CRITICAL ILLUMINATION AND FLICKER FREQUENCY IN RELATED FISHES

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I

The relationships between flicker frequency and mean critical illumination for response to a moved stripe pattern have been determined for the sunfish *Enneacanthus* (Wolf and Zerrahn-Wolf, 1935–36 *b*; Crozier, 1935–36; Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *b*, *d*). When log I_m is expressed as a function of F the curve is a double S-shaped affair; each part is describable with excellent fidelity by a logistic (or by a probability integral). The curve is not describable by the equation which has been employed to graduate the data of human flicker fusion (Hecht and Verrijp, 1933–34; Hecht, Shlaer, and Smith, 1935); the behavior of the curve as a function of temperature is also inconsistent with the stationary state conception of the basis for the shape of the graph (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *c*, *d*). By means of the logistic equation for independent rod and cone contributions, the two parts of the composite flicker recognition curve have been separated (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *d*). To test the propriety of this procedure, and also to examine further the nature of the variability of the critical illumination, it was planned to obtain the F-I_m curve for several other kinds of fresh water teleosts. Suitably chosen hybrids of these forms might then be tested. The expectation was that the flicker recognition curves would not be the same in position or in shape in the different fishes. It could then be ascertained whether the same method of dissecting the curve into rod and cone contributions gives a rational result of the same sort in the several cases. The possibility obtains that a genetic basis could be indicated for the differences to be discovered. Similar considerations apply to the data of variation of performance in the response to flicker.

Three additional forms have been carefully examined by the procedure described in our previous papers; these and the *Enneacanthus* (*Lepomis*) earlier studied provide four quite different curves in which log I_m is given as a function of F. The analysis of these data, given in the present paper, appears to provide a very precise kind of confirmation of the correctness of the proposals we have already made in our treatment of the data on *Enneacanthus*.

The flicker response property of a given animal can be measured or expressed only by defining this property in terms of a system of coordinates—it is the shape and position of the whole curve which is significant. Properties of the curve as a whole alone will permit comparisons of flicker sensitivity among different forms. The curve is reproducible among individuals of the same type, and differs in different types (species); it is therefore a constitutional property. The adequacy of a formulation of the shape of the curve, or of a method of interpreting its shape, can therefore be tested by means of experiments designed to reveal whether elements essential to the proposed interpretation behave in inheritance as other constitutional properties are known to do. The general situation has already been developed in the similar analysis of the geotropic performance of rats (Crozier, 1929; 1935; Crozier and Pincus, 1929–30 *a*, *b*; 1931–32 *a*, *b*; 1935–36). This may very well involve, of course, something more than simple matters of dominance and single factor differences.

The fishes involved[1] were (*a*) a clonal strain of the swordtail ("Green Helleri") *Xiphophorus helleri*; (*b*) a clonal strain of the Black Platy ("Golden Helmet"), *Platypoecilius maculatus;* and (*c*) an inbred strain ("Black Helleri") derived from F_1(*a*) ♀ × (*b*) ♂ by backcrossing fertile F_1 females to (*a*) ♂. The Black Helleri stock is uniform and stable. (*d*) In addition, a clonal strain of Red Platy (*P. maculatus rubra*) has been available for comparison, as well as (*e*) a stock of *P. variatus* and (*f*) the F_1 generation hybrids between (*a*) and (*e*)[1]. We are under obligation to Dr. C. P. Haskins for assistance in obtaining these stocks. We have also tested (*g*) another species of sunfish, *Eupomotus gibbosus*, for comparison with our *Enneacanthus*. The possibility exists that different species and lines of *Platypoecilius* and

[1] For data of the status and interrelations of these types *cf.* Bellamy (1924), Gordon, (1927, 1931), and Fraser and Gordon (1929).

of sunfish, as well as others, may exhibit essentially similar flicker response curves, with or without significant differences in the variability of performance. Changes encountered in the progeny of species crosses would then be of added significance.

Our main purpose has been to discover whether (1) different flicker recognition curves would be encountered in the several species, and if so whether (2) the forms of the specific curves could be modified as result of crossing. We are not in position to discuss the mode of inheritance of the changes actually found; with slightly different material which it is proposed to utilize subsequently, it may be possible to do this with some detail and precision. Without being required, however, to provide a scheme of a mechanism of factorial inheritance in this case, we do obtain evidence which definitely points to the hereditary determination of properties of the curve of critical illumination as a function of flicker frequency. There is also secured a rather neat justification of our procedure for the separation of rod and cone components of the curve.

II

PROCEDURE

The observational procedure was that previously described in detail (Wolf and Zerrahn-Wolf, 1935–36*b*; Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *a*,*b*,*c*,*d*). Determinations of critical illumination were made at fixed flicker frequencies F, at temperature 21.5°C. For each type of fish the same 10 numbered individuals were used throughout the tests. At each F 3 readings were taken on each individual, previously dark adapted. The average of these was taken as I_1, the mean of the 10 I's = I_m, from which P.E.$_{I_1}$ was computed.

The basis of the measurements resides in the behavior of the fish. With any one form, the variation in I_1 among individuals is much greater than among the three measurements on each fish (except at $F_{max.}$). The values of P.E.$_{I_1}$ therefore enable one to study the variation of performance as a function of intensity, F, species, and ultimately of other variables. The variation of performance is not a matter of persistent individual differences within a given stock. The relative sensitivities of the 10 individuals in one stock, in any one test, may be expressed by rank order numbers in the sequence of increasing intensities required to obtain response. These relative sensitivity positions show no correlation in successive tests. The mean rank order numbers are distributed quite at random (Table I). This corresponds in all details with what we have already learned in our measurements with the sunfish (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *b*,*d*). The basis for this interpretation of the variation of I_1, and for the method of averaging

employed, has been tested in several ways. The presumption is, that by taking three successive readings with each individual fish (*a*) the chance of gross error is reduced, (*b*) disturbances possibly introduced by handling the fish and transferring it to the observation chamber are minimized and, (*c*) the average of three such readings should reduce the error of estimation of I_c, the critical illumination, be-

TABLE I

A summary of mean rank orders of sensitivity in sets of measurements with three types of fishes, showing the chance distribution of relative sensitivities in each type, as seen in successive sets of measurements.

	Mean of individual average rank order Nos.	Maximum departure of an individual mean	Differences between extreme individual means
Xiphophorus helleri (31 sets; $n = 10$ individuals)	5.47±0.490	2.86 × P.E.$_1$	2.09 × $\sigma_{dif.}$
Platypoecilius maculatus nigra (22 sets; $n = 10$)	5.50±0.659	2.69 × P.E.$_1$	3.0 × $\sigma_{dif.}$
Black Helleri (hybrids) (21 sets; $n = 10$)	5.50±0.917	2.75 × P.E.$_1$	3.1 × $\sigma_{dif.}$

TABLE II

Comparison of variation of I_1 as obtained in parallel tests with a set of ten Little Sunfish (*Eupomotus gibbosus*) at two flicker frequencies (F) in which (A) each I_1 is the average of three successive readings with each individual and (B) the readings are taken in three successive series of one observation on each fish; I_m is not significantly different in A and B, but in the latter case P.E.$_{I_1}$ is slightly but significantly lower. See text.

F =	6		35	
	log I_m	log P.E.$_{I_1}$	log I_m	log P.E.$_{I_1}$
Method A	$\bar{5}$**.2378**	$\bar{7}$.7837	**0.1335**	$\bar{2}$.3282
Method B	$\bar{5}$**.2435**	$\bar{7}$.5680	**0.1345**	$\bar{2}$.2028

cause the fish will be in about the same intrinsic reactive state. If instead of following this procedure, three sets of single readings on each fish are taken in succession, P.E.$_{I_1}$ calculated in the same way should then be *less* than that obtained for the same individuals under the same conditions by the first process, although larger if calculated from the mean of the 30, because the reactive condition of each fish presumably fluctuates more in the longer period over which its

readings are spread—the observations tend therefore to be less clumped in their distribution. Results from such a test are given in Table II. The fishes used were 10 Little Sunfish (*Eupomotus gibbosus*); they were examined by the routine method at four flicker frequencies (*cf.* Table III), and then at two of these by the method of testing each fish in succession, running through the set 3 times; between tests each fish was in the thermostat in the dark. Analysis by variance test of the individual records shows the same kind of variation among successive readings on one fish as among equivalent readings on different fishes, whereas by our standard procedure the variation is predominantly from fish to fish, although this variation

TABLE III

Comparisons of mean critical illuminations (I_m) at 21.5°C. for various types of fishes at several flicker frequencies (F); showing that the various pure types of *Platypoecilius* agree as regards log I_m and log P.E.$_{I_1}$ (*cf.* Fig. 1); and that two species of sunfish agree as concerns I_m, but differ significantly as to P.E.$_{I_1}$.

Species	F				
	6	9	20	30	35
Black Platy (n = 10)	$\bar{3}.2480$ $\pm\bar{5}.8606$	$\bar{1}.3535$ $\pm\bar{3}.9244$	0.1216 $\pm\bar{2}.5050$	0.4553 $\pm\bar{1}.0077$	
Red Platy (n = 4)	$\bar{3}.2721$ $\pm\bar{5}.8392$	$\bar{1}.3699$ $\pm\bar{3}.9112$	0.1415 $\pm\bar{2}.7622$	0.4704 $\pm\bar{2}.6522$	
P. variatus (n = 4)	$\bar{3}.2725$ $\pm\bar{5}.9240$	$\bar{1}.3608$ $\pm\bar{3}.7337$	0.1248 $\pm\bar{2}.5112$	0.4643 $\pm\bar{2}.5606$	
Sunfish *Enneacanthus** (n = 12)	$\bar{5}.2385$ $\pm\bar{6}.4814$	$\bar{3}.7983$ $\pm\bar{3}.0934$	$\bar{1}.2591$ $\pm\bar{2}.2480$		0.1418 $\pm\bar{1}.1106$
Little Sunfish *Eupomotus* (n = 10)	$\bar{5}.2378$ $\pm\bar{7}.7837$	$\bar{3}.7835$ $\pm\bar{4}.0969$	$\bar{1}.2620$ $\pm\bar{3}.5519$		0.1335 $\pm\bar{2}.3282$

* Data from Wolf and Zerrahn-Wolf, 1935–36*a*, and Crozier, 1935–36.

is randomly distributed as regards the individuals when the whole lot is repeatedly tested (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *a*, *c*, *d*). In the second case, as expected, I_m is identical but P.E.$_{I_1}$ is definitely and significantly less than in the first; but the rectilinear relationship between P.E.$_{I_1}$ and I_m is not distorted. The sunfish has for this test the convenient advantage that the latitude of scatter of P.E.$_{I_1}$ (a measure of $\sigma_{\text{P.E.}_I}$) is less (at this temperature) than with several of the other forms we have studied.

The response upon which the measurements rest is a swimming movement in which the fish follows the rotating stripes. The critical illumination, I_c, is the threshold intensity, obtained by smoothly increasing the intensity from a very

low level until the fish just begins to move with the revolving vertical stripes. (Description of apparatus in Crozier, Wolf, and Zerrahn-Wolf, 1935-36 *a*, *b*.) Quick, abrupt increases of illumination may give a "shock reaction" which has nothing to do with the responses indicating discrimination of light and dark bars. Fishes used in the experiments are kept in the laboratory for a certain length of time, under a regular regime, in aquaria each containing a single individual. Not infrequently the first set of measurements is more irregular, and its mean less reproducible, than is true with subsequent sets. For this reason, and to check the possibility of progressive changes due to alterations of behavior of the fishes, or to education of the observer in the operations of measurement with them, the readings are taken in a random succession of magnitudes of the fixed flicker frequencies; and it is arranged that duplicate sets of determinations are made at certain points. The values of I_m, the mean critical intensities, and the associated indices of dispersion of I_1, provide a basis for evaluating the inner coherence of the data obtained with each type of animal used.

The observations, and their dispersions, may be influenced by (1) properties of the fishes and (2) by properties of the observer and apparatus; the relation between (1) and (2) determines the recorded measurements. Observers and apparatus being the same, the functioning of the observer may nonetheless be influenced in an important way by specific differences in the deportment of the fishes. The types used in the experiments now discussed show characteristic differences in behavior, not correlated with sex. These differences are important for the interpretation of the variation data (sections III and V).

Xiphophorus was hyperactive, "jumpy," in the first trials, and very reactive to minor vibrations in the apparatus. After several days of preliminary tests they were quieter. The reaction at a critical illumination is sharp and clear; the fish swims with the moving stripes, except quite rarely when there occurs a backward motion, opposite in direction. Frequently responses cease after a first "jump," even if the intensity is increased beyond the threshold; the fish then stays close to the wall of the container, with a sinuous flexure of the body and rapid fin movements. On the whole, the responses were "better," from the standpoint of the observer, at low flicker frequencies than at high.

Platypoecilius maculatus, var. *nigra* and var. *rubra*, were quite similar to one another, but more sluggish than the swordtails, and less reactive than *P. variatus*. The Black Platy and the Red exhibit a pronounced "jerk" at the critical illumination and swim with the moving stripes in a path parallel to the wall of the container. At high flicker frequencies the initial movement is often followed by assumption of a position pressed against the wall of the container, with maintained rapid movements of the fins.

Platypoecilius variatus is more reactive, and should probably prove on the whole a better experimental animal than the other two types. Good swimming movements, following the moving stripes, are observed at the critical illumination even with high flicker frequencies. At low flicker frequencies the response is often like that of a galvanometer needle, the fish staying in the center of the container but

turning with the moving stripes and at the same speed. This behavior is like that of the sunfish.

Black Helleri—the hybrid type previously described—shows at first the jumpiness characteristic of the swordtail, but the reactions at threshold illumination for response to flicker are later less sharp. The general behavior is much like that of the Platys.

The Little Sunfish (*Eupomotus gibbosus*) is really indistinguishable in its reactions from *Enneacanthus* (Wolf and Zerrahn-Wolf, 1935–36*b*; Crozier, Wolf, and Zerrahn-Wolf, 1935–36*a*).

These characteristic differences must be expected to play some part in the determination of the dispersions of I_c and of I_1; the extent of their significance cannot be precisely foreseen, and remains to be examined experimentally, as we have already remarked (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *d*). The indication thus far is that differences such as those recorded are of relatively minor significance. This becomes of special interest when the dependence of P.E.$_{I_1}$ upon I_m (or the reciprocal dependence) is considered for various forms (section V).

III

For the purposes of our analysis it was desirable to ascertain (1) if fishes of the same generic type give similar flicker response curves, and (2) if fishes of types quite different structurally and ethologically give flicker responses which are quantitatively diverse. The major portion of this paper is occupied with the second point. The physiological significance and the presumptive utility of the establishment of diverse flicker response curves for genetically disparate stocks, including the weight to be placed upon the results of genetic tests, in a sense depends upon the outcome of attempts to discover if genetically similar types provide rather closely comparable flicker curves. The phrase "genetically similar types" must be understood as used in a broad, loose sense—in the sense, namely, that varieties and species of *Platypoecilius* for example are more like one another than they are like the swordtails or the sunfishes. Changes in the flicker response curve which appear, then, in progeny resulting from the cross-breeding of two contrasting types cannot be regarded as induced by minor or secondary genetic differences, or as superficial accidents, but must be interpreted as due to rather deep-seated and significant processes genetically determined.

We have compared the critical illuminations for response to flicker at four spaced flicker frequencies, two in the rod portion of the curve and two in the cone portion, for three types of Platy (including two species as ordinarily recognized), and two species of sunfish. The results are given in Table III. It is apparent that there are minor and doubtfully significant differences in the values of I_m among the three Platy forms, and only small differences between the values for the two sunfishes. The variation data we will deal with in a later section. The general concordance of the measurements for the Platy forms is such as to indicate comparative uniformity in the character of the respective flicker response curves;

and the same is true of the two sunfishes. It is to be understood that more complete determinations of the forms of the curves, like those obtained for the other species we are to consider, would very probably establish minor varietal and specific differences; but in contrast to the differences between the curves for sunfishes, Platys, and swordtails, such differences are of slight consequence. The indication is that the intratype differences appearing in Table III are comparable to the changes brought about in one individual by altering the temperature (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *c*, *d*). They might be obscurely correlated with ethological factors, or even determined by the sizes of the eyes. The generic differences between flicker curves with which we shall have to deal are of a different order.

IV

We have determined with all possible care, and over the greatest manageable range of flicker frequencies, the curve of I_m as a function of F for a group of *Xiphophorus helleri* (*X*), another of *Platypoecilius maculatus nigra* (*P*), and for the Black Helleri hybrids. The latter had been obtained from backcrosses of certain $F_{1\ X \times P}$ females to *X* males (see section II). Each group contained 10 individuals; in each case the same 10 individuals were used throughout; no individual differences appeared in a group (section II). The results are collected in Table IV. We shall discuss first the curves for *Xiphophorus* and *Platypoecilius* comparing them with our curves for the sunfish *Enneacanthus* (Wolf and Zerrahn-Wolf, 1935–36*b*; Crozier, 1935–36; Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *a*, *d*); the curve for the hybrids is considered in section VI.

Fig. 1 shows certain very definite differences among these three flicker response curves. The differences are not obliterated if the flicker frequency is in each case plotted as a percentage of the respective maximum F, or if intensity is so considered. The differences involve matters of rod and cone $F_{max.}$, slopes of cone and rod portions, and position on the *F-I* grid.

We have shown that at 21.5° the curve for *Enneacanthus* can be, as to its cone portion, fairly well described by the equation which has been used to describe human flicker fusion data (Hecht, Shlaer, and Smith, 1935), but that the fit is not really adequate and in particular systematically fails at other temperatures (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *d*). This equation (Fig. 2) also fits fairly well

TABLE IV

Mean critical illuminations, I_m (millilamberts) for response to flicker, as a function of flicker frequency (F), in homogeneous groups of *Xiphophorus*, *Platypoecilius*, and extracted backcross hybrids of these (Black Helleri, H), with the probable errors (P.E.$_{I_1}$) of the dispersions.

F	*Xiphophorus helleri*		*Platypoecilius maculatus nigra*		Hybrids	
	log I_m	log P.E. I_1	log I_m	log P.E. I_1	log I_m	log P.E. I_1
per sec.						
1			$\bar{6}.2911$	$\bar{8}.8502$		
2	$\bar{7}.9379$	$\bar{8}.5393$	$\bar{6}.8445$	$\bar{7}.2472$	$\bar{6}.4690$	$\bar{7}.0785$
3	$\bar{6}.3420$	$\bar{7}.0969$	$\bar{5}.3579$	$\bar{7}.9183$	$\bar{6}.9547$	$\bar{7}.5827$
4	$\bar{6}.6052$	$\bar{7}.2299$	$\bar{5}.9042$	$\bar{6}.1386$	$\bar{5}.3906$	$\bar{7}.9760$
5	$\bar{6}.8193$	$\bar{7}.4324$	$\bar{4}.8977$	$\bar{5}.2485$	$\bar{4}.2669$	$\bar{5}.0784$
	$\bar{6}.8172$	$\bar{7}.2250$	$\bar{4}.8911$	$\bar{5}.2558$		
6			$\bar{3}.2470$	$\bar{5}.7999$	$\bar{4}.9440$	$\bar{5}.5780$
	$\bar{5}.3721$	$\bar{7}.9653$	$\bar{3}.2489$	$\bar{5}.9212$		
	$\bar{5}.2826$	$\bar{7}.7148$				
	$\bar{5}.3539$	$\bar{7}.8341$				
7	$\bar{5}.8399$	$\bar{6}.2390$	$\bar{2}.1367$	$\bar{4}.3115$	$\bar{3}.4603$	$\bar{4}.2074$
	$\bar{5}.6078$	$\bar{7}.9353$				
	$\bar{5}.6964$	$\bar{7}.9857$				
8			$\bar{2}.8251$	$\bar{3}.3579$	$\bar{2}.0546$	$\bar{4}.5320$
	$\bar{4}.3191$	$\bar{6}.9312$				
	$\bar{4}.3265$	$\bar{5}.0042$				
9	$\bar{3}.2469$	$\bar{5}.8295$	$\bar{1}.3535$	$\bar{3}.9244$	$\bar{2}.6316$	$\bar{3}.1471$
	$\bar{3}.2378$	$\bar{5}.8374$				
10	$\bar{2}.3164$	$\bar{4}.8594$	$\bar{1}.5967$	$\bar{2}.2185$	$\bar{2}.8913$	$\bar{3}.4250$
12	$\bar{2}.6651$	$\bar{3}.0909$			$\bar{1}.3296$	$\bar{2}.0065$
15	$\bar{2}.8918$	$\bar{3}.2650$	$\bar{1}.9056$	$\bar{2}.2253$		
16					$\bar{1}.6557$	$\bar{2}.1072$
20	$\bar{1}.1900$	$\bar{2}.1055$	0.1216	$\bar{2}.5050$	$\bar{1}.9375$	$\bar{2}.5064$
	$\bar{1}.1772$	$\bar{3}.3939$	0.1106	$\bar{2}.5552$	$\bar{1}.9330$	$\bar{2}.2862$
25	$\bar{1}.4708$	$\bar{3}.9719$	0.2911	$\bar{2}.8185$	0.1741	$\bar{2}.8874$
	$\bar{1}.4598$	$\bar{3}.9677$				
30	$\bar{1}.7571$	$\bar{2}.1169$	(0.3838) 0.4553	($\bar{2}$.9631) $\bar{1}$.0077	0.4502	$\bar{2}.8120$
35	0.1520	$\bar{2}.9002$	0.6676	$\bar{2}.9849$	(0.8445) (0.8354)	($\bar{1}$.8874 $\bar{1}$.0849
38	0.4488	$\bar{2}.9271$				
40	0.7213	$\bar{1}.2224$	$\bar{1}.0390$	$\bar{1}.0637$	$\bar{1}.4065$	0.0916
42	$\bar{1}.3057$	$\bar{1}.5398$			$\bar{2}.1691$	0.3228
	$\bar{1}.2785$	$\bar{1}.5864$				
42.5					$\bar{2}.2838$	0.3581
43	$\bar{2}.2174$	0.2309	$\bar{1}.3290$	$\bar{1}.6012$		
	$\bar{2}.2177$	0.3955				
46			$\bar{2}.2953$	0.1021		

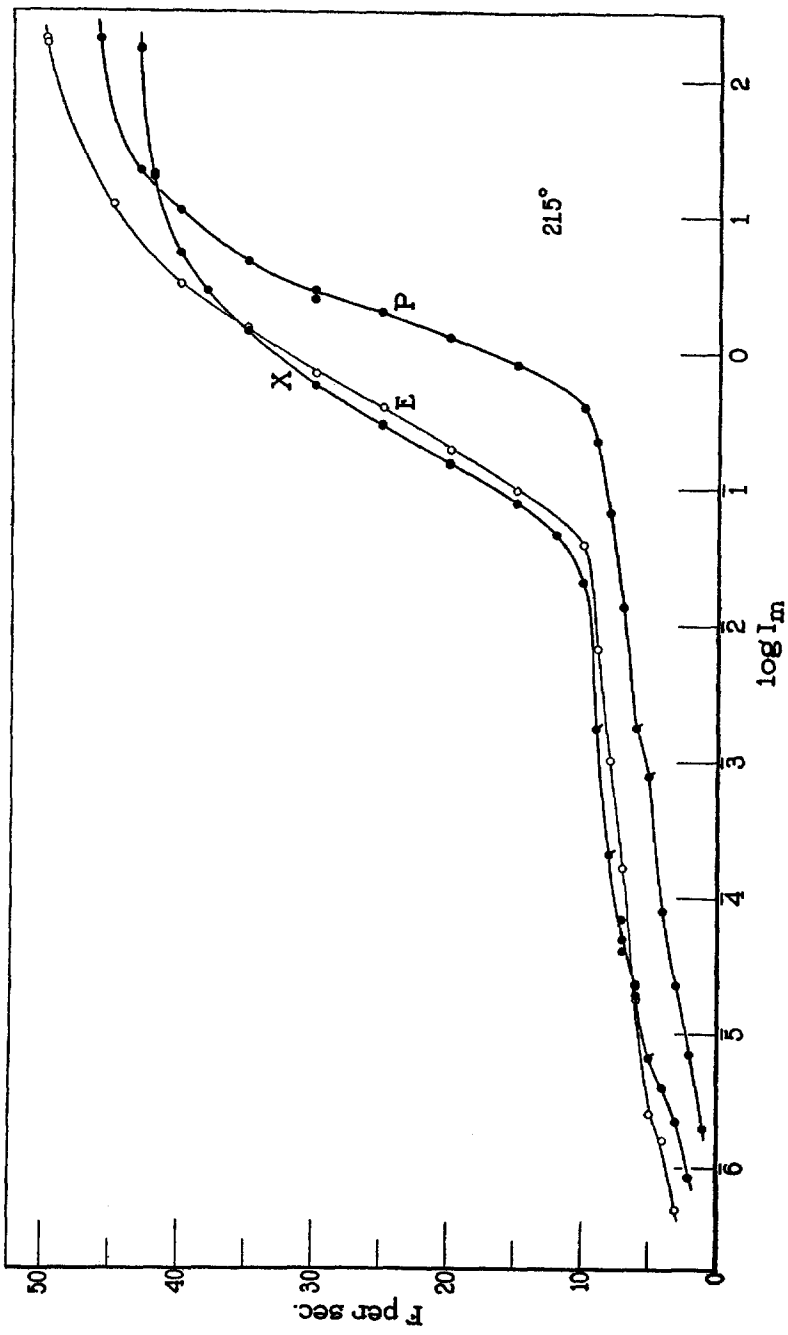

FIG. 1. Mean critical illumination I_m for reaction to flicker, at 21.5°C., as a function of flicker frequency F (flashes per second), for *Xiphophorus* (*X*), *Platypoecilus* (*P*), and *Enneacanthus*. Data in Table V; the points with tags are coincident duplicate determinations of I_m; data for *Enneacanthus* from previous papers. (The one aberrant point on the *P* curve is from the first experiment (see Table V) with these fishes.)

the data on *Xiphophorus*, but cannot be used for the *Platypoecilius* data without the impossible assumption that the photochemical process is of about the 1.7th order. There would also be involved a

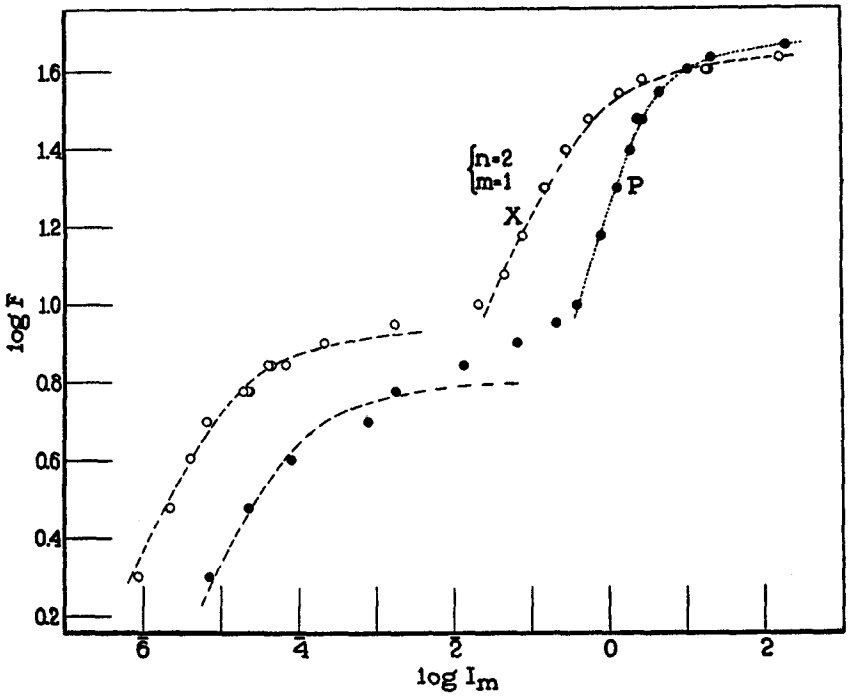

FIG. 2. Critical illumination, log I_m, as a function of log F, to illustrate results of fitting by the stationary state equation (Hecht, Shlaer, and Smith, 1935): $KI = F^n/(F_{max}-F)^m$, with $n = 2$, $m = 1$. For *Xiphophorus* (*X*) the fit is passable (as for *Enneacanthus* at this temperature), both with rods and cones; for *Platypoecilius* (*P*) the fit for the rod portion is not sufficient, while the cone part (dotted) would require fractional exponents. See text.

special assumption regarding the manner of overlap of rod and cone contributions to the complete curve.

On the other hand we have shown (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *a*, *d*) that the rod and cone portions of the *Enneacanthus* curve are each accurately described by the logistic

$$F = F_{max.}/(1 + e^{-p \log I/I_i}),$$

with different values of $F_{max.}$, p, and I_i for the two parts and with a particular sort of addition of critical F's in the region of overlap. The value of p is independent of temperature.

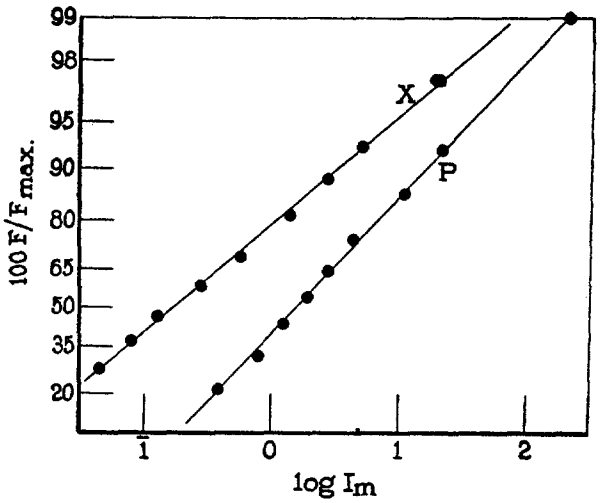

FIG. 3. The upper (cone) segments of the F-I_m curves for *Xiphophorus* (*X*) and *Platypoecilius* (*P*) on a log logistic grid; these segments are presumed not to be complicated by the addition effects due to rods.

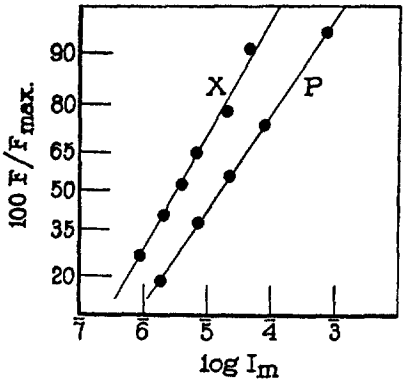

FIG. 4. The rod segments of the F-I_m curves for *Xiphophorus* (*X*) and *Platypoecilius* (*P*) on a log logistic grid.

This formulation applies precisely to the curves for *Xiphophorus* and *Platypoecilius* (Figs. 3 and 4). It also applies to the data from our

hybrid group (H), discussed subsequently. For convenient comparison the descriptive constants for the several forms have been collected in Table V. It is apparent that there is no necessary association between values of $F_{max.}$, p, and I_i.

The logistic has been used for these curves partly as a matter of convenience, because it efficiently describes the data in diverse instances, because of the nature of the dispersions of I_1, because it permits testable extrapolations, because it gives a transformation in which the F-I_m and F_m-I curves become identical (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *d*), and because it is susceptible to suggestive interpretation. In the cases to which it does not completely apply (bee, and *Anax*: Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *b*, *c*) there

TABLE V

Constants for the description of the flicker response curves of four types of fishes. The equation used is that of the logistic

$$F = F_{max.}/(1 + e^{-p \log I/I_i})$$

	Rods			Cones		
	$F_{max.}$	p	I_i	$F_{max.}$	p	I_i
1. *Enneacanthus*	8.0	1.83	0.000,003	50.5	1.57	0.372
2. *Xiphophorus*	7.7	1.95	0.000,003	43.1	1.90	0.177
3. *Platypoecilius*	5.4	1.38	0.000,016	46.5	2.29	1.62
4. Black Helleri hybrids ex. (2) and (3).	5.4	2.00	0.000,006	43.1	1.90	0.832

appear to be structural factors which interfere with its application at low flicker frequencies. It is to be noted, however, that a probability integral of course fits the data about as well—perhaps even better, and might ultimately prove theoretically useful.

The curves for sunfish, swordtail, and Platy (Fig. 1) differ in form, proportions, and position, although their morphology is similar. The two branches of each curve must therefore be assumed due to homologous features of the several fishes. The connection between the rod and cone parts of each curve shows distinct differences in form. This gives opportunity to test the efficacy of the method we have used to separate the rod contribution from that due to the cones (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *a*, *d*). We have given reasons for assuming that the complete rod flicker curve, if obtainable separately,

should rise to a flat maximum and then decline to zero; the cone curve, starting at zero, is in terms of F added to that for the rods. The obvious "bump" on the flat portion of the composite curve (Fig. 1) is due to the entrance of the cone contribution. If this idea be correct, the logistic extrapolation must give a consistent result with flicker curves of very different shapes. The extrapolated curves are plotted in Fig. 5. They clearly do approach zero at the proper place in each case. This is also true with the hybrid curve discussed in section VI.

The decline of the rod contribution to the recognition of flicker which is contemplated in this treatment, and required by the theory

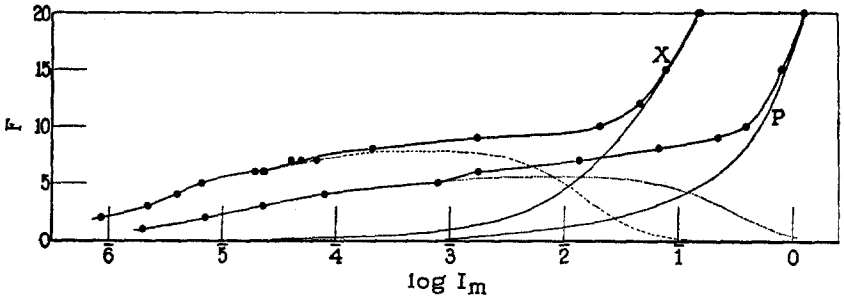

FIG. 5. The lower portions of the F-log I_m curves for swordtail (X) and Platy (P), showing the extrapolation of the logistics fitted to the cone parts (Fig. 3). The difference curves are shown dotted. See text.

of the temperature effect upon the flicker curve, is to be understood as due to the declining number of rod elements, or rod-connected central elements, in which after-image decay is fast enough to permit their participation in the recognition of flicker with the intensities at which a sufficient number of rod + cone elements can so function to an extent required to excite response at the given fixed values of F. This number should exhibit a reversed cumulative population distribution as a function of increasing intensity. The difference curves plotted in Fig. 4 do in fact exhibit this character, as Fig. 6 shows.

In the case of *Xiphophorus* the intensity at which the cone contribution begins to be perceptible exhibits a fluctuation at different times (Fig. 1; Table IV) which is not encountered with the three other forms

tested. The reality of this fluctuation has been checked by means of paired sets of observations at two flicker frequencies, one of these being in the region of the cone curve's start, the other at a nearby level. Very precise concordance was always obtained, except at $F = 6$, 7, and 8; at $F = 5$ or 9, new determinations of I_m showed the sort of agreement which experience has demonstrated to be characteristic at other parts of the *Xiphophorus* flicker curve, in that for *Platypoecilius*, in the hybrids, and in the four curves determined for *Ennea-*

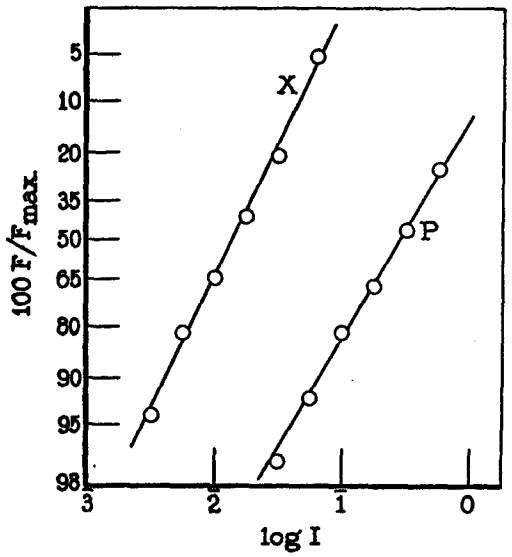

FIG. 6. The difference curves computed (graphically) to depict the decline of rod contribution to the composite flicker curve (Fig. 5) are described by a logistic; the exponent is not the same as for the rising branch of the rod curve—for *Xiphophorus* (X), $p = 2.10$; for *Platypoecilius* (P), $p = 3.44$.

canthus. The differences at $F = 6, 7, 8$ (greatest at $F = 6$) are statistically significant; the scatter of the individual sets of measurements is not affected, as the plot of P.E.$_{I_1}$ *vs.* I_m shows no disturbances at this level (*cf.* Fig. 7). The fluctuation therefore signifies a real fluctuation in the threshold intensity for cone function in flicker recognition. This may be compared with day-to-day fluctuations in the mode of junction of rod and cone dark adaptation curves for a human eye; the intensity for threshold discrimination which supplies the measure of

the progress of dark adaptation depends upon the activation of a threshold number of elements, and thus upon the intensity at which the curve of the population distribution of cone excitabilities begins to rise. The term "element" is here necessarily defined in terms of units capable of involvement, under the given conditions, in the determination of the index response. It is significant that the one case we have encountered of unsteadiness in the values of I_m occurs at the one place in a flicker curve where on general grounds it might be expected. A certain interest would attach to the investigation of this case with attention to the experimental modification of the onset of the cone curve, as by nutritive disturbances or state of adaptation, and also to the determination of F_m as a function of fixed intensities; for the moment this must be postponed.

V

Variability

In the measurements with *Xiphophorus* and *Platypoecilius* the dispersions of I_1 at the various flicker frequencies are related to the mean critical intensities for response (I_m) by the same rule found with bee, *Anax*, and sunfish.[2] P.E.$_{I_1}$ is directly proportional to I_m; but with the fishes the proportionality factor is different, and the origin is shifted, in the region of cone function. The regularity and the properties of this relationship supply an important demonstration of the inner consistency of each of the groups of measurements. We have to inquire as to its meaning for the theory of the response to flicker.

The data for *Xiphophorus* and *Platypoecilius* are given in Fig. 7. The equations for the lines of central tendency, up to the "break," are respectively: $\text{P.E.}_{I_1} = kI_m \pm B\,\text{P.E.}_{I_1}$,

$$\begin{aligned} \textit{Xiphophorus} &- \text{P.E.}_{I_1} = 0.383\, I_m \pm 0.544\ \text{P.E.}_{I_1} \\ \textit{Platypoecilius} &- \text{P.E.}_{I_1} = 0.362\, I_m \pm 0.469\ \text{P.E.}_{I_1} \\ \textit{Enneacanthus} &- \text{P.E.}_{I_1} = 0.389\, I_m \pm 0.402\ \text{P.E.}_{I_1} \end{aligned}$$

where k is the proportionality factor and B is the "spread coefficient" given by the width of the ribbon in Fig. 7. (This might be expressed

[2] Crozier, 1935–36; Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *b*, *c*, *d*.

as $[1 + B]$ P.E.$_{I_1} = kI_m$.) The best fitting lines were drawn in the arithmetic center of the P.E.$_{I_1}$ bands; as found previously, this gives a symmetrical distribution of the observations on either side of the

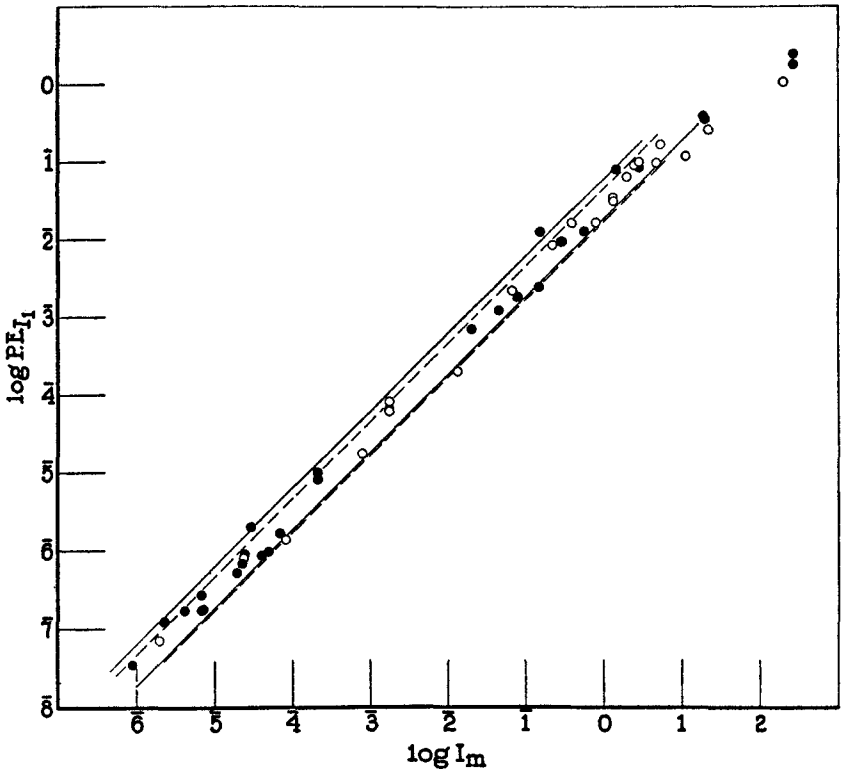

FIG. 7. The variation of I_1 is a rectilinear function of I_m, *i.e.* log P.E.$_{I_1}$ = log I_m + log K, for *Xiphophorus* and *Platypoecilius*; above a certain intensity (as in sunfish) the proportionality constant changes, in the region of exclusively cone function, and a constant must be added. Details discussed in the text.

central line (*cf.* Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *b*, *c*, *d*; Upton and Crozier, 1936; Crozier and Holway, 1937).

A break presumed to be associated with the establishment of exclusively cone function in the determination of I_m was also clear in the data for *Enneacanthus* (Crozier, 1935–36; Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *d*). Above a certain intensity P.E.$_{I_1}$ is no longer

directly proportional to I_m, but to I_m + a constant (or, to pass through the origin, a constant must be subtracted from the observed values of P.E.$_{I_1}$). The intensities at which the breaks occur can be stated only approximately, in view of the breadth of the band which includes the observations, but for the three fishes these were at about

I_{mb} = 0.10 millilambert for *Enneacanthus*,
= 3.16 millilamberts for *Xiphophorus*,
= 2.00 millilamberts for *Platypoecilius*.

The break is less clear in the Platy graph (in the hybrids subsequently discussed it is scarcely apparent at all), so that the magnitude of the corrective constant can be given only in a very approximate way; above the break intensities I_{mb} the values of P.E.$_{I_1}$ must be diminished by

0.10 for *Enneacanthus*,
0.080 for *Xiphophorus*,
0.054 for *Platypoecilius*.

The question naturally arises as to whether the proportionality factors k, the antilog intercept constants in Fig. 7, differ significantly. We have assumed that the variability of critical illumination measures essentially a property of the reacting mechanism of the animal, and we have discussed its relation to temperature upon this basis. It may be important for such a view that at any given intensity the values of P.E.$_{I_1}$ are rather similar in the various forms which have been tested. Considerations directly arising from the quantitative properties of P.E.$_{I_1}$ as obtained in the measurement of diverse visual functions with the same organisms (Crozier, 1935–36; 1936), by means of tests fundamentally involving intensity discrimination in all cases, can be understood only on the assumption that the dispersions of the measurements of critical intensities, and the dependence of these dispersions upon I_m, are due to a fluctuating property of the reacting organism and not to experimental error in the ordinary sense; they are not identical at given intensity. We have noted previously (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *d*) that in the sunfish uncontrolled conditions may introduce consistent temporary changes in P.E.$_{I_1}$ without altering I_m at fixed flicker frequencies; these are correlated with obvious departures from the customary mode of behavior of the fishes. At the

same time, alteration of temperature produces diverse effects upon P.E.$_{I_1}$ and upon I_m, of identical sort in two quite different organisms (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *c,d*) and not correlated with obvious changes in the character of the motor response. These findings indicate that a probably interesting outcome would result from systematic attempts to modify P.E.$_{I_1}$ experimentally, such as we have pointed to in our preceding paper. In the meanwhile, however, it is desirable to review and codify the findings in our routine determinations of the variation of critical illumination for response to flicker. It is not to be lost sight of that naïve applications of probability theory to such dispersions may be quite inappropriate. In an ordinary physical measurement, as of the precise length of a steel bar by micrometer setting, under continuous constant conditions, the mean of the measurements is determined by the actual length and the individual settings are made under the "restoring agency" of the observer's appreciation of an incorrectness of setting. In measurements of critical illumination or of critical flicker frequency the observer is called upon to make a setting with respect to the index response, not to make a photometric match of intensities; three such readings on an individual are averaged to give I_1, and ten I_1's to give a mean. The mean I_e from a series of such readings has no meaning with respect to an individual determination, and σ_1 is merely an index of scatter. A clear discussion of these matters which is unusually helpful is given by Whitehead (1934). The contributions respectively made to the total scatter by (*a*) the observer and the process of observation, and (*b*) the intrinsic variability of the reacting organism, must be disentangled by suitable experimental tests. The behavior of the readings when subjected to a variance analysis test (*cf.* section II) only partially resolves the problem. In crudest form the alternatives are (1) that the properties of P.E.$_{I_1}$ as measured are a valid reflection of properties of the fluctuation of the reacting mechanism of the reacting organism, and (2) that they depend upon properties of observer *plus* procedure. It is to be inquired if the latter notion gives an adequate interpretation of the facts.

It could be expected on the first view (although not as a *necessary* consequence) that for quite different organisms the values of P.E.$_{I_1}$ might differ markedly at a given I_m, in measurements of the same type

of visual response—particularly if the respective curves of critical illumination as a function of flicker frequency should be strikingly different. Fig. 6 might be thought to indicate that such expectation is not really substantiated. The conclusion might then be, either that P.E.$_{I_1}$ reflects merely the error of observation, predominantly due to circumstances outside the reacting animal; or that, though proper to the organism, P.E.$_{I_1}$ is too nearly alike in different animals to be an analytical key to differences in excitability. (It is to be remembered, however, that regardless of the mechanism of determination of P.E.$_{I_1}$ it describes an attribute of the data which governs in an important way the uses which can be made of them when comparisons are made with the requirements of an interpretive theory of the response to flicker.)

The differences visible in the variability functions for *Enneacanthus*, *Xiphophorus*, and *Platypoecilius* are real enough, in the sense that their consistency makes them quantitatively significant. But it might be that they could arise through differences in the training of the observer, so that his appreciation of the reaction signal has improved; or they might be due to small, constant, characteristic, differences in the relation of the diverse reacting animals to the observer. The first contention is scarcely reasonable, however, because in the course of a month or more of intensive work with a single reacting species there is quantitatively no change in the magnitudes of P.E.$_{I_1}$ at given flicker frequencies; this has been repeatedly determined by means of reduplications of tests at the beginning and at the end of an experimental run; consequently there is no evidence that training of the observer has influenced P.E.$_{I_1}$. Moreover, this would quite fail to account for the self-consistent, characteristic properties of the spread coefficient k which measures the proportionate scatter of P.E.$_{I_1}$.

A distinction must certainly be made between (1) *variation of performance* and (2) *variation of intensity* of excitatory agency required to effect a given level of performance (Crozier, 1935). In the former it is clearly demonstrated by the analyses of geotropic orientation in young rats of inbred strains that variability of performance, as a function of intensity or of mean performance, is a constitutional property of the reacting animals, and is independent of the observer's errors of measurement (*cf.* Crozier, 1935) and of influences which

modify both performance and variation of performance as a function of constant external conditions. We can get a clue to the operation of the distinction between (1) and (2) in the case of our flicker experiments by considering the difference between (*a*) the variation of critical flicker frequency as a function of fixed intensities and (*b*) the variation of critical intensity as a function of fixed flicker frequencies. It has been shown that the two kinds of variation are mutually interdependent (Crozier, 1935; 1935–36; 1936; Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *a*, *b*). The former, (*a*), corresponds to fluctuations in *performance*; it has been interpreted as due directly to organic fluctuations in the mechanism basic to discrimination (by response) between the effects of flashes and the effects expressed as their after-images (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *c*). The latter, (*b*), corresponds to fluctuations in the frequency of a heart beat, for example, as a function of the temperature, for such fluctuations reflect variations in the potential (*intensity*) of the chemical process the velocity of which determines the interval between exhibitions of a fixed level of performance, namely the discharge of the pacemaker relaxation oscillator governing the frequency of beats (*cf.* Loeb, 1900; Hoagland, 1935; Crozier, 1929).

We may consider the general properties of these two classes of variations or fluctuations, as empirically obtained in diverse instances, in order to demonstrate (α) that the relation of P.E.$_{I_1}$ to I_m may be very similar, quantitatively, for diverse organisms in which the respective relations of I_m to a common independent variable may be quite unlike; and (β) that only slight differences between P.E.$_{F_1}$ for two organisms may result when pronounced differences between P.E.$_{F_1}$ occur. These expectations follow from the requirements of the idea that the observed variation is a property of the reacting organism. Hence approximate equivalence of P.E.$_{I_1}$'s at the same values of I_m for organisms with very different flicker curves cannot be accepted as evidence of common determination through common observational errors introduced by the uniformity of participating observer and procedure in each case.

A consistent conception of the observed relationships can be obtained from the standpoint that the variation encountered is basically a property of the reacting organism. The flicker response experi-

ments have the advantage that one can examine P.E.$_I$ and P.E.$_F$ concurrently in the same organism; and that the critical frequency F, with the dimensions of a speed, exhibits certain additive properties (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *d*) which help to justify its interpretation as a measure of the intensity (potential) of the inner driving force responsible for the index reaction, whereas I_m measures the mean exciting flux required to achieve this inner potential.

The relation of P.E.$_{I_1}$ to I_m has been obtained for (i) diverse series of measurements with the same organism and (ii) homologous series of measurements with different organisms. Expressed in the same units, P.E.$_{I_1}$ for the bee, as a function of I_m, is lowest in the case of flicker response,[3] 0.3 log unit higher in the visual acuity measurements, and the same when parts of the eyes are opaqued[4]; nearly a full log unit higher in measurements of the time-course of dark adaptation[5]; higher still (as a function of I_2) in measurements of intensity discrimination[6]; in the last instance P.E.$_{\Delta I}$ is directly proportional to ΔI, and is the same fraction of ΔI regardless of the widths of the stripes—although decreasing the width of the illuminated bars moves the values of I_1 and of I_2 (where $\Delta I = I_2\text{-}I_1$) to a higher level of intensities. These differences are quite significant statistically, and their order of increasing magnitude is not related to the chronological order of the experiments; the observer was the same person, the responses all involved discrimination of intensities exhibited on alternating stripes of a barred pattern. The order of increasing magnitudes of P.E.$_{I_1}$ does, however, correspond to certain differences in type of excitation involved in each experiment.

On the other hand, in flicker response experiments the relation of mean P.E.$_{I_1}$ to I_m for bee[3] and dragonfly larva (*Anax*)[7], at the same temperature, is practically identical, although the organisms are different and the index response used is very far from being the same in the two cases. The variation data for several fishes agree rather closely with those for bee and *Anax*, but in the same organism the

[3] Wolf, 1933–34; Crozier, 1935–36.

[4] Hecht and Wolf, 1928–29; Crozier, 1935–36.

[5] Wolf and Zerrahn-Wolf, 1935–36 *a*; Crozier, 1935–36.

[6] Wolf, 1932–33 *a*, *b*; Wolf and Crozier, 1932–33; Crozier, 1935–36.

[7] Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *b*, *c*.

proportionality factors for rod and for cone branches of the curve are quite different.

From such facts it is impossible to conclude that there is a determination of the quantitative dependence of P.E.$_{I_1}$ upon I_m through the activity of the observer. At the same time it is not proved that this is untrue, either.

An analogous situation arises from the investigation of temperature characteristics. The general fact encountered is that if heart beat frequencies are measured at various temperatures, or breathing movements, or frequencies of locomotor movements, the same number of determinations (on the basis of the same number of beats) being made at each temperature, then the latitude of variation tends to be about 8 to 10 per cent of the mean and is independent of temperature.[8] (In some cases[9] the latitude of variation may be less, although this depends partly upon the number of beats involved in a count.) There is no possibility of regarding this kind of variation as due to the observer or to the observational method. Yet it is of the same order of magnitude in quite different organisms, in which the absolute frequencies of the events observed differ enormously and for which the temperature characteristics are very unlike; in these respects it corresponds exactly to the properties of P.E.$_{I_1}$ as a function of I_m in the various flicker response curves we have obtained for different organisms under the same conditions. The parallel extends even to the occurrence of differences in the coefficient of variation on either side of a break in the curve connecting mean frequency of activity with independent variable; good cases have been recorded in temperature curves[10] which exactly correspond to changes in the ratio of P.E.$_{I_1}$ to I_m in the composite flicker response curves of fishes; in neither case do these changes in proportionality constant invariably occur, yet the difference in this respect between *Anax* and the fishes is real and striking.

The point has been made in the treatment of temperature characteristic data that the latitude of variation of a frequency for a given activity in the same individual may be the same fraction of the mean

[8] Crozier, 1929; 1935; Crozier and Stier, 1924–25; Crozier and Federighi, 1925; Pincus, 1930–31; Crozier, 1934–35.

[9] Crozier, Pincus, and Renshaw, 1934–35.

[10] Crozier and Stier, 1926–27; Crozier, 1934–35.

frequency independently of conditions other than temperature which modify either the mean frequency at given temperature or the temperature characteristic.[11] This closely parallels the general fact that two flicker response curves may be quite different while only slight differences appear in the plot of P.E.$_{I_1}$ as a function of I_m. On the other hand, our various Platy types give essentially the same flicker response curve, and the P.E.$_{I_1}$-I_m curves are indistinguishable, while the two sunfish genera (Table III) give F-I_m curves which are indistinguishable although for these two fishes the values of P.E.$_{I_1}$ differ by a factor of 10.

(With reference to the plot in Fig. 7 it is to be remarked that the difference between, for example, the variation data for *Xiphophorus* and Platy seems slight, owing to the scale and the use of logarithmic coordinates. The difference really amounts (for the lines of central tendency) to a consistent factor of 1.06; for others it is larger; if the figures could be adequately presented on an arithmetic grid, their divergence would be obvious. The "second order differences" are thus really considerable.)

The absolute values of P.E.$_{I_1}$, as a function of I_m, have about the same order of magnitude in very different sensory effects; for example, in measurements of auditory intensity discrimination (Upton and Crozier, 1936), in judgments of differences in lifted weights,[12] and in tests of visual functions with the human eye. Second order differences of the sort already noted occur in these cases also.

Another aspect of this matter is revealed by the interpretation of the Weber fraction $\Delta I/I_1$ (Crozier, 1935–36; 1936) as a function of I_1. From the flicker response curve and the visual acuity test curve for the same organism (the bee), the fraction may be computed on the assumption that $\Delta I = k\sigma_{I_2}$. The agreement is quantitative—despite the fact that the two curves are of different shapes and that

[11] The evidence for this independence has been extended in recent observations (Crozier) on the modification of temperature curves for pulse frequency (f) in *Amblystoma* larvae; it is found that σ_f is uniformly in direct proportion to f, and with the same proportionality constant although age and simple experimental treatments may profoundly modify pulse frequency at constant temperature and the form of the temperature-frequency curve.

[12] Holway, 1936.

P.E.$_{I_1}$ as a function of I_m is distinctly different for the two tests. This result, which also agrees quantitatively with the directly measured values of $\Delta I/I_1$, bespeaks an inner coherence of these data due to a property of the reacting animal and is incomprehensible on any other basis.

The point to this review of the general situation is, that the proportionality of P.E.$_{I_1}$ to I_m appears in a great diversity of situations, and that, grossly speaking, the proportionality constant is usually of the same order of magnitude. Closer examination discloses significant differences (second order differences, perhaps, but consistent and not to be ignored). Neither these differences nor the general order of magnitude of the variation is really to be accounted for in a reasonable way by referring it to error of observation. Its general magnitude is determined by some common feature of biological organization, its specific differences reflect specificities in the mechanisms concerned in particular events under scrutiny. It does not in any sense support "indeterminism" (*cf.* Crozier, 1929; 1935; *cf.* Cohen, 1936). Unquestionably it also includes error of observation in the classical sense, but the data do not belong in the category of measurements of physical quantities where the numerical observations are subject to a restoring (anti-dispersive) constraint. The separation of these constituents can only be achieved by experimental procedures, of the sort already indicated in studies of the precision of tropistic orientation.[13]

It remains to consider our second point (β), namely the mechanism whereby a general correspondence may be brought about between P.E.$_{I_1}$ and I_m even in the cases where the F-I_m curves and the values of P.E.$_{F_1}$ are very different. This consideration is important for the evaluation of the notion that P.E.$_{I_1}$ may be merely observational error and not an intrinsic property of the tested organism.

Take first the flicker response curves for *Anax* and *Enneacanthus* (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *a*, *b*). The discussion might with equal propriety have been begun by dealing with the curves of mean critical flicker frequency as a function of intensity, rather than (as we have done) with the F-I_m data. If we really have to do with error of manipulation, then (since the technic employed in the 2 cases was identical), P.E.$_{F_1}$ should be the same at identical values

[13] Crozier and Pincus, 1929–30*a*, *b*.

of F_m for the two organisms. Yet the P.E.$_{F_1}$ for the sunfish is uniformly about 100 per cent greater. Despite this difference, if from the width of the band formed by $F_m \pm$ P.E.$_{F_1}$ as a function of I one calculates the expected magnitudes of P.E.$_{I_1}$ as a function of I, it turns out that the two sets agree in the manner and to the extent which we have already noticed in the directly determined quantities. (The reverse calculation is discussed in Crozier, 1935–36; Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *a, b.*) One cannot attribute both the unlikeness of the P.E.$_{F_1}$ series and the similarity of the P.E.$_{I_1}$ data to common experimental error in each series; on the contrary, although including this factor, they are fundamentally both determined by a property of the reacting organism; this property, expressed as a variability of performance under constant conditions, carries with it the necessity for fluctuation in the potential of an exciting energy required to effect a given amount or extent of performance.

The theory of response to flicker which we have considered regards marginal recognition of flicker as a phenomenon of intensity discrimination. The effects discriminated are: (1) that due to the (average) action of a flash of light, and (2) that represented by the after-action (after-image) persisting during the intervals of no light (Crozier, 1935–36; Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *c, d*). We may suppose that recognition of flicker requires the establishment of a constant percentage difference between the total effect of a flash and the after-image effect. Variation in the number of active elements (N_a) concerned in producing the effect of a flash, from one time to another, will necessitate variations in the I_1 required to give recognition (reaction) at a fixed flicker frequency. Fluctuation in number of active elements will be due to fluctuation in the marginal portion of the excited fraction of the total elements concerned. When the intensity of light is higher, larger numbers of receptive elements are activated. The additivity of the critical flicker frequency, and the essential equivalence of F and N_a at constant temperature, has been discussed in an earlier paper (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *d*). The relation between log I_m and number of elements excited, or F, is described by a sigmoid curve. Its first derivative is a bell-shaped frequency distribution of excitability thresholds. The number of marginally excitable elements, responsible for the fluctua-

tion in effect, will be thus given by $dN/d\log I$, where N is the total number of excitable elements, and will pass through a maximum as $\log I$ increases. This corresponds with the properties of P.E.$_{F_1}$ because, at fixed I, P.E.$_{F_1}$ measures the fluctuation in the effect of a flash (*i.e.*, the action of the intensity $\times$ the duration of the flash is constant for a given end result). With (at any moment) a slightly larger number of excited contributing elements, the duration of a flash of given intensity need not be quite so long to produce the same magnitude of effect and therefore the same intensity-decay curve in the after-image, and so the same difference between the two. On this basis the integration of P.E.$_{F_1}$ over the whole range of intensities should give a proportionate measure of the total number of excitable elements. The integration is made graphically, from the curves drawn through the measurements of P.E.$_{F1}$ as a function of $\log I$ (in: Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *a*, *b*). Σ P.E.$_{F_1}$ as a function of $\log I$ should then be parallel to F as a function of $\log I$. The simplest method of demonstrating the correctness of this expectation is obtained by plotting Σ P.E.$_{F_1}$ against F. For the *Anax* data the result is exhibited in Fig. 8. The rectilinear relationship is striking; the deviation of the last point ($F_{max.}$) is due to instrumental error at the highest intensity used, as with the case of the sunfish (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *a*, *b*). The data for *Enneacanthus*, treated in the same manner, are given in Fig. 9. As already implied from other relationships, the proportionality constant should differ for rods and cones. The break in the relationship comes exactly at the point identified as that at which the rod contribution fades out of the picture (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *d*). This empirically verified conception is therefore embodied in the expression

$$\text{P.E.}_{F_1} = k_1 dF/d\log I = k_2 I dF/dI. \qquad \text{(I)}$$

(When $dF/dI = 0$, at $F_{max.}$, the variation encountered is of another sort, as already indicated.)

With fixed F and an all-or-nothing index of response, the presumed condition for response (other factors constant) being a certain percentage difference between sensory effects due to light flash and to after-image, then I_1 must vary from time to time in such a manner that

$$\text{P.E.}_{I_1} = k' \text{P.E.}_{F_1} dI/dF, \qquad \text{(II)}$$

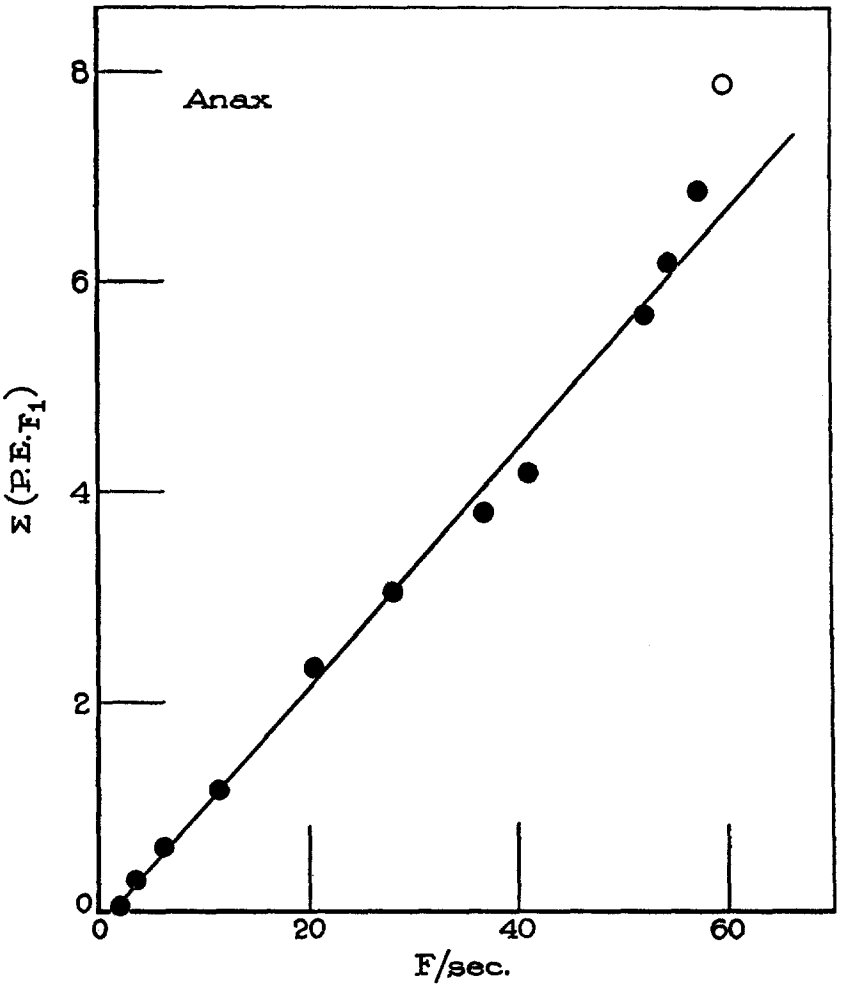

FIG. 8. For reasons given in the text it was expected that the integration of P.E.$_{F_1}$ as a function of log I should provide a quantity, Σ (P.E.$_{F_1}$), proportional at each value of I_m to the associated flicker frequency F. Data upon *Anax* larvae show that this is correct; the two highest points deviate because of the entrance of instrumental errors of observation at the level of maximum critical flicker frequency. The original curve of P.E.$_{F_1}$ *vs.* log I is in: Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *b*; the integration was made graphically, and the units are arbitrary. It was assumed that $F = 3$ is the lower limit; this F is really a little too high; the lower points are therefore subject to a correction which would increase Σ P.E.$_{F_1}$ and (even with the inevitable roughness due to graphic procedure) increase the straightness of the plot up to *ca.* $F = 50$.

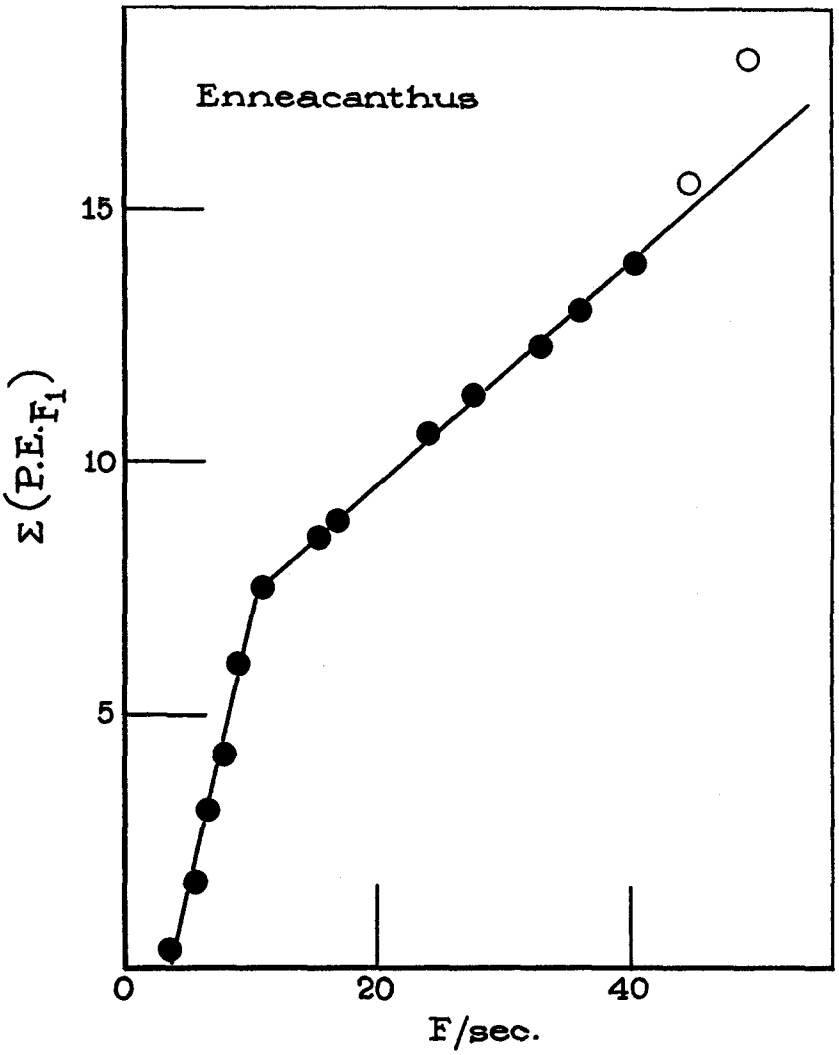

FIG. 9. Similar to Fig. 8, but based upon observations with the sunfish *Enneacanthus* (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *a*). The abrupt break coincides precisely with the dropping out of the rod contribution to the determination of critical flicker frequency as determined by independent criteria (*cf.* Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *a*, *d*). In each zone, respectively of predominant rod function and cone function, $d(\Sigma[P.E._{F_1}])/dF$ is constant.

since P.E._{F_1} measures the spontaneous fluctuation of excitability at fixed I and dI/dF is the mean equivalent change of I per small change of F; the equation is thus dimensionally balanced.

From these 2 equations,

$$\text{P.E.}_{I_1} = C\frac{(dF)(dI)(I)}{(dI)(dF)} = CI. \tag{III}$$

This is the relation of P.E._{I_1} to I_m which has been obtained experimentally. The proportionality constant C contains no reference to the flicker frequency or to the shape of the F-I curve. Hence P.E._{I_1}, as a function of I, should be independent of F. We find empirically that the constant C has the same general order of magnitude for different organisms, although their F-I curves may differ profoundly.

This can be approached in another manner, more general, by a procedure for which we are indebted to Dr. Charles P. Winsor. We have a situation in which the observed result (response to flicker) is conceived to be a function of three variables, F, I, and Z, where F and I are measured and Z is a random variable (due to the organism).

$$f(F, I, Z) = 0. \tag{1}$$

Either F or I may be, experimentally, the independent variable:

$$F = \phi(I, Z) \tag{2}$$

or

$$I = \psi(F, Z) \tag{3}$$

From (2), (3), we have approximately

$$\sigma_F = \frac{\delta\phi(I, \bar{Z})}{\delta Z}\sigma_Z = \frac{\delta F}{\delta Z}\sigma_Z \tag{4}$$

$$\sigma_I = \frac{\delta\psi(F, \bar{Z})}{\delta Z}\sigma_Z = \frac{\delta I}{\delta Z}\sigma_Z. \tag{5}$$

From (1) we obtain

$$dF = 0 = \frac{\delta f}{\delta F}dF + \frac{\delta f}{\delta I}dI + \frac{\delta f}{\delta Z}dZ,$$

and

$$\frac{\delta F}{\delta I} = -\frac{\delta f}{\delta Z}\Big/\frac{\delta f}{\delta F}; \qquad \frac{\delta I}{\delta Z} = -\frac{\delta f}{\delta Z}\Big/\frac{df}{dI} \tag{6}$$

by putting $dI = 0$, $dF = 0$, and solving for dF/dZ, dI/dZ, which are then the required partials.

From (4), (5), and (6)

$$\frac{\sigma_F}{\sigma_I} = \frac{\delta F}{\delta I} \tag{7}$$

which is the result already obtained. It therefore follows that, given experiments in which F or I is the independent variable, we should be able to predict the scatter of the dependent variable when the experiment is performed with the reverse arrangement.

If in addition it is to be assumed that the recorded measurements of F and I are subject to scatter independent of Z, in other words to experimental error in the customary sense, then

$$F = \phi(I, Z) + \epsilon \tag{8}$$

$$I = \psi(F, Z) + \eta. \tag{9}$$

These give

$$\sigma_F^2 = \left(\frac{\delta\phi[F, \bar{Z}]}{\delta Z}\right)^2 \sigma_Z^2 + \sigma_\epsilon^2 \tag{10}$$

$$\sigma_I^2 = \left(\frac{\delta\psi[I, \bar{Z}]}{\delta Z}\right)^2 \sigma_Z^2 + \sigma_\eta^2 \tag{11}$$

and

$$\frac{\sigma_F^2 - \sigma_\epsilon^2}{\sigma_I^2 - \sigma_\eta^2} = \left(\frac{\delta F}{\delta I}\right)^2 \tag{12}$$

This is analogous to (7), but no *a priori* assumptions can be made as to σ_ϵ and σ_η; hence the calculation of σ_F from σ_I or the reverse cannot be undertaken. At the same time, if (7) is adequate it follows that the ϵ, η terms do not enter significantly; this amounts to the conclusion that the variation dealt with is practically free from experimental error in the usual sense. This is entirely consistent with the fact that duplicate determinations of I_m agree much more closely than their σ_I's would warrant (save in the exceptional and specifically accounted for case of the threshold for cones in *Xiphophorus*, already discussed).

We are therefore in position to conclude that a consistent account of the characteristics and the interrelationships of the variabilities of our measurements can be given from the standpoint that this variability is a property of the reacting organism.

VI

Black Helleri Hybrids

Our desire has been, in part, to prepare ground for a genetic analysis of differences such as the curves in Fig. 1 reveal, and which can be

rather simply characterized by descriptive constants assembled in Table IV. The desirability and the importance of such analysis derives from two sources, which from the standpoint of logical experimentalism may be regarded as distinct: (1) it is the necessary procedure for the characterization of the really significant differences, namely *functional* differences, with which genetic theory must ultimately deal; it implies functional formulation, and the genetic properties of such formulations require to be investigated; and (2) it provides a relatively simple and efficient means of deciding whether constants in quantitative formulations have a definite significance, a reality apart from the descriptive efficacy given by curve fitting, and thus may be hopefully regarded from the viewpoint of theoretical interpretation as to mechanism. The complexity of a biological phenomenon, since its properties are specifically reproducible, must be subject to and governed by a determinate control. The complexity increases the labor of experimental inquiry and the difficulties of interpretation, but the possibility of genetic manipulation presents a unique and counterbalancing advantage.

At the moment we are concerned, however, not so much with data for a genetic theory of the determination of type differences in flicker curves as with the demonstration that specific and meaningful changes in these curves may be brought about by cross-breeding. The chief evidence for this position is at present in our data upon the flicker responses of the backcross hybrids derived from *Xiphophorus* × *Platypoecilius nigra*. Nothing very definite can be said as to the possible factorial interpretation of the results, nor is this necessary for our purpose. Similarities with the outcome of analogous, but (as the nature of the case permitted) more simply designed experiments based upon the geotropic performance of young rats,[14] are not, however, to be overlooked. The importance of the results, for the present, is in their demonstration that quantitative features of the flicker curves are transmissible from the two stocks involved in the cross, whatever the mechanism may be. The peculiar combination of properties found in these hybrids also gives a new situation in which to test the method already used for the separation of the rod and cone components, as well as to test the variability relations.

[14] Crozier and Pincus, 1935–36 and citations there given.

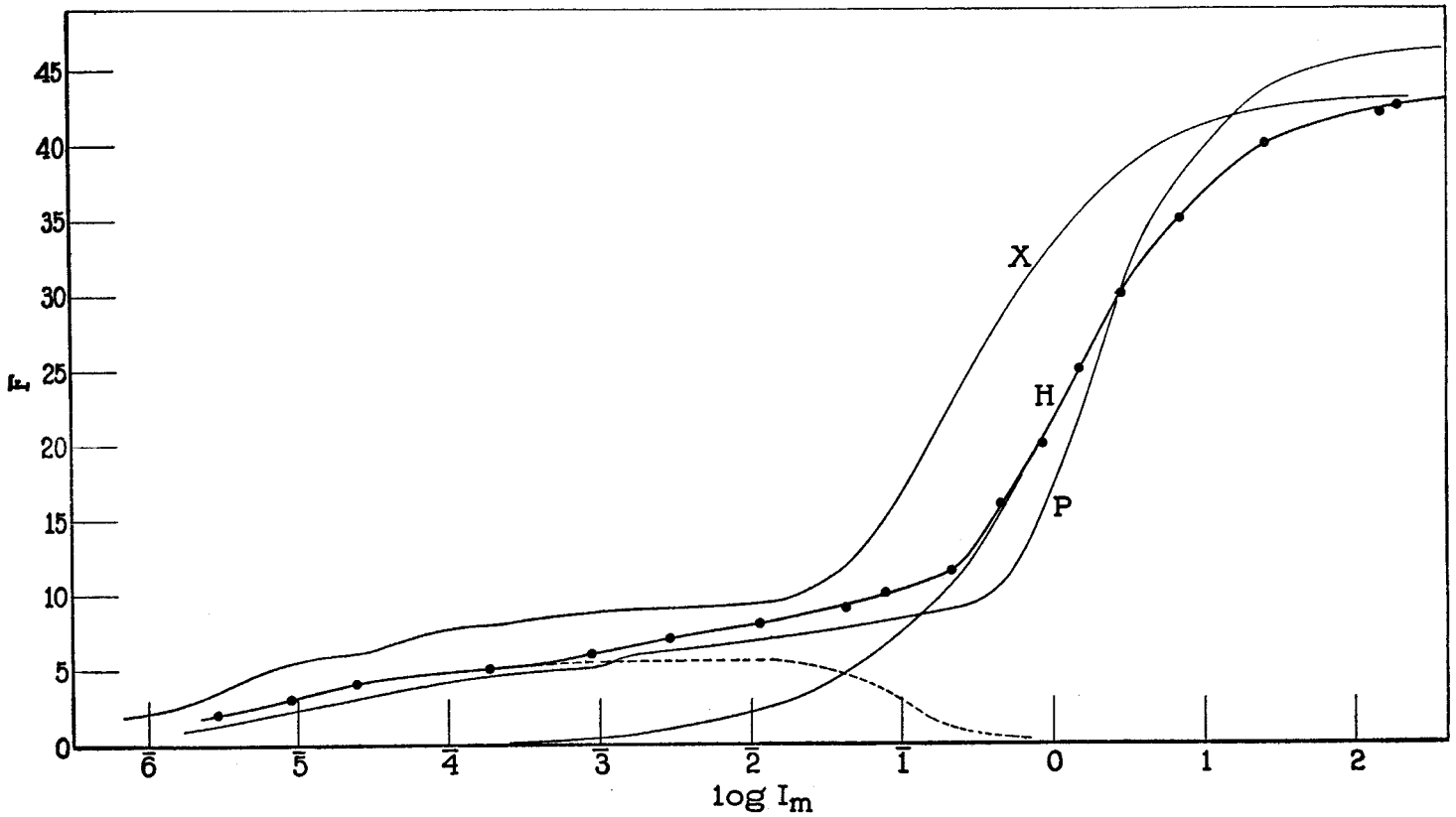

FIG. 10. Mean critical illumination I_m as a function of F for a homogeneous group of backcross hybrids between swordtail (X) and Platy (P). See text. The curves for X and P are transferred from Fig. 1. The cone segment logistic (Fig. 8) is extrapolated to give the presumptive level of intensity for the entrance of activity due to cone excitation. The difference curve has been plotted as in Fig. 5.

The values of I_m and of P.E.$_{I_1}$ for the hybrid stock are given in Table IV; I_m as a function of F is plotted in Fig. 10. A certain re-

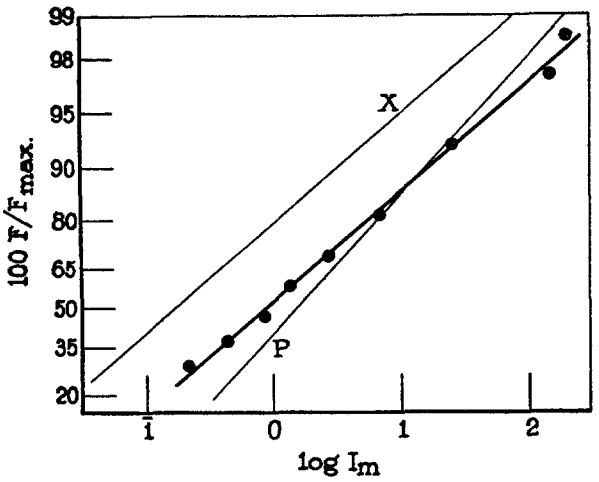

FIG. 11. The cone segment of the flicker response curve for the Black Helleri hybrids gives the same logistic exponent, $p = 1.90$, as does the cone curve for the swordtail parental stock.

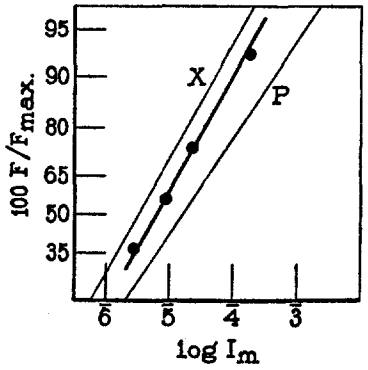

FIG. 12. The rod curve for the Black Helleri hybrids has an exponent p equal to that for the *Xiphophorus* parent ($p = 2.00$), although the maximum rod flicker frequency is almost exactly that for the other parental stock, *Platypoecilius*.

semblance to the curve for *Xiphophorus* is obvious, but the whole curve has been moved to higher levels of critical intensity—in fact, into a

position rather similar to that occupied by the graph for the *Platypoecilius* ancestor. These considerations can be made more definite by the examination of the constants in the descriptive equations for the respective flicker curves (Table V). Comparisons are possible because the logistic also describes the data for the hybrids (Figs. 11 and 12). With our apparatus it was not possible to obtain accurate settings of intensity at higher intensities than antilog 2.5 millilamberts; we were

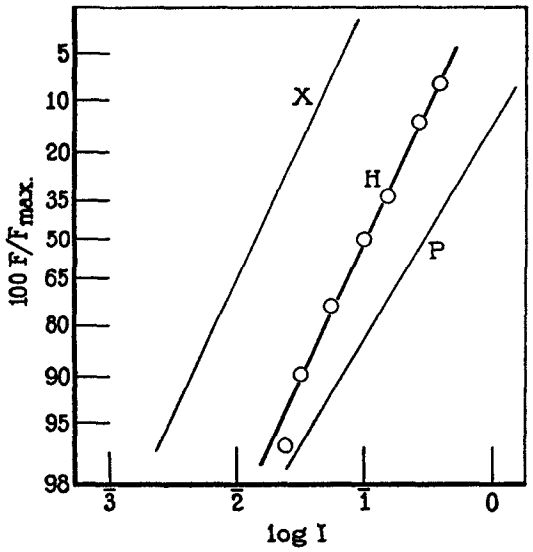

FIG. 13. The declining curve of rod contribution to the composite flicker response curve for the Black Helleri hybrids is fitted by a logistic; p differs from that for the rising rod curve, as in the other fishes investigated, but agrees (as does the p for the ascending curve) with the corresponding constant for the swordtail parent (X).

therefore unable to get quite up to the maximum flicker frequency for the hybrids. The fit of the data to the logistic with $F_{max.} = 43.1$, as with *Xiphophorus*, is so good, however, that this maximum may be safely assumed. It is clear that for the cone curves the exponent p and the value of $F_{max.}$ are identical with those for *Xiphophorus*, while for the rod portions p is like that in *Xiphophorus* but $F_{max.}$ is practically that of the Platy stock. The declining branch of the rod curve,

obtained as before by difference on the basis of the extrapolated cone logistic, has also the exponent characteristic of the declining curve for *Xiphophorus* rods (Fig. 13). This adds to the evidence for lack of influence of the cone function upon the declining contribution of

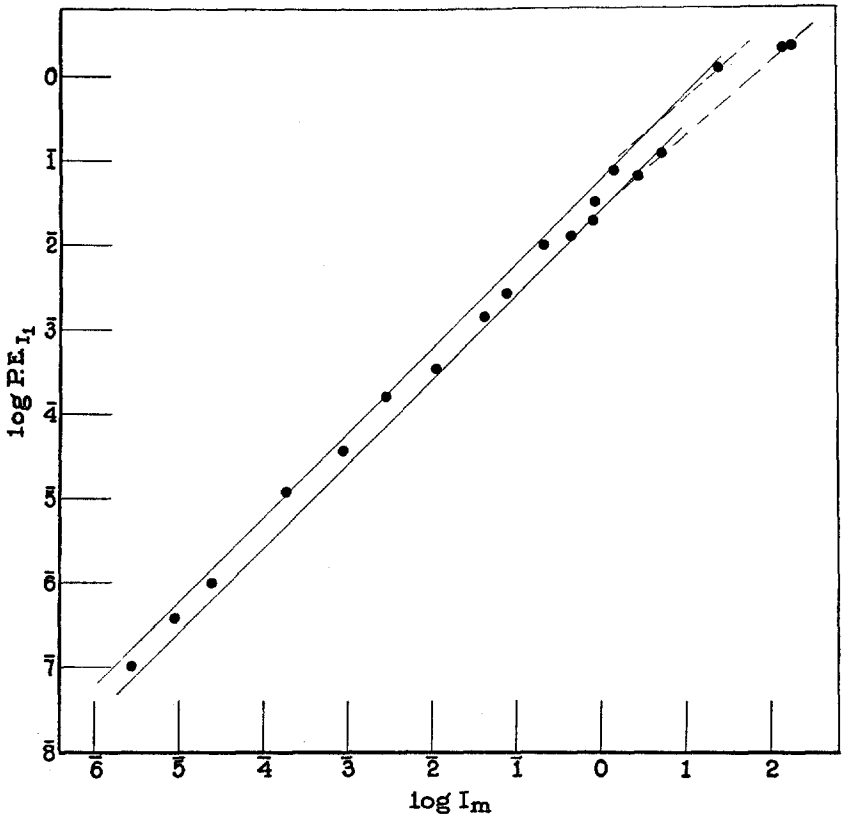

FIG. 14. The variation of I_1 for the Black Helleri hybrids exhibits the same law of relation to I_m as in the other fishes tested (*cf.* Fig. 7).

rods, since the two components have been, with respect to *Xiphophorus*, shifted to different degrees.

The important thing is that as result of the crossbreeding a new type of flicker curve has been built up—in which it might be said that the shape characteristics are derived mainly from one stock but the gross level of effective intensities derives from the other, as well as

the rod $F_{max.}$. The difference between the *Xiphophorus* and hybrid curves is not similar to the change produced by alteration of temperature (Crozier, Wolf, and Zerrahn-Wolf, 1935–36 *d*). The values of I_i for rods and cones, the intensities for $F = 0.5\ F_{max.}$, are not equally affected in the new combination, so that the mode of junction of the rod and cone curves has a different aspect. The extrapolation of the logistic for the cone curve, however, approximates $F = 0$ at precisely the right level of intensity for correlation with the bump on the composite curve (Fig. 10).

The variability of I_1 is exhibited in Fig. 14; there is no distinct break in the plot of P.E.$_{I_1}$ *vs.* I_m, although it is possible that measurements at still higher intensities would show something of the sort. The relation between P.E.$_{I_1}$ and I_m is described by

$$(1 \pm 0.402)\text{P.E.}_{I_1} = 0.374\ I_m.$$

The proportionality constant is just the mean of those for swordtails and Platys (see section V); the spread coefficient of P.E.$_{I_1}$ is lower than for either of the parental stocks.

VII

Comparison of the flicker response curves for the four fishes thus far studied in some detail permits conclusions relative to certain points in the theory of visually excited responses, which may be commented upon briefly.

Differences between these curves as obtained at one temperature involve not merely shifts in position on the intensity axis, but also changes in scale and in the proportions of constituent parts. The same type of quantitative formulation is, however, applicable to all of them. Rod and cone activity constituents derivable from two parent stocks serve to synthesize the result obtained in a hybrid stock produced from these parent lines.

The "elements of action" involved in controlling the responses must be presumed to be of central nervous location. These elements are clearly correlated, however, with discrete retinal properties. Of these the most conspicuous is the separability of rod and cone functions as required by the duplicity doctrine (Kohlrausch, 1922; von Kries, 1929; Hecht, 1934). For convenience we may speak of rod thresholds,

populations of rod and cone effects, and the like, without its being assumed or implied that properties of the flicker curves are directly representative of the excitabilities of the corresponding retinal elements. With this reservation as to the use of the terms rods and cones, we note that in our group of flicker response curves (i) higher rod threshold does not necessarily mean a smaller population of rod effects, (ii) or a more rapidly rising rod curve, (iii) or a higher cone threshold, (iv) or a steeper cone curve; and that (v) there is no correlation between the steepness (p) of a cone curve and its maximum critical flicker frequency, or (vi) between p and general intensity level. Threshold, $F_{max.}$, p, and I_i are separately and independently determined.

This body of evidence, coupled with the analyses of the individual flicker response curves, supplies a proof of a new kind that the basic requirement of the duplicity theory is correct: the retinal functions of vertebrates involve the distinct operations of two groups of elements, rods and cones.

VIII

SUMMARY

Flicker response curves have been obtained at 21.5°C. for three genera of fresh water teleosts: *Enneacanthus* (sunfish), *Xiphophorus* (swordtail), *Platypoecilius* (Platy), by the determination of mean critical intensities for response at fixed flicker frequencies, and for a certain homogeneous group of backcross hybrids of swordtail × Platy (Black Helleri).

The curves exhibit marked differences in form and proportions. The same type of analysis is applicable to each, however. A low intensity rod-governed section has added to it a more extensive cone portion. Each part is accurately described by the equation

$$F = F_{max.}/(1 + e^{-p \log I/I_i}),$$

where F = flicker frequency, I = associated mean critical intensity, and I_i is the intensity at the inflection point of the sigmoid curve relating F to log I.

There is no correlation between quantitative features of the rod and cone portions. Threshold intensities, p, I_i, and $F_{max.}$ are separately and independently determined.

The hybrid Black Helleri show quantitative agreement with the *Xiphophorus* parental stock in the values of p for rods and cones, and in the cone $F_{max.}$; the rod $F_{max.}$ is very similar to that for the Platy stock; the general level of effective intensities is rather like that of the Platy form. This provides, among other things, a new kind of support for the duplicity doctrine. Various races of *Platypoecilius maculatus*, and *P. variatus*, give closely agreeing values of I_m at different flicker frequencies; and two species of sunfish also agree. The effect of cross-breeding is thus not a superficial thing. It indicates the possibility of further genetic investigation.

The variability of the critical intensity for response to flicker follows the rules previously found to hold for other forms. The variation is the expression of a property of the tested organism. It is shown that, on the assumption of a frequency distribution of receptor element thresholds as a function of log I, with fluctuation in the excitabilities of the marginally excited elements, it is to be expected that the dispersion of critical flicker frequencies in repeated measurements will pass through a maximum as log I is increased, whereas the dispersion of critical intensities will be proportional to I_m; and that the proportionality factor in the case of different organisms bears no relation to the form or position of the respective curves relating mean critical intensity to flicker frequency. These deductions agree with the experimental findings.

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