

# TEMPERATURE AND CRITICAL ILLUMINATION FOR REACTION TO FLICKERING LIGHT<sup>1</sup>

## V. XIPHOPHORUS, PLATYPOECILIUS, AND THEIR HYBRIDS

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### I

For various kinds of animals there are found<sup>2</sup> pronounced quantitative and qualitative differences in the dependence of visual excitability upon temperature. It has also been shown<sup>3</sup> that specific values of certain parameters descriptive of the form of the visual response contour (for reaction to flicker) behave as heritable invariants in cross-breeding experiments involving teleosts of the genera *Xiphophorus* and *Platypoecilus*. The question arises, do these constants differ in their dependence upon temperature?

Parameters which are heritable must be considered to express determinate properties of distinct assemblages of elements. The fact that the function efficiently describing the relation of critical intensity to flash frequency is a probability integral<sup>4</sup> in  $\log I$  raises a number of significant queries. The use of this particular function is required for two reasons: it describes the course of the data, thus far uniquely well; and its parameters ( $F_{max.}$ ;  $\tau' = \log I$  at inflection; and  $\sigma'_{\log I}$ ) have been shown to experimentally exhibit appropriate properties.  $F_{max.}$  and  $\sigma'_{\log I}$  are the measures which show genetic invariance numerically. They are expressions of essentially statistical attributes of the assemblages of elements concerned. When temperature is varied they are found to remain constant. Hence it cannot be assumed that reaction velocities are directly concerned in their deter-

<sup>1</sup> Previous reports in *J. Gen. Physiol.*, 1936-37, **20**, 393, 411; 1938-39 *a*, **22**, 311; 1938-39 *b*, **22**, 487; 1938-39 *c*, **22**, 795. *Proc. Nat. Acad. Sc.*, 1938, **24**, 216; 1939 *a*, **25**, 78; 1939 *b*, **25**, 171; 1939 *c*, **25**, 176.

<sup>2</sup> *Proc. Nat. Acad. Sc.*, 1938, **24**, 216; 1939, **25**, 78. *J. Gen. Physiol.*, 1938-39 *a*, **22**, 311; 1938-39 *b*, **22**, 487; 1938-39 *c*, **22**, 795.

<sup>3</sup> *J. Gen. Physiol.*, 1937-38, **21**, 17; 1938-39, **22**, 463. *Proc. Nat. Acad. Sc.*, 1937, **23**, 516; 1938, **24**, 542.

<sup>4</sup> *Proc. Nat. Acad. Sc.*, 1937, **23**, 71. *J. Gen. Physiol.*, 1938-39, **22**, 311, 451.

mination. The abscissa of inflection, however, is dependent on temperature, and in such a way, quantitatively, as to require the assumption that the excitability ( $1/I$ ;  $F$  fixed) is governed by the speeds of intrinsic reactions common to all the elements concerned in the production of a given reaction contour.

This situation permits a rather definitive test of two distinctly different questions. The first has to do with the proposition<sup>5</sup> that the characteristically duplex curves of visual performance obtained with vertebrates are of a form which permits the deduction of kinetically different modes of excitability for retinal rods and cones. The second concerns the more general conception of the nature of genetic differences. The potent evidence which can be obtained by way of genetic tests of organic invariance<sup>6</sup> indicates that a connection may be theoretically important and empirically significant. We have examined from this standpoint the dependence of response to flicker upon temperature in *Xiphophorus*, *Platypoecilus*, and their  $F_1$  hybrids.

## II

The technic of the tests has been described in other cases.<sup>1</sup> To favor homogeneity of observational conditions the number of animals tested has been reduced to the point where it has been possible to obtain very careful readings of critical intensities upon sets of *Xiphophorus* (*X.*), *Platypoecilus* (*P.*), and  $F_1$  on the same day, under the same general conditions, within a space of several hours. Three observations were taken on each individual at each flash frequency ( $F$ ) used, at each temperature. Observations on any one day were taken at the same temperature. The order of the temperatures used was essentially random, and included adequate check determinations at certain temperatures. Of *X. montezuma*, 5 individuals were used, of *P. maculatus*, 5; and of  $F_1$ , 2. The essential equivalence of individuals in any one of these stocks<sup>1</sup> obviates the necessity of large numbers of individuals, as indeed the present results also prove. This could only be justified, however, on the basis of adequate experience with these animals. The variation of the readings supplies an efficient check. The individuals used had been represented in our earlier experiment with these forms.<sup>7</sup>

Determinations of critical intensities were made after several hours' dark adaptation in a thermostat<sup>8</sup> at the desired temperature. During the comparatively brief interval

<sup>5</sup> Cf. Hecht, S., *Physiol. Rev.*, 1937, **17**, 239, and much earlier work there summarized. *Harvey Lectures*, 1937-38, **33**, 35.

<sup>6</sup> *J. Gen. Physiol.*, 1929-30, **13**, 57, 81; 1931-32, **15**, 421; 1936-37, **20**, 111; 1937-38, **21**, 17; 1938-39, **22**, 463.

<sup>7</sup> *Proc. Nat. Acad. Sc.*, 1938, **24**, 542. *J. Gen. Physiol.*, 1938-39, **22**, 463.

<sup>8</sup> Cf. *J. Gen. Physiol.*, 1932-33, **16**, 757. We are under obligation to the Trustees of the Elizabeth Thompson Science Fund for a grant to one of us which was used for the construction of one of our thermostatic tanks.

out of the thermostat required for an observation the aquarium containing a fish (in 250 ml. water), the temperature of the container, and probably that of the fish, changed a little. The temperature listed in the tables (I and II) has been corrected for this change, so as to give the mean temperature during the period of observation. This never differed by  $>0.3^\circ$  from the adaptation temperature. While we naturally regret this source of inaccuracy, it has been impossible to thermostat the optical system employed for the observations, or the whole dark room. At the same time it is to be noticed that the temperature change in the fish was probably slower than in the aquarium water; and that the quantitative result obtained (Fig. 2) would be by this factor if anything slightly but definitely improved if the correction could really be applied in a more adequate way.

The behavior of the different lots of individuals used is not quite the same. *X. montezuma*, *P. maculatus*, and  $F_1$  (or  $H^*$ ) have been described previously.<sup>7</sup> We have to add certain points concerning relations to temperature, and also some comments on the  $F_2$  individuals obtained from our  $F_1$ 's (cf. Section VI).

*X. montezuma* is less quiet than *P. maculatus*. Quite young individuals are on the whole not so restless, but are difficult to observe at low illuminations, and consequently older individuals were used. Their reactions are clearer at low flash frequencies. With higher temperature the critical reactions become sharper and more vigorous. At lower temperatures the end-point must be approached with particular care to avoid overshooting of the critical intensity. At  $F = 25$  restlessness increases with rise of temperature.

*P. maculatus* is steadier, and at low illuminations the observation of the marginal response to flicker presents no difficulties at any temperature. At  $F = 25$ , and particularly at higher temperatures, the platys tend to push against the cylinder wall, nose down, and may exhibit a general activity—due in large part to the light—upon which the forced reaction to discriminated flashes is superimposed during quiescent intervals.

The  $F_1$  hybrids are more like *X.* than like *P.* in their behavior. Even after 6 months under observation they are comparatively "wild;" readings are taken during periods of quiescence. At higher temperatures restlessness is greater, at  $F = 25$  especially.

$F_2$  hybrids were obtained (several broods) from 1  $F_1$  ♂ and 2  $F_1$  ♀♀. The age differences in the lot used were 5 to 6 months (lengths 14 to 30 mm.). They are much more quiet than  $F_1$ . All tests with these were made at  $21.5^\circ\text{C}$ . Up to  $F = 10$  responses were quite clean and sharp, mostly as a motor jerk in the direction of rotation of the stripes. Above  $F = 10$  these animals stay at the periphery of the jar and respond by moving slowly (but with rapid fin motions) in the direction of passage of the stripes; the responses are more difficult to recognize. In general the behavior is much more like platy than like *montezuma*, in contrast to  $F_1$ , but this may easily be due in part to the fact that they were subjected to the laboratory routine from an earlier age.

It is to be noted that small and large individuals of any one of these types are quantitatively indistinguishable in their values of critical intensities. This was most elaborately shown with  $F_2$  (Section VI), but was found also for *X. montezuma*.

The summarized observations are given in Tables I and II. Our plan was to secure data at a low flash frequency within the purely "rod" section of the duplex flicker response contour, and also at a much higher frequency within the purely "cone" segment of the curve (cf. Fig. 1). For this purpose

TABLE I

Mean critical intensities and the dispersions of the individual values (as log millilamberts) for response to visual flicker at the low flash frequencies (per second) indicated, for *X. montezuma*, *P. maculatus*, and their  $F_1$  hybrids, at various temperatures; flash cycle with light and dark intervals equal. For *X.* and *P.*,  $N = 5$  individuals each; for  $F_1$ ,  $N = 2$ ; 3 readings on each individual at each temperature. (Values in parentheses are from parallel tests with other lots of individuals.)

$t^{\circ}\text{corr.}$	$F = 4.0$		$F = 2.5$		$F = 3.0$	
	<i>X. montezuma</i>		<i>P. maculatus</i>		$F_1, X. \times P.$	
	log $I_m$	log P.E. <sub>1I</sub>	log $I_m$	log P.E. <sub>1I</sub>	log $I_m$	log P.E. <sub>1I</sub>
12.7	$\bar{6}.8913$	$\bar{7}.1577$	$\bar{5}.4098$	$\bar{7}.8028$	$\bar{5}.0846$	$\bar{7}.6850$
15.4	$\bar{6}.7793$	$\bar{7}.0569$	$\bar{5}.3023$	$\bar{7}.4554$	$\bar{5}.0330$	$\bar{8}.9227$
17.2	$\bar{6}.7065$	$\bar{8}.8945$	$\bar{5}.2222$	$\bar{7}.3699$	$\bar{6}.8935$	$\bar{7}.0733$
19.2	$\bar{6}.6522$	$\bar{8}.9018$	$\bar{5}.2028$	$\bar{7}.6761$	$\bar{6}.8710$	$\bar{8}.7894$
21.5	$\bar{6}.5942$	$\bar{8}.8892$	$\bar{5}.1274$	$\bar{7}.5987$	$\bar{6}.8197$	$\bar{8}.3665$
	$\bar{6}.5936$	$\bar{8}.9199$	$\bar{5}.1202$	$\bar{7}.6028$	$\bar{6}.7543$	$\bar{8}.8189$
	( $\bar{6}.5891$ )	$\bar{8}.7059$ )			$\bar{6}.7487$	$\bar{8}.3705$
	( $\bar{6}.6052$ )	$\bar{7}.2299$ )			$\bar{6}.9534$	$\bar{8}.8235$
	$\bar{6}.4823$	$\bar{8}.9287$	$\bar{6}.9827$	$\bar{7}.1813$	$\bar{6}.6981$	$\bar{7}.1383$
23.9			$\bar{5}.0255$	$\bar{7}.5883$		
27.2	$\bar{6}.4108$	$\bar{8}.7950$	$\bar{6}.8764$	$\bar{8}.9577$	$\bar{6}.5441$	$\bar{8}.7633$
29.7	$\bar{6}.3126$	$\bar{8}.6640$	$\bar{6}.8073$	$\bar{7}.1562$	$\bar{6}.5222$	$\bar{7}.0043$
32.7	$\bar{6}.2462$	$\bar{8}.6842$	$\bar{6}.7786$	$\bar{8}.6406$	$\bar{6}.5046$	$\bar{8}.6850$
35.6	$\bar{6}.1697$	$\bar{8}.3257$	$\bar{6}.6111$	$\bar{8}.8539$	$\bar{6}.3222$	$\bar{8}.6630$

TABLE II

As in Table I, but at flash frequency  $F = 25$  per second.

$F = 25$

$t^{\circ}\text{corr.}$	<i>X. montezuma</i>		<i>P. maculatus</i>		$F_1, X. \times P.$	
	log $I_m$	log P.E. <sub>1I</sub>	log $I_m$	log P.E. <sub>1I</sub>	log $I_m$	log P.E. <sub>1I</sub>
12.7	$\bar{1}.7646$	$\bar{2}.0966$	$0.5831$	$\bar{1}.0726$	$0.3003$	$\bar{2}.9697$
15.4	$\bar{1}.6717$	$\bar{2}.0730$	$0.4735$	$\bar{2}.9373$	$0.1661$	$\bar{2}.3893$
17.2	$\bar{1}.5786$	$\bar{3}.6603$	$0.3804$	$\bar{2}.8353$	$0.0856$	$\bar{2}.2679$
19.2	$\bar{1}.5345$	$\bar{3}.8193$	$0.3495$	$\bar{2}.7761$	$0.0430$	$\bar{2}.2536$
21.5	$\bar{1}.4571$	$\bar{3}.5317$	$0.2896$	$\bar{2}.6109$	$\bar{1}.9999$	$\bar{2}.0367$
	$\bar{1}.4639$	$\bar{2}.1501$	( $0.3002$ )		$\bar{1}.9880$	$\bar{2}.5750$
	( $\bar{1}.4700$ )	$\bar{3}.8653$ )	( $0.2833$ )			
	( $\bar{1}.4598$ )	$\bar{3}.9677$ )	( $0.3107$ )		$0.0020$	$\bar{3}.9943$
	( $\bar{1}.4708$ )	$\bar{3}.9719$ )	( $0.2911$ )	$\bar{2}.8185$ )		
23.9	$\bar{1}.3769$	$\bar{3}.6897$	$0.2030$	$\bar{2}.2894$	$\bar{1}.9046$	$\bar{2}.2191$
27.2	$\bar{1}.2617$	$\bar{3}.7205$	$0.0962$	$\bar{3}.9925$	$\bar{1}.8211$	$\bar{3}.7356$
29.7	$\bar{1}.1940$	$\bar{3}.5861$	$0.0233$	$\bar{2}.2403$	$\bar{1}.7361$	$\bar{3}.6516$
32.7	$\bar{1}.1326$	$\bar{3}.4407$	$\bar{1}.9357$	$\bar{2}.1278$	$\bar{1}.6575$	$\bar{3}.6399$
35.6	$\bar{1}.0342$	$\bar{3}.3699$	$\bar{1}.9916$	$\bar{3}.8785$	$\bar{1}.5641$	$\bar{2}.3653$

we chose for  $X$ .,  $P$ ., and  $F_1$  respectively the levels  $F = 4, 2.5,$  and  $3$  (cf. footnote 7), and for all of them  $F = 25$ . The fact that for each of the three forms the difference  $\Delta \log I_m$  between mean critical intensities at the high and low flash frequencies is independent of temperature shows directly that the form of the  $F - \log I_m$  contour is independent of temperature.<sup>1</sup> With the sunfish *Enneacanthus*<sup>8a</sup> we discovered that for the low- $F$  segment of the curve there occurs a dislocation with respect to the upper (cone) part, at ca. 20°C. With the fishes herein concerned this is not found.

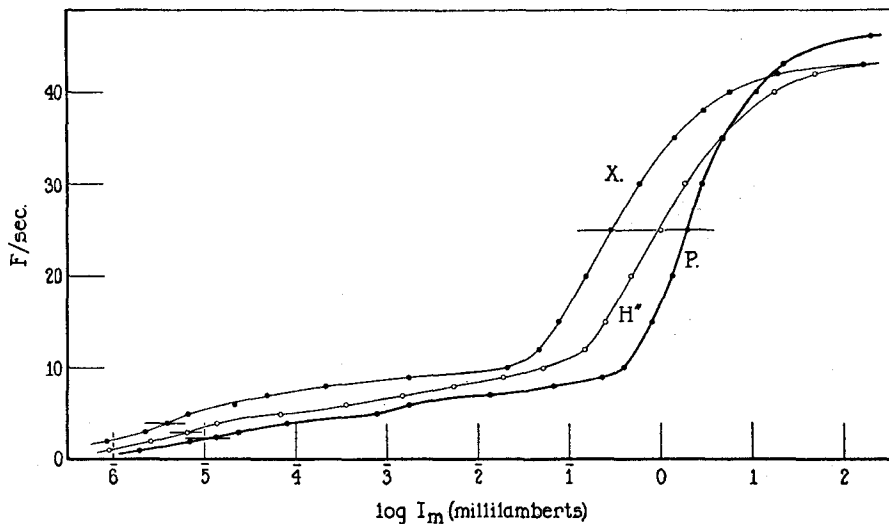


FIG. 1. Flicker response contours for *X. montezuma* ( $X$ .), *P. maculatus* ( $P$ .), and their  $F_1$  hybrids ( $H^*$ ). Temperature, 21.5°; flash cycle with light time = dark time; cf. Crozier, W. J., and Wolf, E., 1938-39, *J. Gen. Physiol.*, **22**, 463. The horizontal lines indicate the levels of  $F$  at which observations of dependence on temperature were made (Tables I and II).

### III

The data of Tables I and II are plotted in terms of the Arrhenius equation  $\ln k = \exp. (-\mu/RT) + c$ , in Fig. 2. For this purpose it has been considered that the proper measure of excitability is to be taken as  $1/I_m$ . The fact that  $F$  vs.  $\log I_m$  is the fundamental descriptive function<sup>4</sup> is no obstacle.  $F$  vs.  $\log I_m$  is a summation curve of neural effects produced; at a given level of  $F$ , with other relevant conditions fixed, these effects are elicited by flash intensity  $I$ . The capacity of intensity  $I$  to arouse effects

<sup>8a</sup> *Proc. Nat. Acad. Sc.*, 1939, **25**, 78. *J. Gen. Physiol.*, 1938-39, **22**, 487.

adequate to occasion response is presumably governed by the velocities of metabolic events in the neural units concerned. If a particular kind of

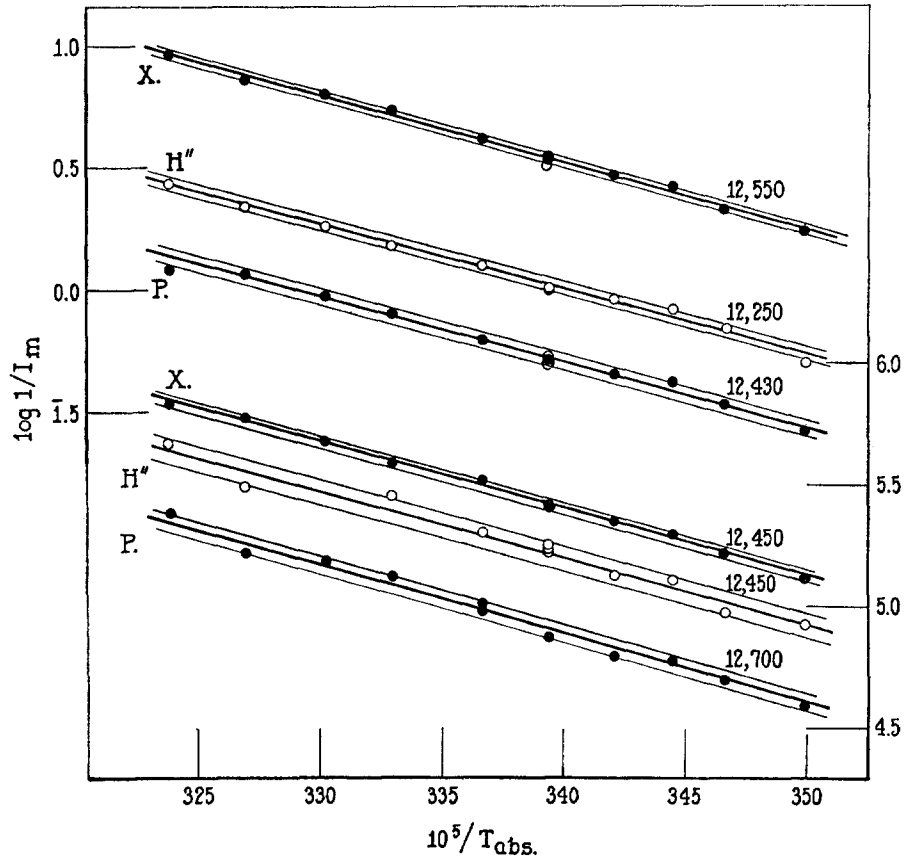


FIG. 2. Data in Tables I and II, plotted to show rectilinearity in terms of

$$\ln(1/I_m) = \exp.(-\mu/RT) + C,$$

for each flash frequency. The slopes of the lines, and consequently  $\mu$ , are not distinguishably different; consequently  $\mu = 12,400$  is independent of  $F$ .

The three upper lines refer to the cone parts of the  $F - \log I$  curves, the three lower to the rod parts (*cf.* Fig. 1).

The constant relative scatter of  $1/I_m$ , of course, depends in part on the number of observations in each series, but there are indications that it is intrinsically *lower* for  $X$ . than for  $P$ .

velocity plays a controlling rôle, and if it is of a physicochemical type sufficiently simple, it may be expected to reveal itself through the dependence of critical flash intensity on temperature. This intensity will vary

inversely with the velocities of the excitability process if its reciprocal is a rational measure of "excitability." The simplicity or complexity of the governing processes is something to be determined from the properties of the data, not by anterior convictions about the nature of events in organisms. The graphs in Fig. 2 show that  $1/I$  is described by the Arrhenius equation and that for  $X$ .,  $P$ ., and  $F_1$   $\mu$  for  $1/I_m$  is demonstrably equivalent, both in the rod and in the cone segments of the  $F$ -log  $I$  contour.

The values of the temperature characteristic  $\mu$  in Fig. 2 are, from above downward, 12,550; 12,400; 12,430; 12,450; 12,600; 12,700 (mean = 12,510  $\pm$  30). These values do not differ significantly. They are obtained from the slopes of the lines adjusted to give the best reasonable descriptions of the dispositions of the points, with particular reference to the fact that in each series of readings  $\sigma_r$  is directly proportional to  $I_m$ , independent of temperature. If, because of lag in drift of internal temperature during observations at temperatures much above or much below that of the room, the values of  $t_{corr}^\circ$  in Tables I and II have been over-corrected, the rectilinearity of the graphs in Fig. 1 would be somewhat improved and the value of  $\mu$  would be slightly less (not below 12,300).

## IV

For different animals<sup>1</sup> thus far investigated the values of  $\mu$  obtained for  $1/I_m$  are quite diverse. This is in correspondence with the fact that one must presume sunfish, turtle, dragon-fly nymph, and similarly unlike forms to be the embodiments of quite dissimilar physicochemical systems. Our genera  $X$ . and  $P$ . (both placed by taxonomists in the family *Poeciliidae*) are less unlike in this respect, since they can interbreed and produce fertile offspring. In the other forms mentioned not only are the values of  $\mu$  different, and also different from the 12,500 for  $X$ . and  $P$ ., but also the types of physicochemical organization of visual excitability revealed by the occurrence of critical temperatures.<sup>1</sup> These facts provide a forceful empirical argument for the analytical significance of  $\mu$  as a quantitative index of temperature dependence. One aspect of this matter deserves renewed comment. The argument we have used implies that for response at a low  $F$  the velocity of the process for which  $I$  is responsible is required to be much less than at a high  $F$ . In the present observations  $\mu$  is independent of the magnitude of this presumptive velocity, over a range of  $I_m$  corresponding to a factor of 250,000 (*cf.* Tables I and II; Fig. 1)—much greater even than in our earlier observations on *Enneacanthus*.<sup>8a</sup>

This sort of homogeneity can result if the constant  $\mu$  has the kind of meaning envisaged in the hypothesis that it represents the activation

energy of a governing catalyst,<sup>9</sup> and if the organic elements concerned in the eventuation of the index response constitute a truly homogeneous population (aside from statistical dispersion). An objective verification of this requirement is given by the dispersions of the measurements of critical intensities. No persistent differences in the excitabilities of the individuals in any one set were detectable; this is the condition already found in other work with fishes.<sup>1</sup> Fig. 3 shows that the relation between  $P.E._{I_1}$  and  $I_m$  is rectilinear, when change of  $I_m$  is induced at fixed levels of  $F$  by altering

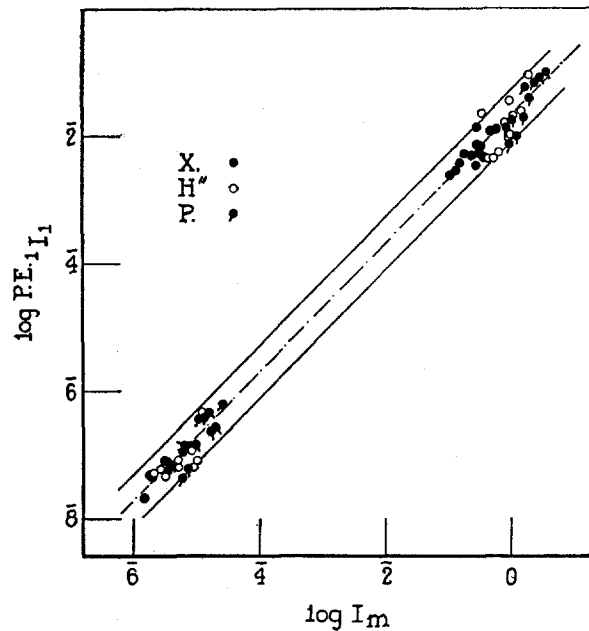


FIG. 3. The variation of  $I_1$  is directly proportional to  $I_m$  for  $X$ .,  $P$ ., and  $H''$  when temperature is varied at fixed levels of  $F$  (the log-log slope = 1).

the temperature. The proportionality constants are identical with those found<sup>10</sup> in previous observations with these forms at constant temperature. When it is independently demonstrable that in experiments of this kind more than one process is involved in the control of the critical intensity<sup>11</sup> the variation of  $I_m$  when temperature is the variant does not exhibit these properties. Since the three sets of individuals providing the data in Fig. 3

<sup>9</sup> *J. Gen. Physiol.*, 1938-39, **22**, 311; cf. 1924-25, **7**, 189, etc.

<sup>10</sup> *J. Gen. Physiol.*, 1938-39, **22**, 463.

<sup>11</sup> *J. Gen. Physiol.*, 1938-39, **22**, 795.



are not homogeneous, we, of course, find that the division of the band into halves with equal numbers on either side of a central line requires the bisection of the log P.E. span, not of arithmetic P.E. This corresponds to the effect of independent fluctuations of a visual performance function as a whole in series of determinations with one individual over a period of time.<sup>12</sup>

For the sunfish *Enneacanthus* we found for  $1/I_m \mu = 8,200$  (12 to 20°) and  $\mu = 14,400$  (20 to 30°); for *Pseudemys* (turtle),  $\mu = 27,000$  (11 to 29.5°) and  $\mu = 12,400$  (29.5 to 36°); incomplete experiments with *Fundulus* gave (above 20°)  $\mu = ca. 12,600$ ; with *Anax* there can be distinguished a controlling process with  $\mu = 19,200$  (8.5 to 35°). The forms of the  $F - \log I$  contours for these animals are quite different. The types of dependence on temperature also differ, as shown by the occurrences of critical temperatures. In this respect the control of visual excitability resembles that for such properties as the frequency of the heart-beat in various animals, and is not inconsistent with the view that the excitability measured is governed by the speeds of common cellular metabolic processes. With the exception of  $\mu = 8,200$  at lower temperatures with *Enneacanthus*, the temperature characteristics thus far found are not those typically associated with respiratory oxidations. The indication is that with such forms as *X.* and *P.* over a considerable range the level of respiratory oxidations in the neural elements concerned might affect the value of  $I_m$  with  $t^\circ$  constant, but without modifying its temperature characteristic.

That in all these cases rise of temperature lowers  $I_m$ , and that the values of  $\mu$  are very much higher than permits assigning them to photochemical reactions, is quite consistent with the conception that the determination of marginal response to flicker is dependent on central nervous discrimination<sup>1</sup> between the results of flashes and their after effects. We are not called upon to give a specific interpretation for the value of  $\mu$  obtained in the present experiments, but it may be pointed out that  $\mu = 12,200+$  has been typically found for frequencies of various (non-respiratory) movements of arthropods,<sup>13</sup> and also in connection with heart-beat frequencies for which  $\mu = 8,300, 14,400, 20,000,$  and  $27,000,$  have also been found.<sup>14</sup> No interpretation of the specific causation of  $\mu = 12,400$  is required for our present purposes, but merely the fact that it is a valid constant (Fig. 2) and that its value is so high.

<sup>12</sup> *Proc. Nat. Acad. Sc.*, 1937, **23**, 23; 1938, **24**, 130.

<sup>13</sup> *J. Gen. Physiol.*, 1926-27, **7**, 123, 565; 1928-29, **9**, 49; 1929-30, **10**, 227.

<sup>14</sup> *E.g.*, *J. Gen. Physiol.*, 1927-28, **11**, 349.

## V

For the interpretation of the shapes of flicker response contours it has been held desirable to push to the plausible limit the assumption that they are governed by the kinetics of processes limiting or determining primary peripheral excitation. The logical necessity for this is not especially notable, since it should be obvious that the responses providing the basis for the measurements are not actually given by the retina but are determined by the performance of the animals as reacting systems. Descriptions of the forms of the rod and "cone" branches of the flicker response contours for vertebrates have been given in terms of photochemical reaction systems presumed or deduced to characterize differences between the excitatory capacities of retinal rods and cones. These differences provide, for any one kind of animal, an "internal" situation posing a test of these general ideas. The essential point is that for such forms as sunfish (*Enneacanthus*), *Xiphophorus*, and *Platygoecilius* it is necessary to assume on this theory<sup>5</sup> that the photochemical pseudostationary state in the rod elements is specifically different from that in the cones, as we have already pointed out.<sup>16</sup> As deduced from the supposed applicability of Talbot's law to the situation at marginal flicker, with the assumption that the velocities of photochemical and dark processes are critically unbalanced at the point of marginal response, the equation for a flicker contour takes the form<sup>16, 5</sup>

$$pKI = F^n / (F_{max} - F)^m$$

where  $p$  = the percentage light time in the flash cycle,  $K$  is a composite constant, and  $n$  and  $m$  are the apparent orders respectively of the dark and light processes. The equation fails to predict even the correct direction of the shift of the curve with change of temperature,<sup>17</sup> let alone the form of the temperature function. It makes erroneous predictions as to the dependence of the position and other properties of the flicker contour when the light time fraction is altered.<sup>18</sup> Nevertheless it is desirable to show that in a purely qualitative sense this equation fails to interpret the flicker response contour in any single type of vertebrate.

<sup>15</sup> *J. Gen. Physiol.*, 1935-36, **20**, 363, 411; 1937-38, **21**, 17.

<sup>16</sup> Hecht, S., Shlaer, S., and Smith, E. L., Intermittent light stimulation and the duplicity theory of vision, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1935, **3**, 237.

<sup>17</sup> *J. Gen. Physiol.*, 1936-37, **20**, 393, 411; 1938-39, **22**, 311, 795. *Proc. Nat. Acad. Sc.*, 1938, **24**, 216; 1939, **25**, 78.

<sup>18</sup> *J. Gen. Physiol.*, 1937-38, **21**, 313, 463. Ross, R. T., *J. Gen. Psychol.*, 1938, **18**, 111.

The question cannot be decided by curve fitting to observations under fixed conditions. One reason is that for duplex performance curves (a) the rod curve is usually much too small, and in some cases (e.g. man, newt<sup>19</sup>) too complexly involved with the cone segment, and (b) the larger cone part cannot be directly tested in the lower 20 per cent of its range. For such duplex contours it has been found necessary to conclude that the mechanism of excitability includes systems of processes in which the orders (i.e.,  $n$  and  $m$  in the equation) of the reactions differ for rods and cones, in the same animal. If the dependence of critical intensity upon temperature proves to be quantitatively the same, however, for rod and cone curves it cannot be assumed, nor on any ground asserted, that the physicochemical basis for visual performance is different in the rod and cone elements. This is not the same thing by any means as stating that they are identical. If the physicochemical basis for primary excitation of retinal rods and cones is different in essential particulars, this difference is not involved in the differing shapes of the two branches of the flicker response contour. And the chemical kinetics of the primary excitation reactions therefore cannot be deduced from the form of the curve.

For *Enneacanthus*,  $X$ ,  $P$ ,  $H'$ , and  $H''$ , the application of the photo-stationary state equation provides a passable description of the rising phase of the  $F - \log I$  contour.<sup>20</sup> For *Triturus* (newt)<sup>19</sup> and certain others it does not. Nor in any case does it give anything like an acceptable interpretation of the intermediate region of the  $F - \log I$  curve where the putative rod and cone branches overlap. The upper cone segments are in general rather well described. For those cone segments which are of low slope and much involved in the rod part,<sup>21</sup> the description applies only to the highest levels of  $F$ . For much more completely exposed cone curves the use of the equation gives actually no better a description than is given by a logistic in  $\log I$  (i.e.,  $n = m$ ). For completely isolated simplex visual performance curves, rod or cone, the equation does not fit at all.<sup>22</sup> In these cases values of  $n$  and  $m$  (apparent reaction orders for opposed dark and light reactions) are chosen as 1 or 2. In different animals the values which have to be selected differ for the rod and cone branches. In any one animal they also differ in this way.

<sup>19</sup> *J. Gen. Physiol.*, 1937-38, **21**, 203. *Proc. Nat. Acad. Sc.*, 1938, **24**, 125.

<sup>20</sup> *J. Gen. Physiol.*, 1935-36, **20**, 363; 1937-38, **21**, 17. Hecht, S., 1938, *J. Appl. Physics*, **9**, 156. *Harvey Lectures*, 1937-38, **33**, 35.

<sup>21</sup> Data on *Fundulus*, in course of publication.

<sup>22</sup> *Proc. Nat. Acad. Sc.*, 1938, **24**, 125, 216, 538. *J. Gen. Physiol.*, 1938-39, **22**, 311, 795.

Since the flicker response contours for *Enneacanthus*, *X.*, turtle, *Anax* and the like exhibit differences in their shape constants, and since  $\mu$  for  $1/I$  is likewise different, it could be entertained as a possibility (but not proved) that with these forms, differences in the chemical (including photochemical) kinetics of the excitatory processes are involved in producing the differences in shapes of the contours. The argument is completely independent of any specific assumptions whatever concerning the form which the mass action kinetic equations employed to describe the data may be forced to take. But the same kinds of differences in shape constants occur in the rod and cone segments for a single kind of animal, as in *X.*, *P.*, *H'*, *H''*, and *E.*<sup>23</sup> Yet for each of these cases (excepting *H'*, not tested) the temperature characteristics ( $\mu$ ) for  $1/I_m$  at fixed *F* are identical.

Consequently one is not permitted to assume that in any of these fishes the chemistry of the excitability situation is different in the rod and cone branches of the intensity function. The alternative is to conclude that the excitabilities concerned in determining the quantitative properties of the data are not the excitabilities of the primary rods and cones in the retina. However different the intrinsic basis of photic excitation may be in the several kinds of retinal elements, these differences cannot be characterized from the properties of the two branches of the flicker contour, and the photochemical kinetics of the primary excitation cannot be deduced from its shape.

This conclusion is amply reinforced by the fact that the shape constants for cone and rod parts in *X.* and *P.* differ, in the same manner as for other forms, yet for their quite dissimilar contours  $\mu$  is identical. It cannot be assumed that the chemistry of excitability is different, for either the rod population or the cone, in *X.* and *P.* Moreover, the shape constants are heritable, as the data on  $F_1$  prove, yet  $\mu$  for rod and cone parts of the contour is again the same. Therefore,  $\mu$  is independent of the shape constants.

The general conception that the quantitative properties of data of visual performance are not determined at the periphery,<sup>23</sup> and consequently do not reflect quantitative properties of the primary receptors, is obviously supported by a number of additional facts not otherwise accounted for save by the use of various extra and very dubious assumptions. Thus the result of induced variations in the availability of vitamin A is to synchronously modify both rod and cone thresholds in the same direction;<sup>24</sup> the same is

<sup>23</sup> Crozier, W. J., and Holway, A. H., 1938-39, *J. Gen. Physiol.*, **22**, 341.

<sup>24</sup> Haig, C., Hecht, S., and Patek, A. J., Jr., *Science*, 1938, **87**, 534. Hecht, S., and Mandelbaum, J., *Science*, 1938, **88**, 219. Wald, G., Jeghers, H., and Arminio, J., *Am. J. Physiol.*, 1938, **123**, 732.

true of changes in pressure of atmospheric oxygen.<sup>25</sup> At the same time, retinal adaptation may occur without detectable change in concentration of visual purple,<sup>26</sup> supposedly the basis for the distinction between rod chemistry and that of cones<sup>27</sup> and so far as now known the only basis for the peripheral involvement of vitamin A in photic excitability.<sup>28</sup>

The point has frequently been made that visual performance clearly involves peripheral as well as central factors. This is obvious enough, but the problem at the moment is to decide (a) whether the specific properties of the data, the essential invariant indices of performance capacity, are determined peripherally or not; and (b) which (if any) of the measures of performability have any connection with kinetic chemical features of excitability. It is to be noted that the form of the  $F$ -log  $I$  contour is certainly not specific,<sup>29</sup> as shown by the fact that a probability integral in the log of the independent variable applies to a great variety of dynamically similar but otherwise unlike situations; moreover experimental tests of the three parameters show that they have concordant properties in these cases. This formulation applies where one deals with the contributions summated from many varying elements, and if suitable separate determinations could be made of purely retinal and purely "central" phenomena they would pretty certainly be found to follow the same form of law as a function of intensity. Likewise, as other work has shown,<sup>30</sup> and as general considerations have indicated to be likely,<sup>31</sup> the same formulation is definitely applicable to various kinds of measures of visual performance. For these reasons it is already improbable that the quantitative properties of the data will tell anything about the kinetics of specific types of peripheral excitation. Decision as to the central or peripheral origin of the quantitative properties of the data must depend upon the use of other criteria.<sup>32</sup>

It might still be suggested that some sort of "limiting case" could be found in which, despite the passage of impulses from receptors to muscles involved in obtaining the data, we might have to do with a simple one-to-one correspondence between excited end-organ units and central units. It is to be pointed out, however, that, in such a case, we would still have to deal

<sup>25</sup> Cf. McFarland, R. A., and Evans, J. N., 1939, *Am. J. Physiol.*, **127**, 37.

<sup>26</sup> Granit, R., *J. Physiol.*, 1938, **94**, 430.

<sup>27</sup> Hecht, S., *Physiol. Rev.*, 1937, **17**, 239.

<sup>28</sup> Wald, G., *J. Gen. Physiol.*, 1935-36, **19**, 351; 1937-38, **21**, 795.

<sup>29</sup> *Proc. Nat. Acad. Sc.*, 1937, **23**, 71.

<sup>30</sup> The interpretation of the time and intensity function, and of photic adaptation, to be discussed elsewhere.

<sup>31</sup> *J. Gen. Physiol.*, 1935-36, **19**, 503.

<sup>32</sup> *Proc. Nat. Acad. Sc.*, 1936, **22**, 412; 1937, **23**, 23, 71.

with the fact that the units of the end result are not the same as those of the initial excitation. We are stressing the inescapable circumstance that the units involved are necessarily in terms of  $\Delta E_1/\Delta I$ , where  $E_1$  is primary effect produced and  $I$  is intensity (or other independent evoking variable). If the units of end result  $R$  are  $\Delta R/\Delta I$ , then we have to recognize that if  $E_1$  is in terms of impulses per unit time or something of that sort then when we put

$$\Delta R/\Delta I = K \Delta E_1/\Delta I$$

and eliminate  $\Delta I$  we still must reckon with the complex dimensional constant  $K$ , and proof is required that it is independent of  $I$  and  $E_1$  before we can argue for the direct interpretation of  $R$  in terms of the initiating mechanism of excitation.

## VI

The shape constants ( $\sigma'_{\log I}$ ), maxima, and abscissae of inflection ( $\tau'$ ) for the  $F$ -log  $I_m$  curves differ markedly in the forms  $X$  and  $P$ . The results already considered show empirically that  $\mu$  cannot be determined by or correlated with any of these parameters. The evidence<sup>3</sup> indicates that in  $H'$  and  $H''$  the shape constant for the rod part may be inherited independently of that for the cone part of the curve, and independently of changes in  $F_{max}$  or  $\tau'$ .  $F_{max}$  may also be specifically heritable, or may show modifications. In the hybrids,  $\tau'$  is increased. This could be formally explained as due to a lesser amount of excitable substance (or to a greater amount of an inhibitor) in all the elements of a set. Changes in  $F_{max}$  are clearly to be understood as due to alteration in the total number of elements of induced effect; this does not mean increased numbers of cells, but refers to the sort of increase in  $F_{max}$  (without change of  $\sigma'_{\log I}$ ) which can be produced by shortening the light flash duration in the cycle—although this involves diminution of  $\tau'$ . It would be of some interest in this connection to know whether  $\mu$  would be the same if the percentage light time in the flash cycle were altered. We are more concerned, however, with the heritably invariant shape constants ( $\sigma'_{\log I}$ ) of the  $F$ -log  $I$  contours and their independence of  $\mu$ .

The designation of heritable attributes of organic constitution has been based fundamentally upon the recognition of rather gross differences between properties which behave as alternates in inheritance. The tangle of hypotheses and suppositions which has grown up about the attempt to systematize the analysis of less clear examples has added nothing of an independent character to the theory of the gene. From this standpoint a

gene is a label for the entity which in an operational sense exhibits a particular behavior in breeding experiments. The elaboration of the evidence that in the germinal chromosomes there is found a mechanism which parallels in minute detail the behavior required of the basis of segregation, linkage, crossing over, and spatial interrelationships of heritable units provides empirically a physical explanation of the assortment of the determinants of characteristics; but tells nothing as to the nature of their control. Active realization of the elementary fact that the individual manifestation of organic properties is achieved in the course of individual development has served only to sharpen the difficulty of theoretical genetics. On the one hand we have the numerical results of breeding tests in which developed properties are the markers which permit recognition of the distribution of heritable units; on the other hand we have the known behavior of chromosomal elements. The temptation has been insistent to account for the "nature" of a gene in terms of the physicochemical properties which the size and rationally presumed activities of the chromosomal gene require. The net result, as one finds it at the moment in the body of "physiological genetics," is fairly to be characterized as a mass of curiously unconvincing assumptions concerning the involvements of enzymes and unsatisfying analogies with the physicochemical manifestations of reaction rates.

Experiment has shown that there exist definite properties of individual organisms which are quantitatively reproducible, and heritable in a simple manner, but which could not be recognized at all by means of the customary and by now practically static and crystallized methods of genetic investigation.<sup>33</sup> The observable nature of several of these properties indicates the origin of at least a part of the prevalent confusion in genetic theory. Their investigation plainly suggests the kind of procedure which may aid materially in putting theoretical consideration of these matters upon a more productive foundation. The properties concerned are typically those characteristics of individual organisms which are to be termed capacities to exhibit particular modes of performance. A capacity for performance can be estimated only in terms of measured performance under defined conditions. It is easy to demonstrate in an elementary way that the index

<sup>33</sup> Crozier, W. J., and Pincus, G., 1929-30, *J. Gen. Physiol.*, **13**, 57. *Proc. 6th Internat. Cong. Gen.*, 1932, **2**, 31. *J. Gen. Physiol.*, 1936-37, **20**, 111. The essential point has in certain features been realized by Hogben and others, but not completely (*cf.* Hogben, L., *Nature and nurture*, 1933, New York, W. W. Norton & Co.; and Dickson, H., 1939, *Ann. Bot.*, **3**, n.s., 113.

of such a *capacity* can be obtained only by defining the relationship between measured performance and values of a controlled variable responsible for its manifestation. Consider the situation described by the curves in Fig. 1, and especially at the right hand side of this figure. These portions of the curves are reproduced in Fig. 4. Genetic analyses are usually, in fact almost exclusively, based upon the performance of individuals under constant (*i.e.*, the same) conditions. In the present instance this corresponds to testing the flicker responsiveness of *X.*, *P.*, and *H''* fishes, under fixed

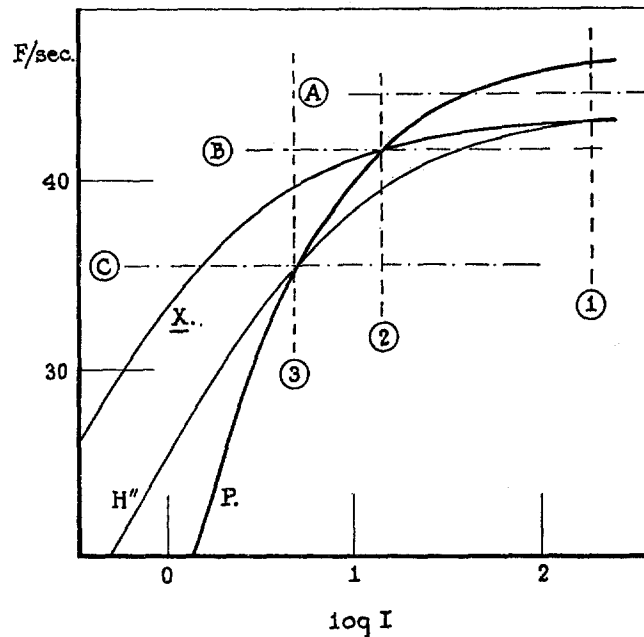


FIG. 4. The upper end of the graphs in Fig. 2, with dashed lines added to indicate the relationships which would appear if observations seeking to compare the responsiveness of *X.*, *P.*, and  $F_1$  were to be made at constant conditions of *F* or of *I*.

conditions of temperature, of  $t_L/t_D$ , and of antecedent dark adaptation, and of either flash intensity or flash frequency (it being impracticable to measure the internal sensory disturbance at the threshold state for just detectable response when it is forced to appear). Since there is no *a priori* guide for the selection of any specific set of conditions we have a right to compare, on the basis of the information in Fig. 1, the results to be obtained by selecting in turn a number of different conditions under which the comparison is to be made. If these in fact lead to contradictory results we



must conclude that this general method of comparison is incapable of leading to significant deductions.

Suppose we had chosen the comparison implied by line *A* in Fig. 4. We would conclude that *P.* and *X.* clearly differ in the sense that *P.* is flicker responsive, *X.* not; and *H''*, their  $F_1$  hybrids, would obviously be like *X.* If instead we had happened to choose as working environment the conditions signified by flash intensity level 1, we would conclude that *X.* responds at a lower flash frequency than *P.*, and that the  $F_1$  hybrids *H''* are quantitatively like *X.*; at flash intensity 3, however, if we had happened to select this, and had at our disposal only the results at this level, we would be compelled to consider that *X.* is typified by a higher critical *F* than *P.* and that *H''* is precisely like *P.*; at position 2, or *B*, *X.* and *P.* would be found indistinguishable but *H''* either lower (in  $F_c$ ) or higher ( $I_c$ ) than either, depending on the criterion for elicitation of performance we chanced to be using. It is clear that only by acquaintance with the whole curve can we hope to institute comparisons between individuals which can be used for interpretative analysis. In this way alone can we avoid the confusion inescapable when arbitrarily fixed conditions circumscribe the exhibition of differences between individuals concerned in breeding experiments. And a large and significant factor of uncertainty exists in any given case of whatever kind when the invariance of the characteristics involved with respect to developmental factors has not been demonstrated. Apart from this, unless the nature of such dependence has been established, we are left with mere markers of the existence of functional differences. These markers, in general, give no information whatever as to the kinetic character of the processes from which they result.

In many cases the recognizable features of performance characteristics necessarily depend upon the integrated or at least collective actions of a number of cells or other units. The summated actions of these units inevitably exhibit properties involving the distribution of contributive capacities among the units present. That this distribution is one of uniformity is excessively unlikely. That it may be random or according to some other fixed rule is to be decided from the data. The problem then arises, how is it possible for an assemblage of units to provide summated effects following a simple law which is physically interpretable? A full discussion of this problem, would take us far from our immediate purpose, because the question is decidedly a fundamental one for many kinds of measurements with biological systems. It is insistent in connection with measurements of the relation between temperature and "velocity" of per-

formance by cell aggregates,—for example, respiration per unit time in cell suspensions; or photosynthetic liberation of  $O_2$ ; or, in the present data, the velocities of processes governing photic excitation.

Such relationships, when dynamically simple so far as the properties of the measurements reveal, must be taken to signify that the same controlling process is at work in each of the elementary units concerned; the effects which it produces are randomly distributed, as if there were a random distribution of amounts of the governing catalyst.

It thus becomes a matter of very considerable importance to obtain instances in which there may be achieved a means of analytically separating

TABLE III

Mean critical intensities for response at various flash frequencies ( $t_L = t_D$ ;  $21.5^\circ$ ) for 17  $F_2$  individuals (three observations on each of the 17 at all points) produced by 1  $F_1$  ♂ and 2  $F_1$  ♀♀ from *X. montezuma* and *P. maculatus*.  $\log I_m$  (millilamberts) and  $\log P.E._{1F_1}$  are not distinguishable from values for  $F_1$  individuals (in Crozier, W. J., and Wolf, E., *J. Gen. Physiol.*, 1938–39, **22**, 463). See Fig. 4.

$F$ per sec.	$\log I_m$	$\log P.E._{1F_1}$
2	$\bar{6}.4440$	$\bar{8}.7989$
3	$\bar{6}.8193$	$\bar{7}.0022$
4	$\bar{5}.1235$	$\bar{7}.5355$
5	$\bar{5}.8298$	$\bar{6}.0777$
6	$\bar{4}.5441$	$\bar{6}.9659$
8	$\bar{3}.7317$	$\bar{5}.8985$
10	$\bar{2}.7330$	$3.2541$
15	$\bar{1}.4043$	$\bar{3}.7954$
20	$\bar{1}.6772$	$\bar{3}.9831$
30	$0.2615$	$2.7479$
40	$1.2365$	$\bar{1}.4629$

the purely statistical parameters from those giving opportunity for kinetic characterization. The present data supply an instance. Certain statistical parameters for rod and cone sections of the *X.* and *P.* curves (Fig. 1) are specific and determinate, as proved directly by the results of the breeding tests. That these parameters ( $F_{max.}$  and  $\sigma'_{\log I}$ ) are essentially statistical is proved by the manner in which they are (nonspecifically) dependent upon retinal area and light time fraction in the flash cycle.<sup>34</sup> The abscissa of inflection ( $\tau'$ ), however, is a simple function of temperature. The descriptive constant in this relationship ( $\mu$ ) is independent of  $F_{max.}$  and of  $\sigma'_{\log I}$ ,

<sup>34</sup> *J. Gen. Physiol.*, 1937–38, **21**, 223, 313, 463.

and is the same in  $H''$  as in the parent stocks  $X$ . and  $P$ . Thus it must be concluded that, since  $F_{max.}$  and  $\sigma'_{\log I}$  can follow one parent precisely in the hybrid offspring, the purely statistical properties exhibited by performance

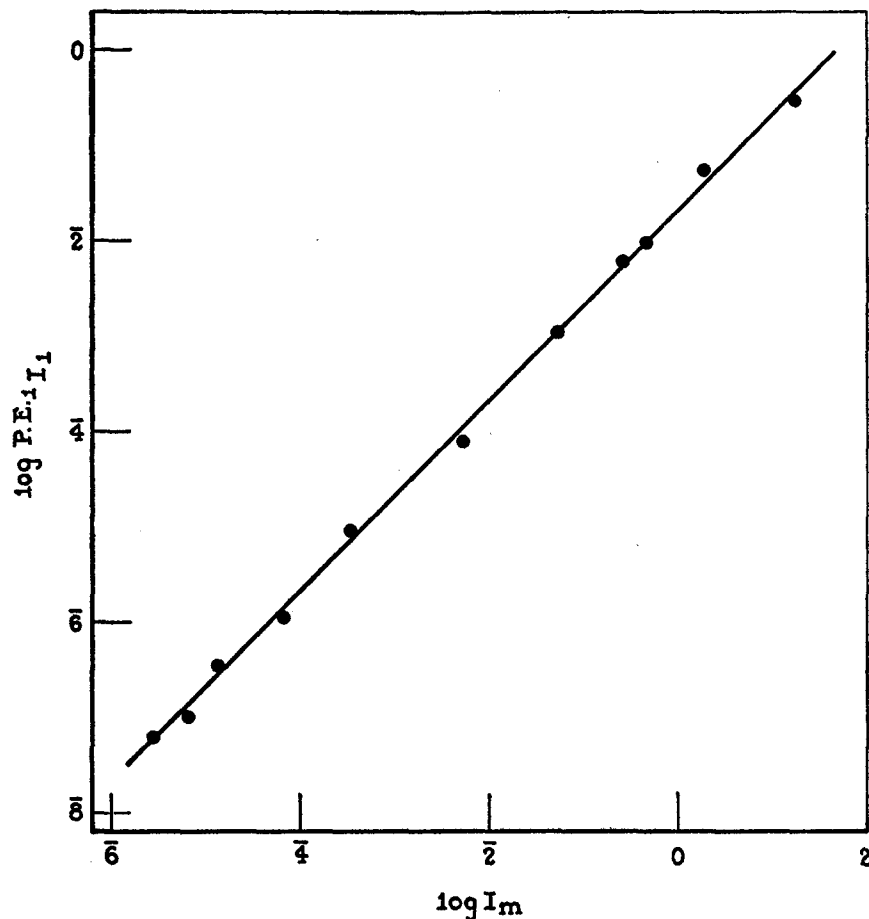


FIG. 5. The variation of  $I_1$  is directly proportional to  $I_m$  in the data on  $F_2$  individuals from  $X. \times P.$ , and the proportionality constant is the same as for  $F_1$  (see text),  $n = 3$ ,  $N = 17$  (*i.e.*, fifty-one readings at each point).

capacity may be inherited without the expression of a chemical difference in the mechanism governing the performance.

The crossing of  $X$ . and  $P$ . undoubtedly brings into play conditions well known to be involved in interspecific and intergeneric crosses. Only certain genetic combinations are viable, and peculiar conditions arise in the sex manifestations, so that frequently such hybrids tend to breed true. Thus

in our earlier experiment<sup>2</sup> backcrosses of  $F_1 \times X$ . gave ( $H'$ ) animals providing a condition of the  $F$ -log  $I$  contour essentially like that of the present  $F_1$  ( $= H''$ ); the minor differences (of  $\tau'$ , and in the shape of the rod branch) could be explained by differences of species in the  $X$ . and  $P$ . parental stocks. So also, several batches of  $F_2$  hybrids produced from 1 ♂ and 2 ♀♀  $F_1$ 's gave data (Table III) not to be distinguished quantitatively from those for  $F_1$  at the same temperature, either in the values of  $I_m$  or of the inter-individual variability. Simple segregations are not necessarily to be looked for, in general, in such material. Color segregations were apparent in the expected way: of 17 individuals, 11 were "gray" and 6 "gold." What is important, rather, is the stability of the descriptive constants introduced as result of the crossing.<sup>35</sup>

## VII

## SUMMARY

For the teleosts *Xiphophorus montezuma*, *Platypoecilus maculatus*, and their  $F_1$  hybrids the temperature characteristics ( $\mu$  in Arrhenius' equation) are the same for the shift of the low intensity and the high intensity segments of the respective and different flicker response contours (critical intensity  $I$  as a function of flash frequency  $F$ , with light time fraction constant, at 50 per cent). The value of  $\mu$  is 12,500 calories or a very little less, over the range 12.5 to 36°. This shows that  $1/I$  can be understood as a measure of excitability, with  $F$  fixed, and that the excitability is governed by the velocity of a chemical process common to both the classes of elements represented in the duplex performance curve (rods and cones).

It is accordingly illegitimate to assume that the different shapes of the rod and cone branches of the curves are determined by differences in the chemical mechanisms of excitability. It is also forbidden to assume that the differing form constants for the homologous segments in the curves for two forms ( $X$ . and  $P$ .) are the reflections of a difference in the chemical factors of primary excitability. These differences are determined by statistical factors of the distribution of excitabilities among the elements implicated in the sensory effect *vs.* intensity function, and are independent of temperature and of the temperature characteristic.

<sup>35</sup> Genetic information about these fishes is referred to in summaries by Goodrich, H. B., *Quart. Rev. Biol.*, 1929, 4, 82, and Goldschmidt, R., *Quart. Rev. Biol.*, 1937, 12, 426. Koswig in particular has used "*X. montezuma*" and *P. maculatus* (*Z. indukt. Abstammungs- u. Vererbungslehre*, 1931, 59, 61), but these observations have been concerned with sex and color factors.

It must be concluded that the physicochemical nature of the excitatory process cannot be deduced from the shape of the performance contour.

The form constants ( $\sigma'_{\log I}$  and  $F_{max.}$ ) for  $F$  vs.  $\log I$  are specifically heritable in  $F_1$ , although  $\mu$  is here the same as for  $X$ . and  $P$ . In an intergeneric cross one cannot in general expect Mendelian simplicity of segregation in subsequent generations, and in the present case we find that  $F_2$  individuals are indistinguishable from  $F_1$ , both as regards  $F$  vs.  $\log I$  and as regards the variation of  $I$  within a group of 17 individuals. The result in  $F_2$  definitely shows, however, that certain specific statistical form constants for the  $F$ - $\log I$  contour are transmissible in inheritance. It is pointed out that there thus is provided an instance in which statistical (distribution) factors in performance characteristics involving the summing properties of assemblages of cellular units are heritable in a simple manner without the implication of detectable differences in chemical organization of the units involved. This has an important bearing upon the logic of the theory of the gene.