate aquaria. They were fed at regular intervals. 2 hours before the start of an experiment they were transferred to cylindrical jars, 10 cm. in diameter and holding 220 cc. of water, and put into darkness. The temperature was 22° ± 0.5°; during an experimental run the temperature was 21.5° ± 0.4°. A jar was placed on the glass-topped table, surrounded by a striped screen, in the apparatus illustrated in our preceding papers (Wolf and Zerrahn-Wolf (1935–36*b*); Crozier, Wolf, and Zerrahn-Wolf (1936–37)). Screens with 5, 10, 20, and 40 opaque vertical stripes (alternating with transparent stripes of equal widths) were used to produce various controlled flicker frequencies (F). The method of control and measurement of F has been described in the preceding paper; the method of controlling and estimating the intensity of illumination was also the same as that previously used; in the present experiments light sources of 100 and 1000 watts were used, without neutral filters; no properties of the data are correlated with a difference in the primary source of light.

After being dark adapted, and subsequent to an interval of adjustment to allow for any disturbance due to handling, the striped cylinder is set into motion at a speed of rotation giving a certain fixed flicker frequency (F). The light is then turned on, with the diaphragm closed. The diaphragm is then slowly opened (by a gear transmission) until the first response to the moving stripes is apparent. The diaphragm opening is then noted; by means of calibration charts the illumination I is obtained from this reading. At each flicker frequency, with each animal, three such determinations are made in succession. From these three a mean intensity (I_1) is gotten for each individual, at each F. The average of the twelve values of I_1 gives the mean intensity I_m. This procedure is used (Crozier, Wolf, and Zerrahn-Wolf (1936–37); and page 367) because it minimizes errors of estimation of I due to the technique and permits an examination of the intrinsic variability of response. With the diaphragms set to give fixed illumination = I_m, the larvae are again examined to determine the critical frequency of flicker for reaction. The striped cylinder is set into rotation at a speed giving a flicker frequency much higher than the critical; the light is then turned on; the larva remains quiet; the flicker frequency is then reduced slowly, until a response is obtained; at this instant the voltmeter connected with the magneto actuated by the driving shaft is read by another observer, and a shutter interrupts the light while the flicker frequency is again set at a high level. A second and a third reading are then taken in the same way. These are averaged for each individual, as in the case of the intensity readings.

The reaction taken to signify excitation by the flickered light consists typically in a swimming movement in the direction of the moving stripes. In certain cases the larva does not swim but lifts the head, turns toward the stripes, and after a few

seconds extends the labium toward the stripes ("catching reaction"). This response occurs at the same levels of F and I as does the swimming movement. The readings used, however, are based upon the occurrence of the initial swimming movement. It is very important that the larva should be quiet before a new reading is attempted; any movement influences the apparent value of the critical I or F.

III

The observations have been treated in the same manner as in our experiments with *Lepomis* (Crozier, Wolf, and Zerrahn-Wolf (1936–37)). The three diaphragm readings at each fixed F are averaged to obtain from the calibration curves the critical intensity for response in each individual. The intensity figures for the twelve individuals are then averaged to give I_m, and the P.E. of the set of twelve is computed. This is done to reduce the adventitious errors of making and reading the intensity settings. The mean P.E. of the separate deviations for the twelve individuals from their respective means is quite small, and amounts to 1.3 to 1.6 per cent of the mean diaphragm reading; this is precisely the mean error of a setting of intensity in the original determination of the calibration curves of the apparatus, and is independent of the intensity and of the individual animal. The individual means differ among themselves to a much greater extent and their dispersions exhibit an orderly relation to the intensity. The use of mean standard deviations of individual departures from individual means of performance, for the analysis of data in genetically homogeneous material, has been illustrated in another case (Crozier and Pincus (1935–36)) where the number of measurements with each individual was larger and each datum was, in the nature of the measurement itself, an "internal average" just as in the case of response to flickering light.

Any one individual does vary from time to time in reactiveness, and it is a major aim to investigate this variation because its properties give a clue to the real meaning of the average critical intensity for the threshold response (movement); but this variation, as will be proved, is at a much slower pace (as a rule) than could be reflected in measurements made in rapid succession. It is quite clear, however, that repeated sets of determinations of I_m at any fixed F, with one individual, would show over a long time precisely the sort of variation

given by the different individuals used at one time. The behavior of the dragonfly larvae differs from that of the fishes used in our previous experiment in one significant detail—the fish is predominantly *quiet* up to the moment of definitive reaction, whereas the *Anax* larvae are often in slight motion,—perhaps crawling, perhaps propelled by the rectal pump. This might be expected to make the readings more variable, since the occasional state of slight motion, or incipient movement could modify the apparent threshold for excitation. There is no evidence in the data, however, that this is a recognizable factor.

It is of some importance that there is no relationship between rank-order position as to relative sensitivity or mean sensitivity and chronological sequence of the tests over the period involved in the experiment. The time-order of intensities used in securing the data of Table V was essentially random. It is given here:

Order of sequence in time.....	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Flicker frequency tested.....	**10**	**5**	**25**	**30**	**35**	**20**	**16**	**12.5**	**8**	**6.72**	**3.33**	**2**	**45**	**55**	**60**	**61**	**58**	**50**	**40**	**25**

It is more important that the readings show no progressive change during the three determinations at each setting. This rules out any disturbance due to adaptation during a test. As the data in Table V show, altering the gear ratio or the stripe system on the rotated cylinder has no influence upon I_m, F_m, or the dispersions of I_1 or of F_1.

It might be thought better to employ the dispersions of the sets of 36 measurements. We have preferred to utilize the method of "internal averaging" because it conveniently minimizes the influence of adventitious errors of measurement. The values of I_m, however, and of P.E.$_{I_1}$, computed on this basis, have exactly the same general properties as those given in Table V.

The data on critical flicker frequency have been treated in a precisely analogous way. Both sets of data demonstrate that there

has been no change of mean sensitivity during the course of the series of measurements; there is thus no evidence for progressive change in the animals, nor in the observer's use of his criteria for the occurrence of response. An interesting subsidiary point has to do with the fact that on two occasions a larva moulted several hours before a test was made; there was no change in its sensitivity before or after this occurrence.

TABLE I

Average rank-order positions (R_I, R_F) of relative sensitivity in 20 sets of determinations of mean critical intensity and of mean critical flicker frequency for response for 12 individual larvae of *Anax*. The rank-order numbers in any one set of measurements (see text) are assigned in the order of *increasing* intensities (I) required to evoke response, and in the order of *decreasing* flicker frequency (F).

Animal No.	Mean relative sensitivity	
	R_I	R_F
1	7.42	6.41
2	7.17	7.23
3	8.07	7.35
4	6.92	5.95
5	8.17	7.45
6	4.10	5.96
7	4.72	5.68
8	6.36	7.48
9	6.51	6.55
10	7.00	5.75
11	5.15	4.85
12	6.85	6.86
Mean.....	6.53 ± 0.781	6.46 ± 0.612

In order to show the necessity for considering the measurements of I_c separately for each individual we may examine the *relative* sensitivities of the 12 individuals in the various sets of measurements. This is best done by means of a rank-order scale. In any one set of tests the individual reacting with the lowest value of I_c is numbered 1, the next 2, and so on. From the group of 20 sets of tests a mean rank-order position is then computed for each individual. In the same way, from the determinations of F_c at fixed values of I rank-

order numbers are assigned (in the sequence of *decreasing* magnitudes of F_c) and mean rank-order numbers again obtained. These are listed in Table I. The *mean* rank-order index of relative sensitivity is distributed in a completely random manner, for R_I and for R_F; the extreme deviates are 3 × the P.E. of the dispersion, and the extreme differences are = 2.3 P.E.$_{Diff.}$ in each case. However, mean R_I shows positive correlation with mean R_F (Table II). But this does *not* signify that certain individuals are consistently more sensitive than others. Determinations of I_1 and of the associated F_1 were made at one intensity on each day (in one afternoon). When the relation of I_1 to F_1 rank-order positions is studied in the data of the

TABLE II

Correlation of mean rank-order positions from intensity determinations (R_I) with mean rank-order positions from flicker frequency determinations (R_F) for 12 individual larvae of *Anax*.

		Mean R_F			
		4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9
Mean R_I	4.0–4.9		2		
	5.0–5.9	1			
	6.0–6.9		1	2	1
	7.0–7.9		1	1	1
	8.0–8.9				2

TABLE III

Correlation between rank-order positions from intensity determinations (R_I) and rank-order positions from F_m determinations (R_F) made shortly afterward (same afternoon). (See Fig. 1.)

		R_F						
		1.5	3.5	5.5	7.5	9.5	11.5	Mean R_F
R_I	1.5	6.50	10.00	6.00	9.25	4.75	5.25	**5.08**
	3.5	10.00	12.50	8.75	7.00	3.75	—	**4.64**
	5.5	10.00	4.50	12.25	7.00	5.25	4.00	**5.76**
	7.5	6.50	5.50	9.50	6.00	8.00	7.50	**6.71**
	9.5	6.00	5.00	7.50	7.50	10.50	8.50	**7.14**
	11.5	4.00	5.00	2.50	3.50	11.50	15.00	**8.31**

same day, the correlation is marked (Table III), and the regression of mean $R_{F_1'}$ upon $R_{I_1'}$ is definite and significant (Fig. 1). The correlation completely disappears when the intensity-sensitivity order on one day is related to the intensity-sensitivity order on the *next* day,

TABLE IV

Exhibiting absence of correlation between rank-order position for intensity determinations (R_I) on one day and rank-order position from flicker frequency determinations (R_F) made on the *next* day.

		R_F			
		2	5	8	11
R_I	2	12	15.50	14.50	16
	5	18.50	10.75	14.75	11.50
	8	13	11.50	19.50	14.50
	11	16.50	15.75	9.25	13.50

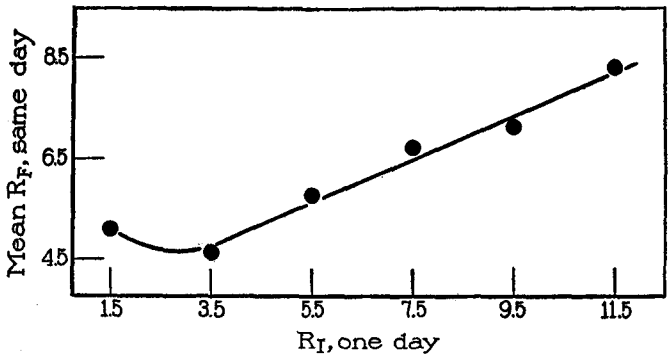

FIG. 1. Mean rank-order number from determinations of F_1 associated with rank-order classes of the same individuals based on determinations of I_1 *in tests made within 2 hours on the same day.* See text.

or to the flicker frequency sensitivity order on the next day (Table IV). The same general relationship appears in two other series of measurements made in a subsequent experiment. This means that the relative sensitivities of the 12 individuals are adequately indicated by their respective averages of I_1 and F_1, and that the individual sensitivities tend to be maintained for a period of several hours, but not for 24 hours. The form of the regression of F_1 rank-order position

upon I_1 position shows that the relatively most sensitive condition of an individual is maintained longer than its least sensitive state. This can be pictured as due to fluctuation of an individual's relative sensitivity with time, in which the cycle of changing sensitivity is not sinusoidal but asymmetrical and rather flat-topped, the rising phase being more abrupt, and with briefer depressions to minimum sensitivity; thus the more sensitive individuals after a given interval which is not too long are found to preserve more distinctly their rank-order positions than do the less sensitive. It is easily seen that if the interval between them is not too long two determinations of sensitivity (R) with a single individual will then be related, on the average, in such a way that for a high value of sensitivity in the first measurement (R') there will be found a slightly lower mean value in the second (R''), for the lowest value in the first determination a distinctly higher value in the second, but that for an intermediate value (on the low R side) R'' must pass through a minimum.

The change in sensitivity with time is completely out of phase in the several individuals—as proved by the random distributions of the mean rank-order positions. Therefore it is not due to the time of day or to other events which might control the individuals as a group. The absolute amplitude of the cycle of changes in sensitivity which must be assumed depends upon the intensity of the light, since the ordinate span of units of sensitivity (1 to 12) has the same kind of meaning as P.E._{I_1} and is proportional thereto. One is consequently justified in supposing that there must exist a connection between the fluctuation in sensitivity (on the rank-position scale) and the variation in I_1 for threshold response to flicker. A line of reasoning has already been suggested according to which response to flickered light depends upon a discrimination between E_x, the effect of a light flash, and $E_x - \Delta E_x$, the persisting effect in the dark interval (Crozier (1935–36)). In the nature of the case both E_x and $(E_x - \Delta E_x)$ are averages, and are functions of I_x; with equal dark and light sectors I_x may be assumed to be about 0.5 I, or at least $= nI$, where I is the acting intensity of the light flash. From the directly ascertained data of intensity discrimination ΔI is proportional to $\text{P.E.}_{\Delta I}$, and so to $\text{P.E.}_{I_{(2)}}$ (Crozier (1935–36)). Hence the fineness of the discrimination which can be made to meet a fixed criterion (threshold response to

flicker) is related to the frequency distribution of the effects produced by the acting intensity, and particularly to the dispersion of these effects. Consequently P.E.$_{I_1}$ calculated from the 12 values of I_1 at each intensity measures the dispersion of "effects" produced by I_m in the states of the reacting organic system open to detection or measurement under the conditions of the present tests. The coherence of the data shows that using these 12 individuals at one time merely amounts to a convenient way of examining 12 randomly distributed reactive states of this system at about the same moment. The behavior of P.E.$_I$, for example, as a function of I_m, is precisely

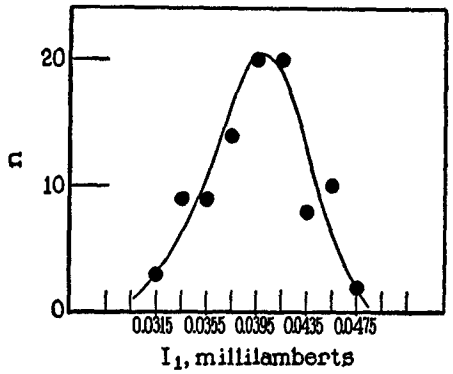

FIG. 2. Frequency distribution of 95 determinations of I_1, the critical illumination for response to flickered light, with flicker frequency fixed at $F = 25$ per second. The distribution is not skewed, shows no preponderance of low intensity readings.

the same as that obtained in cases where single measurements are made upon each of a large number of individuals from a homogeneous population (*cf.* Crozier (1935–36)).

The index of dispersion of I_1 or of F_1 is a reproducible property. The two sets of determinations at $F = 25$ illustrate this. We have also made a series of 12 additional sets of determinations of I_m and of P.E.$_{I_1}$ at $F = 25$, using 6 individuals; the mean values of I_m from these sets was 0.03943 millilambert, agreeing admirably with the values 0.03998 and 0.04061 previously obtained at $F = 25$ (see Table V); P.E.$_{I_1}$ was found to be 0.002735, the previous values (Table V) being 0.002095 and 0.002939. The various determinations of I_1

at $F = 25$ total in all 95. The frequency distribution of these is quite random (Fig. 2); there is no evidence to support the notion that the data are influenced by an "interval of uncertainty" in the flicker effect—as, if this were the case, the mode of the distribution should be well to the left.

The points at the lowest intensities used are from the standpoint of the observer the most difficult to obtain. 6 weeks after the data for mean I at $F = 2$ and $F = 3.33$ were secured the determinations were repeated with the same individuals. This was done primarily because there was a reasonable suspicion that at the times when the first measurements at these frequencies were made the intensity of the source may have been actually too high, by a small amount (due to battery charging on the same circuit), or too variable. It was also desired to see if the observer's technique had improved or changed in the course of several months' day by day occupation with the taking of such readings, so that P.E.$_{I_1}$ might conceivably be affected *if* this quantity does reflect precision of observation rather than primarily the variation in the excitability of the *Anax* larvae. The redetermined values of I_m, and of P.E.$_{I_1}$, at $F = 2$ and 3.33, are entered in Table V, and are given in Figs. 3 and 6 (solid circlets with "tags"). The indication is that the intensity of the light source was really a little high (hence, read as too low) in the case of the figures first determined at these particular flicker frequencies; this is strengthened by the findings in two other series of measurements discussed in the succeeding paper. The slight displacement given by the second set of measurements does not in any way affect the treatment of the data. The variation of I is in excellent agreement (Fig. 6) with the requirements of the plot drawn before these last determinations were made, and shows the absence of any influence upon the magnitude of P.E.$_{I_1}$ due to change in the properties of the observer.

We are interested chiefly in discussing the rational basis for our method of treating the primary data. It is to be noted that the procedure used, based on the properties of the measurements, results in a picture of the variation of performance which is astonishingly consistent with that already obtained in the case of other organisms (*cf.* Crozier (1935–36); Crozier, Wolf, and Zerrahn-Wolf (1936–37)).

IV

The determinations of mean critical illumination (I_m) and of mean critical flicker frequency (F_m) are given in Table V, and in Fig. 3. It is apparent that the F_m curve is continuously higher than that for I_m at fixed F. This is the relationship predicted as a necessary consequence of the fact that the law connecting F and I for threshold

response, as given by the determinations, is not a curve, but a *band* with limits defined by a measure of the probability of the departures

TABLE V

Mean critical illuminations (I_m) at fixed frequencies of flicker (F), with P.E.$_{I_1}$ (computed as in text); and mean critical frequencies of flicker (F_m) ± P.E.$_{F_1}$ at these mean intensities; three determinations on each of the same 12 individuals (*Anax junius*) at each point.

F	log I_m	P.E.$_{I_1}$	F_m	P.E.$_{F_1}$
per sec.	*millilamberts*	*± millilamberts*	*per sec.*	*per sec.*
2.0	$\bar{4}$.4882	0.000,017,52	2.144	0.046,22
	$\bar{4}$.5884*	0.000,020,94		
3.33	$\bar{4}$.9005	0.000,036,85	3.589	0.058,37
	$\bar{4}$.9784*	0.000,050,80		
5.0	$\bar{3}$.3367	0.000,301,8	5.200	0.108,9
6.72	$\bar{3}$.5376	0.000,188,9	6.918	0.074,19
8.0	$\bar{3}$.7308	0.000,435,9	8.168	0.059,47
10.0	$\bar{3}$.8982	0.000,847,5	10.25	0.118,5
12.5	$\bar{2}$.0649	0.000,453,4	13.00	0.139,1
16.0	$\bar{2}$.2830	0.001,443	16.73	0.278,2
20.0	$\bar{2}$.4881	0.001,678	20.80	0.310,6
25.0	$\bar{2}$.6019	0.002,095	26.22	0.356,2
	$\bar{2}$.6086†	0.002,939	26.24	0.363,1
	$\bar{2}$.5958†	0.002,735		
30.0	$\bar{2}$.7449	0.002,729	31.06	0.346,7
35.0	$\bar{2}$.8592	0.002,616	35.99	0.396,7
40.0	$\bar{2}$.9696	0.004,881	41.04	0.357,8
45.0	$\bar{1}$.0874	0.004,644	46.09	0.295,1
50.0	$\bar{1}$.3438	0.010,58	51.19	0.277,5
55.0	$\bar{1}$.7356	0.028,43	56.05	0.232,3
58.0	0.1252	0.073,08	58.69	0.230,4
60.0	0.5759	0.173,4	60.62	0.178,1
61.0	1.8407	6.825	61.42	0.201,7

F = 2, 5 stripe cylinder
F = (3.3–10), 10 stripe cylinder
F = (12.5–16), and †, 20 stripe cylinder
F = (20–61), 40 stripe cylinder

* Repetition of the experiment; the lower values of I_m at F = 2, 3.33, probably due to a fluctuation in lamp current (see text).

F = (2–35) inclusive, with gear ratio 1:1; †and (40–61) inclusive, with ratio 2:1.

of the individual determinations from the means. The relation between the two curves (Fig. 3) is of exactly the same sort as that

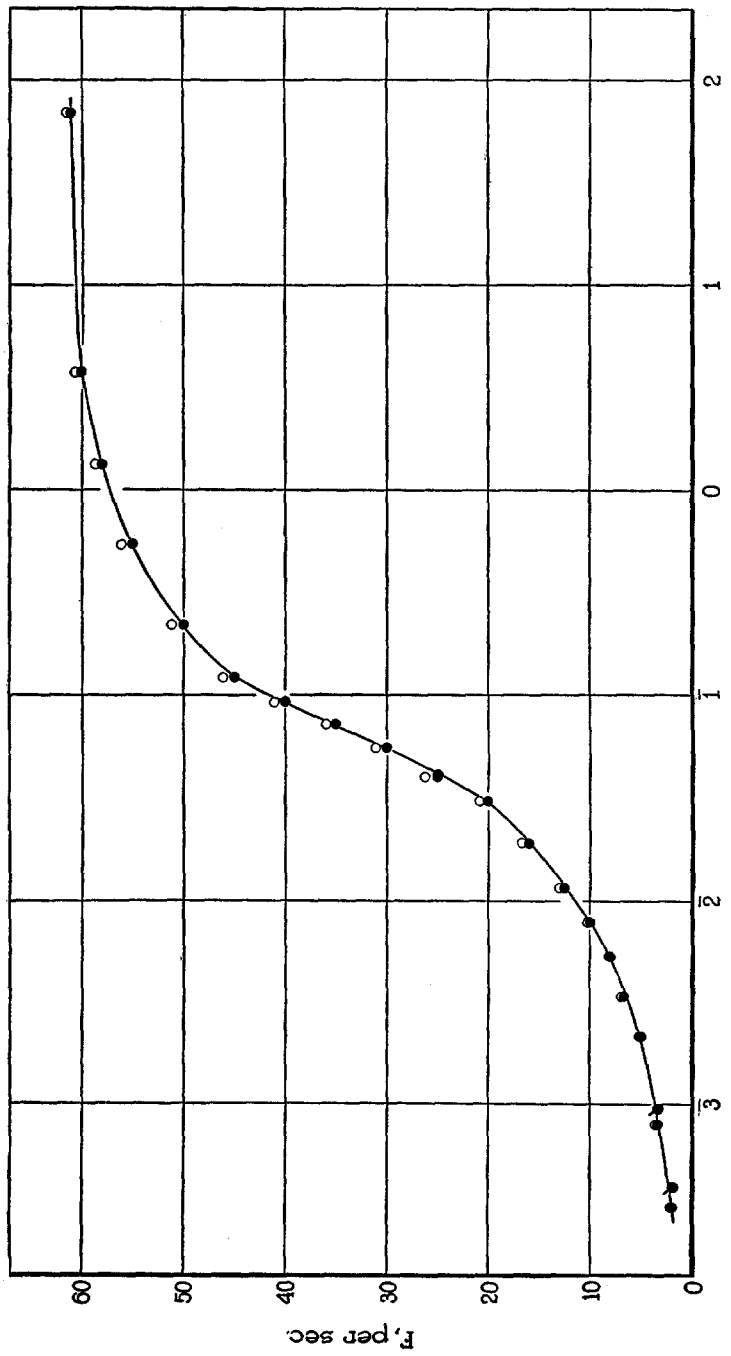

FIG. 3. Solid circlets, log I_m as a function of fixed flicker frequencies. Open circlets, F_m as a function of fixed levels of log I_m. See Table V.

expected from an analysis of the data on *Apis* (Crozier (1935–36)) and found in the experiments on *Lepomis* (Crozier, Wolf, and Zerrahn-Wolf (1936–37)). The present data are superior in the sense that the two curves were obtained concurrently. Calculation from the width of the band describing $I_m \pm$ P.E.$_{I_1}$ as a function of F shows that the discrepancy between them ($F_m - F$) must be expected to pass through a maximum at the inflection point of the F – log I_m curve. Fig. 4 shows that this is found. The intrinsic lack of precision in the determinations of critical flicker frequency at the highest intensities

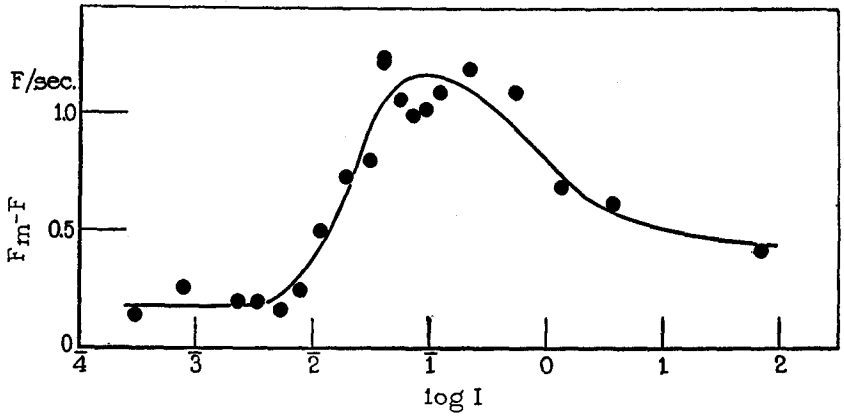

FIG. 4. The vertical separation ($F_m - F$) of the curves based upon (*a*) determinations of mean critical frequency of flicker (F_m) and (*b*) on determinations of mean critical illumination (I_m) at fixed values of F. The experimental findings follow a curve which passes through a maximum in the expected way.

causes the discrepancy to be larger than expected beyond F = about 80 per cent of maximum F, just as in the data on *Lepomis* (Crozier, Wolf, and Zerrahn-Wolf (1936–37)). The difference ($F_m - F$) is almost exactly 3 × the size of P.E.$_{F_1}$, and (see Figs. 3, 4, 5 and Table V) is *consistent*; the horizontal separation is similarly related to P.E.$_{I_1}$.

We have already referred to the fact that P.E.$_I$ is lawfully related to I_m. The data of earlier experiments with *Apis*, and other relevant material, show that P.E.$_I$ is directly proportional to I_m. Fig. 6 exhibits this relationship for the measurements on *Anax*. The several determinations of P.E.$_{I_1}$ are of the same statistical weight; hence

each is subject to a P.E. proportional to its own magnitude; consequently a plot of $P.E._{I_1}$ *vs.* I_m should be in the form of a band with straight margins which diverge as $P.E._{I_1}$ increases (*cf.* Crozier (1929); (1935); etc.). For the lower values only of I_m it is mechanically

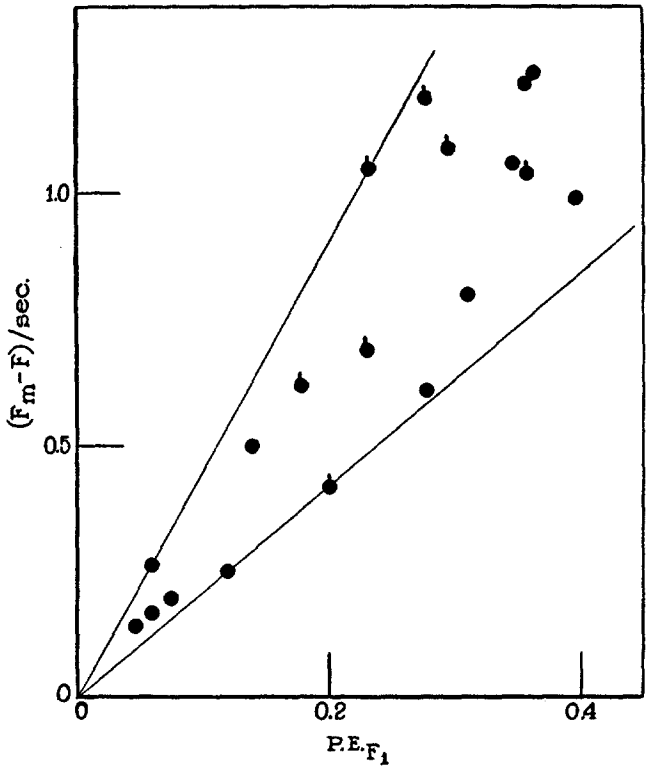

FIG. 5. The separation of the curves based upon F fixed and F_m measured (Fig. 3) is constantly $= 3\ P.E._{F_1}$. Points above the inflection point in the F — log I plot (Fig. 3) carry tags. This separation at any one point could happen once in 20 trials by chance; for the 20 sets of determinations the chance of the separation being accidental is $1:(20)^{20}$.

possible to show this graphically in Fig. 7. As is generally the case (Crozier (1935–36)) the band, regarded as straight, does not quite go through the origin, as theoretically it should; the line of central tendency in Fig. 5 being

$$P.E._{I_1} = 3.89\ (I_m + 0.000{,}008).$$

The "correction," 0.000,008, presumably indicates the magnitude of the constantly present contribution of a source of "error" which is due to the nature of the measurements and is independent of the magnitude

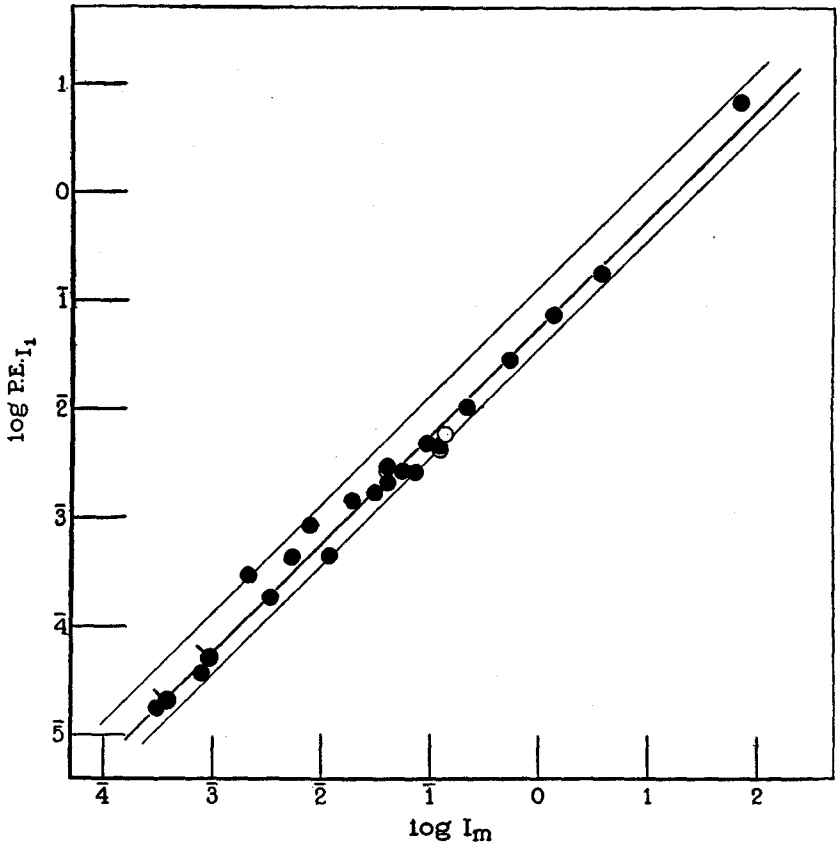

FIG. 6. The dispersions of the measurements of I_1, as a function of log I_m; data in Table V; 3 additional determinations not included in Table V are plotted as open circlets. The points are expected to form a band of constant width. The central line divides the band drawn into two arithmetically equivalent zones (bisecting the abscissa dimension); seven observations are above the line, eight below it, eight on it; this demonstrates a random scatter.

of I. The correction is so small that its effect is not representable in Fig. 6. The proportionality of σP.E.$_I$ to P.E.$_I$ brings it about that in a logarithmic plotting (Fig. 6) the values of P.E.$_{I_1}$, given a large

number of them, should be distributed in a band with parallel edges. The inflection of the log I_m – F curve (Fig. 2) leads to a central concentration of the plotted points in Fig. 6, but the random distribution of the determinations is attested by the fact that when the band in Fig. 6 is divided into two arithmetically equivalent strips by a line through the mid-abscissa of its breadth (I_m being the independent variable) the points are found to be equally distributed on either side of it (Fig. 4). The reproducibility of the determinations of P.E.$_{I_1}$ is shown by the "repeat" determination of the point at $F = 25$, already referred to in Section II, as well as by three supplementary

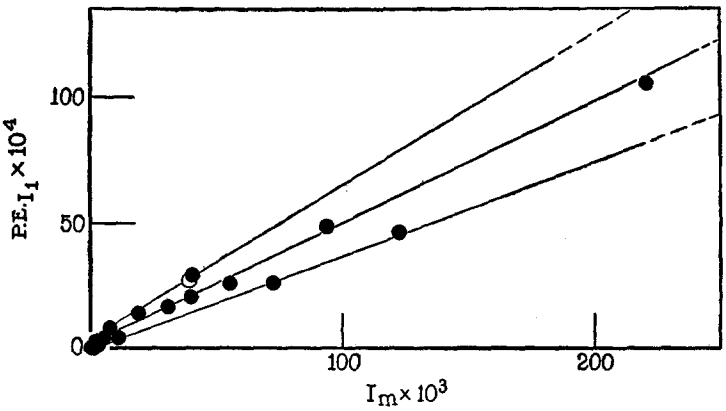

FIG. 7. P.E.$_{I_1}$ as a rectilinear function of I_m. Only the lowest intensities used can be given on an arithmetic plot. The best fitting line misses the origin by not more than $I = -0.000008$. See Fig. 6.

determinations indicated in Fig. 6 by open circlets (not listed in Table V).

Just as, from a knowledge of the values of I_m and of P.E.$_{I_1}$, it is possible to predict the nature of the curve relating F_m to log I, it is also possible to predict the form of the dependence of P.E.$_{F_1}$ upon F_m (or log I) (Crozier (1935); Crozier, Wolf, and Zerrahn-Wolf (1936–37)). This is given by one-half the vertical extent of the band formed by the plotting of $I_m \pm$ P.E.$_{I_1}$; it rises to a maximum in the region of the inflection of the log I curve, then falls (this is not *dependent* on the inflection, since no inflection occurs when F is plotted as a function of I); the high F end is not so low as the left hand limb, because

of the inherent lack of precision, relatively, in the determinations at this end—which means that there is less difference among the measurements with the several individuals, but lower reliability than there "should" be in the averages for a single individual. The curve in Fig. 8 shows the dependence of P.E.$_{F_1}$, as measured, upon log I; and Fig. 9 shows the relation to F_m. Two supplementary determinations, not entered in Table V, are shown as open circlets. The vertical scatter of the points also goes through a maximum, as it should, for reasons identical with those already mentioned in connection with the properties of P.E.$_{I_1}$. (It has sometimes been supposed that in

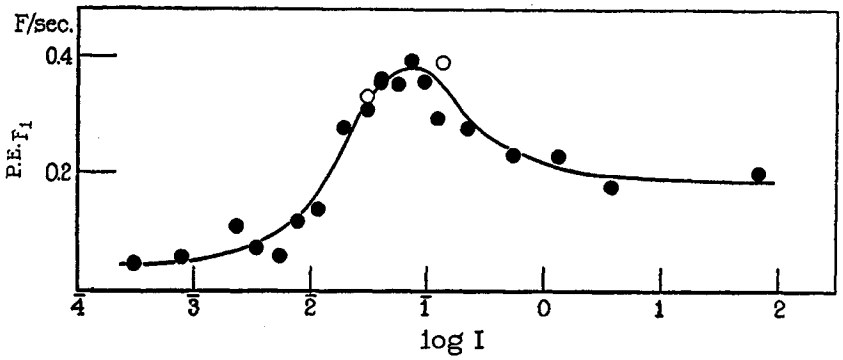

FIG. 8. The dispersion of the measurements of F_1 as a function of log I. The form of the band delimited by log ($I_m \pm$ P.E.$_{I_1}$) as a function of F predicts that P.E.$_{F_1}$ must pass through a maximum near log $I = 2.85$. The descending limb of the curve falls to a level higher than that of the left hand end of the curve, for reasons discussed in the text. Two determinations not included in Table V are plotted as open circlets.

measurements with a biological system the P.E. of the measurement should increase with the magnitude measured, a vague and essentially irrational analogy being made with the process of repeatedly applying a foot rule in ascertaining a length. The fact is, of course, that no such relationship is generally found at all. It is of some value, however, to point to the behavior of P.E.$_{F_1}$ as an instance.)

The correlative behavior of P.E.$_{I_1}$ and P.E.$_{F_1}$ shows that the variation in F_c and in I_c respectively are two aspects of the fundamental organic variation in capacity for exhibiting the threshold response to flicker. A theory of the mechanism of excitation cannot be based upon the

consideration merely of mean critical illuminations as a function of flicker frequencies, or upon measurements of mean critical flicker frequencies; the fact that these two curves are not the same, and differ in the ways we have discussed, suffices in itself to show that the relation between intensity and flicker frequency, for threshold response, must be stated in terms recognized as preserving the primarily statistical properties of the data. Otherwise dimensional incongruities are inevitable.

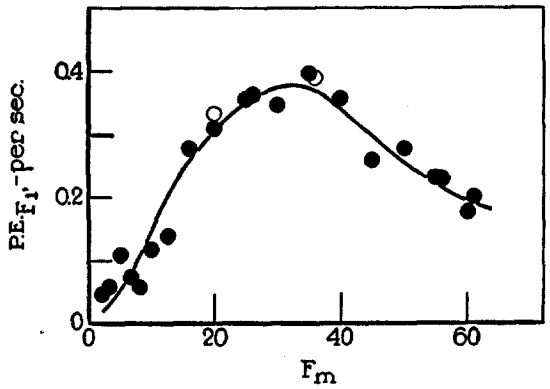

FIG. 9. The dispersions of the measurements of F_1 as a function of F_m. See Fig. 8.

V

The reactions of larval Odonata to flickering illumination have been utilized by Sälzle (1932). His data of *Aeschna* exhibit in a general way the sort of relationship between F and I_m which we have described for *Anax*. The curve is much steeper, however. The reaction employed as test of excitation was the "catching motion" of the labium, directed at a moving spot of light on a revolving dark circle. The recognition of (that is, reaction to) flicker involves therefore in this case not equal durations of light and dark periods, as in our experiments, but a much greater relative duration of the dark interval. It will be shown that this may be expected to make the curve of log I_m as a function of F very much steeper. The shape of Sälzle's curve, consequently, is not primarily due to a difference in the species used nor in the criterion of excitation. Our curve of I_m for *Anax*

is of precisely the same form as that secured in measurements with *Apis* (Wolf (1933–34)) when the light and dark intervals are also equal.

Hecht ((1934); Hecht and Verrijp (1933–34); Hecht, Shlaer, and Smith (1935)) has given a provisional theoretical derivation of the equation for flicker fusion with equal dark and light periods. It is obtained on the assumption that the change of light adaptation proceeding during the light flash and the change of dark adaptation proceeding unopposed during the equally long interruption of light are brought into equality at flicker fusion. This cannot be made applicable to flicker fusion specifically without the introduction of an additional assumption, namely that the flicker frequency at fusion is proportional to the amount of photoproduct during a light flash. The relation of f, the critical fusion frequency, to intensity should accordingly be given by

$$\frac{2k_1}{k_2} = KI = \frac{f^n}{(c - f)^m} \tag{1}$$

where k_1 = the photochemical velocity constant, k_2 = the velocity constant of the regenerative dark reaction, c = a constant, the maximum flicker frequency, and n and m are exponents signifying the orders of the dark and of the light processes respectively.

These equations do not fit the measurements with *Anax*, nor those with *Apis*. There should be no significant difference due to the use of F, the frequency for marginal recognition of flicker, instead of f for flicker fusion. The fit can be tested by means of Fig. 10; we use only the measurements of I_m at fixed flicker frequencies in this illustration. Log F should be a smoothly increasing function of log I, with continually diminishing slope. The slope at the lower end defines exponent n in equation (1), the form of the bend defining m. It cannot be said that with $n = 2$, $m = 1$, there is even an approximation to the data. Increasing m makes the departure worse. Even with $n = 2$ the fit at the lower end is not satisfactory. It is to be remembered that (Fig. 3) there are really two essentially concordant sets of measurements; the departures even at the low end of the graph in Fig. 10 are quite significant. Moreover, the data on *Apis* have the same properties. This cannot be the result of the difference between

"fusion of flicker" as used in obtaining the data described by equation (1) and "marginal recognition of flicker" used in the present cases. This is definitely shown by the fact that our data on *Lepomis* (Wolf and Zerrahn-Wolf (1935–36*a*); Crozier (1935–36); Crozier, Wolf, and Zerrahn-Wolf (1936–37)), based on marginal reaction to flicker, not fusion, are quite well described by equation (1), as Fig. 11 demon-

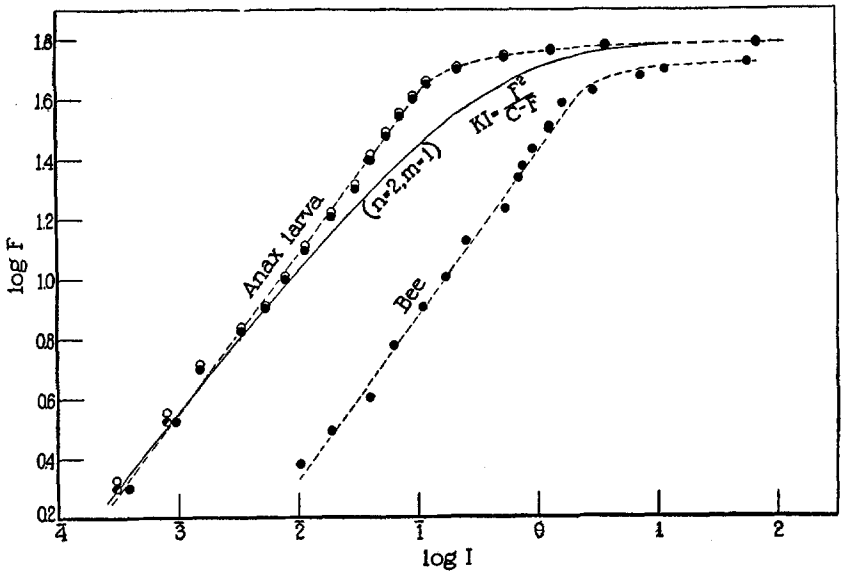

FIG. 10. Mean critical illuminations for response to flicker, as a function of fixed flicker frequencies, for *Anax* larvae and for the bee (*cf.* Wolf (1933–34)). A logarithmic grid is used to exhibit the way in which the data behave with reference to the applicability of the equation $KI = F^n/(C - F)^m$. The continuous curve is drawn with $n = 2$, $m = 1$. The data for *Anax* and for the bee clearly exhibit the same general properties.

strates; in this case, somewhat unusually (*cf.* Hecht, Shlaer, and Smith (1935)), $n = 2$ and $m = 1$ for the cone portion.

The failure of equation (1) to describe the data from the responses of insects to flickered light might be due to a real mechanical difference in the conditions. The curve in Fig. 10 might be regarded as in a measure resulting from the inability of low intensities to stimulate a certain proportion of the ommatidia—say those at the periphery of the eye—within the times permitted even at low flicker frequencies.

Higher intensities might therefore recruit additional elements. The intensity discrimination data on the bee (Wolf (1932–33); *cf.* Crozier (1935–36)) contain no hint of such a complication (*cf.* Hecht (1935), where $n = m = 2$); it is very doubtful that there is any difference in the technic in the two cases—for example, in the relation of the striped field to the bee's eye—which could account for this; and no obvious trace of any such effect appears in the data on dark adaptation as followed by means of tests involving intensity discrimination

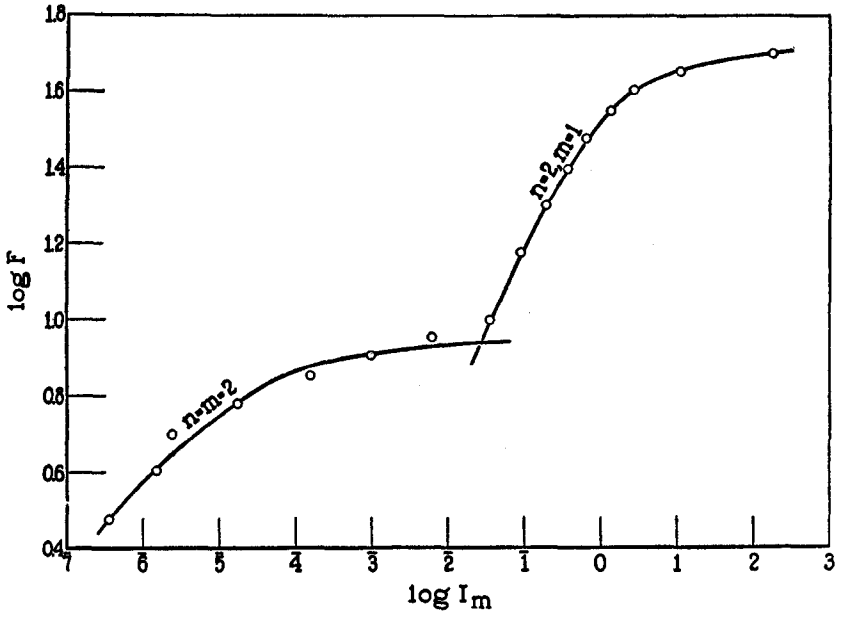

FIG. 11. The data on *Lepomis* (Wolf and Zerrahn-Wolf (1935–36*b*)) are quite well described by equation (1); see text.

(Wolf and Zerrahn-Wolf (1935–36*a*); *cf.* Crozier (1935–36)). The visual acuity data on the bee, however, with normal eyes and with partially covered eyes (Hecht and Wolf (1928–29)), show an identical break at precisely the intensity at which it occurs in the logistic plot (Fig. 13) of the bee flicker data. The difference in the shape of the eye in *Apis* and *Anax* also renders this doubtful. In any case, the possibility is open to test by means of experiments with only parts of the eye functioning; the visual acuity data do not include measure-

ments with only the central area of the eye functioning. It is scarcely desirable to assume more than one class of excitable elements. Perhaps the strongest evidence against this is found in the absence of any indications in the data that the variation of I is a discontinuous function of I_m; in the case of *Lepomis* (rods and cones) this evidence is provided (Crozier (1935–36)), but with *Anax*, as with *Apis*, there is no sign of it. We are therefore reluctant to consider that here there are two kinds of receptor elements with organically distinct ranges of thresholds for excitation.

Even if by means of a correction for an effect of this sort the measurements with *Anax* and with *Apis* could be made to conform to equation (1) it would not of necessity follow that the assumptions leading to this formula are really efficient. It is implicit in our discussion of these matters that an adequate theory of the situation cannot in fact be based upon measurements of average intensities, because the organism's responses exhibit intrinsic variation (*cf.* Crozier (1935–36)). The deduction from other cases involving intensity discrimination is (Crozier (1935–36); and a subsequent paper) that discrimination results from the comparison of the effects produced by two populations of excited elements, or in one population at successive times. Curves of log I_m as a function of F might therefore be expected to have certain of the properties of population curves. The most generally useful such curve is perhaps the "logistic,"

$$Y = \frac{A}{B + De^{-px}}. \tag{2}$$

Equation (1), with $n = m$, is in fact this equation, with

$$F = \frac{F_{\max.}}{1 + be^{-p \log I_m}}, \tag{3}$$

as then

$$p \ln I = \ln \frac{bF}{(F_{\max.} - F)};$$

in each case K in (1) $= (1/b)^p$.

For cone critical frequencies in the human subject Hecht (Hecht, Shlaer, and Smith (1935)) found $KI = f^2/(c\text{-}f)^2$, so that $p = 0.5$.

When equation (1) describes the flicker curve, as in the case of *Lepomis*, the logistic does so equally well (Fig. 12). For the cone portion of these data, where we have made $n = 2$, $m = 1$ in (1), the upper portion at least (all that is open to inspection) is well fitted (Fig. 12) with p in (3) = 0.82 (*cf.* Crozier (1935–36);[1] for the rod portion, $n = m = 2$. For the asymmetrical case, $n = 2, m = 1$, we write $F = F_m/(1 + Fe^{-s \log I})$.

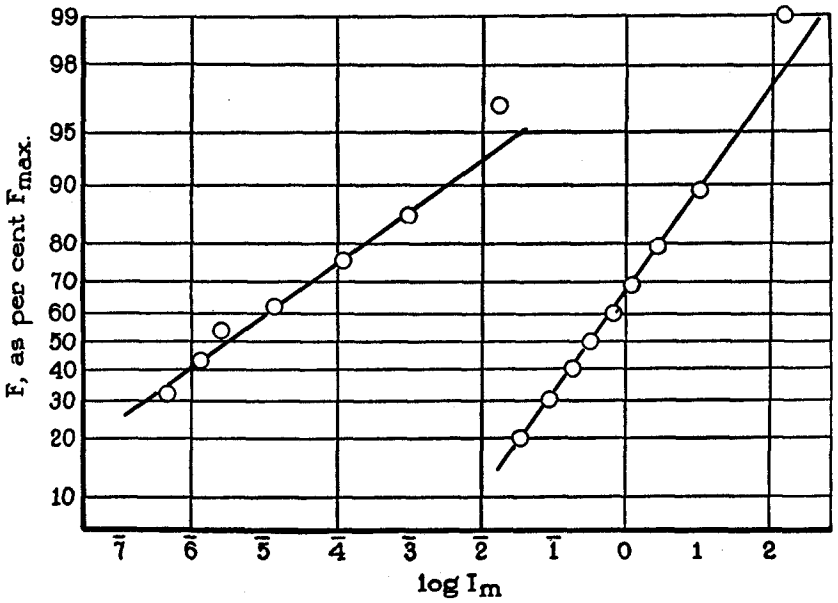

FIG. 12. The data on *Lepomis* are adequately described by the logistic equation; F (as percentage of maximum F) is plotted on a logistic grid (*cf.* Wilson (1921)), as a function of log I. The rod and cone sections are regarded as separate populations; the departure of the highest point could be reduced by adjustment of the assumed value of $F_{max.}$, here taken as the highest F observed. The highest point on the rod curve presumably is a result of overlapping of rod and cone populations.

With the data for *Anax*, and for *Apis*, the case is not quite so simple (Fig. 13). There is again indication of a change in the relationship with increasing intensity (incidentally, the transition does not coincide with, and thus seems not to determine, the maximum in the curve of σF_1). This means that the required form of curve is

[1] p. 519.

asymmetrical; for example, if in the lower portion of Fig. 13, up to log $I_m = \bar{1}.0$, $\sqrt{F}$ is substituted for F the logistic relationship is obeyed very well.

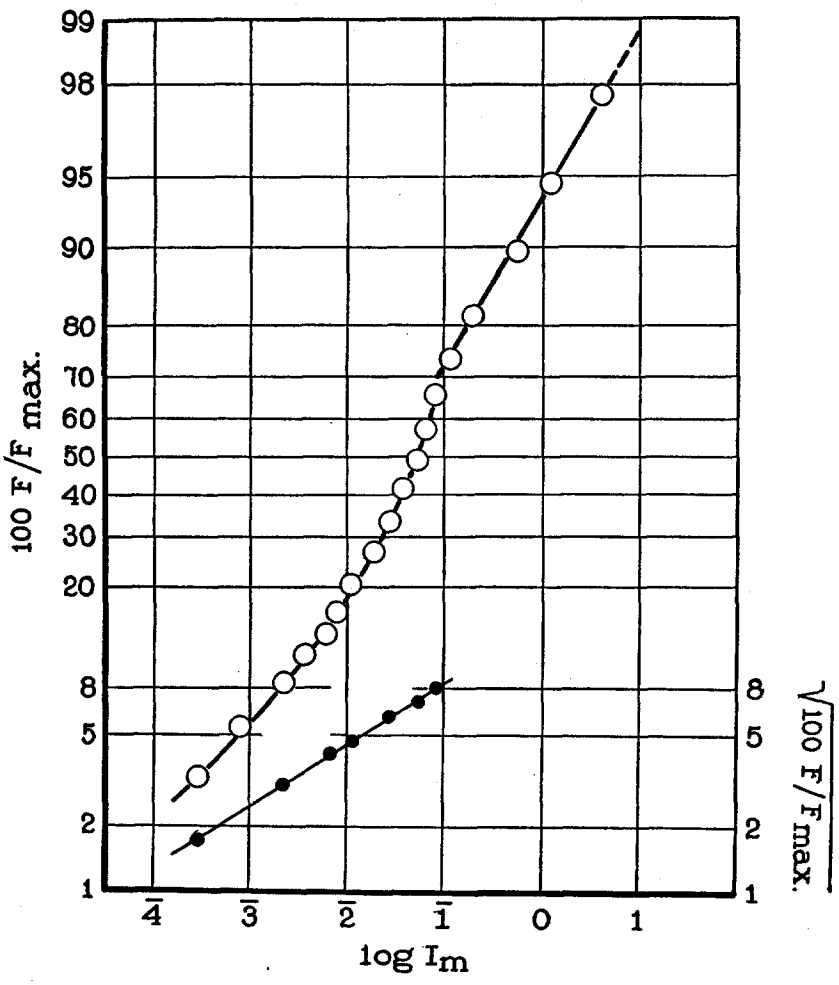

FIG. 13. The data on *Anax* show in terms of a logistic grid a separation into two sections; the upper may be regarded as a passable fit ($p = 2$); the lower becomes so if for $F/F_{max.}$ there is substituted $\sqrt{F/F_{max.}}$. See text.

There is thus no reason to suppose that applicability of equation (1) justifies the conception that critical illumination for flicker, as

a function of flicker frequency, *depends upon* the equality of average rates (or amounts) of change of photoproducts in the retina during light adaptation and dark adaptation respectively occurring in the periods of illumination and of no light. It necessarily *involves* such equality, as in any steady state. What is really made questionable by the "population curve" character of equation (1) is the idea that F $(= 1/t)$ corresponds to the amount of photochemical change in a light flash. In fact, however, it can be shown in another way, quite in keeping with the requirements of the basic notion of comparison between "populations of effects" during the periods of light and of no light, that the formal adequacy of equation (1) does not signify the propriety of the reasoning whereby it was arrived at.

The constants k_1 and k_2 have the meaning of velocity constants; k_1 for the photochemical change (Hecht (1919–20)) must have a low temperature coefficient (even with allowance for effects upon absorption of light), whereas k_2 must have a much larger temperature coefficient (*cf.* Hecht (1926–27); Crozier and Wolf (1928–29)). If we lower the temperature certain specific predictions can be made on the basis of (1). When the maximum value of F is independent of temperature, and this is the experimentally determined fact in the case we shall discuss, then at fixed flicker frequency F the right hand side of (1) will stay constant; but k_1/k_2 must increase with falling temperature; consequently at fixed F the critical illumination must decrease. Our investigation of this situation is described in a succeeding paper; the general result is that instead of decreasing, the critical intensity *rises* with falling temperature. Consequently k_1 and k_2 (and thus K) cannot have the meanings called for in the derivation of equation (1). It may also be noted that, from the standpoint of (1), the animal at higher temperatures should be *less* light adapted on exposure to light; consequently, at fixed I, F should fall (*cf.* also Lythgoe and Tansley (1929)); actually it rises. The facts thus far considered are, however, in agreement with the requirements of the idea that for flicker fusion there must be established a relationship between F and I such that the average effect of a light flash (or the total effect during the light interval) just fails to be distinguishable from the effect due to or expressed as the after image of the flash. Hecht has pointed out (Hecht, Shlaer, and Smith

(1935)) that the interval between flashes may be occupied with the effects governed by removal of photoproducts produced during the flash, not to be confused with the process governing the rate of dark adaptation. It is known that in a general way, and over an interval of some seconds, the brilliance of an after image declines; these phenomena are complex, but measurements at least show that when the intensity is gauged by contrast with measured intensities (*cf.* Lasareff (1923); Kravkov (1924)) the brilliance declines logarithmically with time.

These measurements involve time intervals longer than those concerned in critical flicker frequencies. Data do exist, however, making it possible to push back the decay curve to very brief time intervals. Fry (1934) measured the photic energy required in the human eye to match the after image of a flash of fixed energy as a function of time after cessation of the flash. His figures show that the energy required for a "match," under such conditions that the after image of the first flash persists, declines logarithmically as a function of time (0.0025 to 0.125 second); also that it declines less rapidly when twice the area is initially excited. Fry (1934) interpreted his data as indicating inhibition of the effect of a flash by the action of a succeeding flash; this seems to be an unnecessary assumption and indeed without any real foundation. These measurements have been utilized by Piéron (1935) to support the theory of "meta contrast" (Stigler (1910); (1918)). Stigler appealed to Exner's conception of the metaphotic image, which Piéron rejects because of the delay in subjective recognition of the onset of a luminous flash; it does not appear to have been realized that these delays in recognition play no part where successive flashes are involved—as indeed the clear demonstration (Rubin (1929); Piéron (1935)) of the erroneous nature of the interpretation of the measurements of "*Empfindungszeit*" (Frölich, 1929) makes evident.

There are phenomena of photic response in insects which indicate at least delay in the expression of the effect of a flash (Mast (1912)). It may also be safely assumed that the establishment of the effect produced by a flash does not instantaneously attain its full value, particularly when the flash involves movement of a bar or sector of light. For purposes of qualitative illustration and exploration, then, we may assume a scheme of the possible relationships which calls for a decay of the after effect of a flash which is dependent upon (1) the level of effect attained by a flash, a function of its intensity and of its duration, and (2) the control of the rate of decay by temperature; and which regards the establishment of a certain difference between the integrated effect of a flash and of its after effect as necessary for

marginal recognition of flicker. The increase of I at fixed F, when the temperature is lowered, is a necessary outcome of this formulation. Certain other consequences of this position, akin to that advocated by Exner (1870) and by Grünbaum (1897–98), will also be dealt with in a succeeding paper.

VI

SUMMARY

Curves relating flicker frequency (F) to mean critical illumination (I_m) for threshold response to flickered light, with equal durations of light and no light intervals, and relating illumination (I) to mean critical flicker frequency (F_m) for the same response, have been obtained from homogeneous data based upon the reactions of dragonfly larvae (*Anax junius*). These curves exhibit the properties already described in the case of the fish *Lepomis*. The curve for F_m lies above the curve of I_m by an amount which, as a function of I, can be predicted from a knowledge either of the variation of I_m or of F_m. The law of the observable connection between F and I is properly expressed as a band, not as a simple curve.

The variation of I_m (and of F_m) is not due to "experimental error," but is an expression of the variable character of the organism's capacity to exhibit the reaction which is the basis of the measurements. As in other series of measurements, P.E.$_I$ is a rectilinear function of I_m; P.E.$_F$ passes through a maximum as F (or I) increases. The form of P.E.$_F$ as a function of I can be predicted from the measurements of P.E.$_I$.

It is pointed out that the equations which have been proposed for the interpretation of curves of critical flicker frequency as a function of intensity, based upon the balance of light adaptation and dark adaptation, have in fact the character of "population curves;" and that their contained constants do not have the properties requisite for the consistent application of the view that the shape of the $F - I$ curve is governed by the steady state condition of adaptation.

These curves can, however, be understood as resulting from the achievement of a certain level of difference between the average effect of a light flash and its average after effect during the dark interval.

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