

## THE FLICKER RESPONSE CONTOUR FOR THE ISOPOD ASELLUS

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### I

The form of the curve exhibiting the relation between flash frequency ( $F$ ) and intensity of flash ( $I$ ) critical for response to visual flicker is peculiar in the case of various arthropods previously studied.<sup>1</sup> For sundry vertebrates<sup>2</sup> the curve of  $\log I$  vs.  $F$  is either a simple probability integral or else (typically) a composite of two such curves, corresponding to the duplexity of the visual action system.<sup>3</sup> For such forms as bee, dragon fly larva, and fiddler crab,<sup>1</sup> however, the lower portion of the curve increases in slope too rapidly for this formulation. All of these animals have large convex optic surfaces. The deformation of the  $F - \log I$  graph was accordingly attributed<sup>4</sup> to the existence of mechanical conditions affecting reception of light by these eyes. At lower intensities certain of the ommatidia, especially around the periphery of the eye, do not receive sufficient energy to enable them to contribute to the determination of response to flicker within the time permitted by the flash cycle. At higher flash frequencies (and higher critical intensities) the chance of these disadvantaged units being significantly implicated should be much greater, and their effect should therefore be detected. In keeping with expectation, the asymmetry is not affected by changing the temperature over a wide range;<sup>5</sup> but, again as predicted, it is *reduced* by increasing the proportion of light time in the flash cycle.<sup>6</sup>

<sup>1</sup> Cf. Crozier, Wolf, and Zerrahn-Wolf, 1937-38c.

<sup>2</sup> Wolf and Zerrahn-Wolf, 1935-36; Crozier, 1935-36; Crozier, Wolf, and Zerrahn-Wolf, 1936-37a, d; 1937-38a, d; 1937; 1938c, d. 1938a, b; 1938-39; 1937-38b; Crozier, 1937; and footnote 15.

<sup>3</sup> Crozier, Wolf, and Zerrahn-Wolf, 1938a, e.

<sup>4</sup> Crozier, Wolf, and Zerrahn-Wolf, 1936-37b; 1937-38c, e.

<sup>5</sup> Crozier, Wolf, and Zerrahn-Wolf, 1937-38c.

<sup>6</sup> Crozier, Wolf, and Zerrahn-Wolf, 1937-38e.

## II

A rather direct test of the validity of these considerations should be obtained by determining the flicker frequency *vs.* critical intensity relationship for an arthropod with smaller, flatter eyes—particularly if the ommatidial axes do not diverge too greatly in each eye. The more or less incidental information available with respect to the eyes of isopods<sup>7</sup> indicates that aquatic forms such as *Asellus* should give material for a crucial examination of the point. Our own sections of the eye of *Asellus aquaticus* (histologically a difficult object) show that each eye consists of roughly four rows of rather well separated ommatidia with (save at the edge) approximately parallel axes. Each row shows about 25 ommatidia. It is crudely estimated that each eye is made up of about 125 (or fewer) ommatidia. Its surface is not markedly curved, as in such forms as *Anax*.

The apparatus producing flickered light has been described in our earlier papers.<sup>2</sup> The tests with *Asellus* were made by determining critical intensities at fixed flash frequencies. Observations were made under conditions such that the light time in a flash cycle was equal to the dark time (*i.e.*,  $t_L/t_D = 1$ ). The same type of thermal adaptation and control of temperature (21.5°) was used as in previous experiments with other forms.<sup>2</sup>

The data obtained with *Asellus* show none of the “distorted” asymmetrical features thus far encountered with other arthropods. The  $F - \log I$  curve, like that for the rod-free turtle *Pseudemys*, is a simple, symmetrical function over its whole range. Especially when considered together, these two curves permit a really significant test of the acceptable analytical form of the flicker response contour, because the whole range of the function is open to examination. This has not been possible with vertebrates exhibiting duplexity of visual properties, since only the upper 80 per cent or so of the “cone” curve has been available and the “rod” curve is comparatively minute as well as complicated<sup>2</sup> by its overlapping with the lower (concealed) end of the cone curve.

*Asellus* were kept individually in the cylindrical dishes of thin glass in which their visual responses were tested. To secure precise reactions a good foothold

<sup>7</sup> Parker, 1891; Peabody, 1938.

must be provided. On a glass surface struggling motions may appear at the beginning of a response, and the specific reaction to the moving stripe system cannot then be easily recognized. Filter paper accurately cut to fit the bottom of the dish was therefore used to give a surface for easy creeping. *Asellus* is very reactive to vibrations and to disturbance of the water. After being put in place in the apparatus they usually run about for a time. Light may influence this restlessness. During a test the animal must be quiescent when the light is admitted. If it is found to be moving, the diaphragm is closed. Observation is then re-begun until the isopod is found to be quiet.

At the threshold reaction to flicker the isopod may dash to the periphery of the jar and then continue crawling in the direction of the movement of the stripes. If it is already at the wall of the container and facing in the direction of the motion of the stripes, the initial response is usually a short dash, which may or may not be continued around the dish. In some cases circus movements are observed in the center of the jar. Frequently recognizable response is obtained when the animal is already in motion, in the form of a sudden change of direction. However, if the isopod is at first facing against the motion of the illuminated stripes the reactions are not clear; the turning movements as result of reaction to the flicker are too slow and not adequately sharp for use as an index, although the critical illumination as then observed is clearly of the same order of magnitude as in the other cases. There is no difference between the critical illumination for response to flicker, at any flash frequency, for the non-moving *Asellus* whether in the center of the jar or in contact with the wall,—although in the latter instance there is opportunity for a stereotropic factor which might influence the reading.

### III

The data obtained with *Asellus* are given in Table I. The figures are averages obtained from the same 10 individuals at each flash frequency ( $F$ ). The various levels of  $F$  tested were used in practically random order, and there are no detectable drifts from day to day during the course of the experiment. For reasons which have been given in earlier papers,<sup>2-4</sup> the readings were taken in the following way: At each  $F$ , three observations of the critical illumination in a flash are obtained in succession on an individual; the mean of these =  $I_1$  for that animal; the mean value of the  $I_1$ 's for the ten numbered isopods =  $I_m$ ; the P. E. of the dispersion of the  $I_1$ 's = P. E.  $r_1$ . These values are (as their logarithms) entered in Table I. Readings are taken at two flash frequencies on each day. In tests made at different  $F$ 's the numbered individuals are used in (different) random orders of succession.

The values of  $I_1$ , for a given  $F$ , can be arranged in an ascending order. The rank-order numbers (1-10) indicating decreasing sequence of excitabilities may then be examined to establish the degree of homogeneity in the group of individuals for which the  $I_1$  readings are averaged. Homogeneity of the group is important for the understanding of the meaning of the data.<sup>8</sup> In the absence of demonstrable homogeneity the data refer to an average performance which may well

TABLE I

Mean critical intensities, and dispersions, at various flash frequencies,  $F$  per sec., for response of *Asellus aquaticus* to flashing light, as  $\log I_m$  and  $\log P.E._{I_1}$  (millilamberts). Light time and dark time equal in the flash cycle (*i.e.*,  $t_L/t_D = 1$ ). Temp. = 21.5°. The same 10 individuals used at each  $F$ , with three readings on each averaged to give  $I_1$  for each individual;  $P.E._{I_1}$  is the dispersion of these means.

$F$ per sec.	$\log I_m$	$\log P.E._{I_1}$
1	$\bar{3}.2533$	$\bar{5}.5464$
2	$\bar{3}.6235$	$\bar{5}.9860$
4	$\bar{3}.9023$	$\bar{4}.2151$
7	$\bar{2}.2874$	$\bar{4}.8238$
10	$\bar{2}.5136$	$\bar{3}.1206$
15	$\bar{2}.7863$	$\bar{3}.0232$
20	$\bar{1}.0414$	$\bar{3}.4284$
25	$\bar{1}.2790$	$\bar{3}.6871$
30	$\bar{1}.5149$	$\bar{2}.0519$
35	$\bar{1}.7252$	$\bar{2}.0414$
40	$\bar{1}.9939$	$\bar{2}.5205$
42.5	$0.2185$	$\bar{2}.8005$
45	$0.4442$	$\bar{2}.7317$
47.5	$0.8700$	$\bar{2}.8562$
50	$1.0871$	$\bar{1}.4749$
51	$1.5331$	$\bar{1}.8689$

have no ascertainable meaning. A homogeneous group is one in which the individuals are nearly enough alike for the purposes of the experiment so that they are demonstrably interchangeable. This must be established from the data. This kind of demonstration is curiously absent in studies of the sensory performance of human subjects. Unless it can be established, merely increasing the number

<sup>8</sup> Crozier, 1935-36; 1936.

of individuals in the tested group serves only to obscure more completely the nature of the measured phenomenon. At the same time, the discovery in the data of minor persisting heterogeneities can serve to prove the adequate refinement and precision of the methods of observation. It is really impossible to influence subjectively the observations, under the conditions of these experiments, in such a way as to produce accidentally specific correlations of the kinds which the readings disclose.

The mean rank-order numbers for our ten *Asellus* are randomly distributed in time, and show no correlation in pairs of tests made successively on the same individual. The procedure is delicate enough to detect this effect when it is real (*cf.* *Anax* data;<sup>9</sup> turtles<sup>10</sup>). The two individuals whose mean rank-order numbers depart most widely on either side of the mean are Nos. 5 and 8. For these the respective mean rank-order numbers with their P. E.<sub>m</sub>'s are:  $7.09 \pm 0.769$  and  $3.84 \pm 0.648$ . The difference is 3.8 times its probable error, and is thus barely on the verge of possible significance. This is borne out by the fact that the mean critical intensities for No. 8 are on the average (12 cases out of 16) slightly greater (by *ca.* 4 per cent) than those for No. 5 determined at the same time. The evidence of consistency is of interest as reinforcing the indications of the rank-order analysis, and as objectively showing the reliability of the observational procedures employed. This is the general result in all the investigations we have made, with various forms, when an essentially homogeneous population has been available. When *Asellus* Nos. 5 and 8 are excluded, the relative rank-order positions of the remaining 8 are distributed in an entirely random way. The slight but persisting individualities of Nos. 5 or 8 have no detectable influence on the form of the curve which the data give, and the group of 10 is accordingly treated as, from a practical standpoint, homogeneous.

The variation of  $I_1$ , measured by P. E.<sub>1</sub>, obeys the rule observed with other forms: P. E.<sub>1</sub> is directly proportional to  $I_m$  (Fig. 1; data in Table I). As with other invertebrates for which flicker response

<sup>9</sup> Crozier, Wolf, and Zerrahn-Wolf, 1936-37*b*, *c*; 1937-38*c*, *e*.

<sup>10</sup> Crozier, Wolf, and Zerrahn-Wolf, 1938-39.

data have been obtained,<sup>1, 4, 11</sup> there is no "break" in the plot of  $P. E. I_1$  vs.  $I_m$ . This is also observed for the measurements with the turtle *Pseudemys*,<sup>10</sup> in which one encounters the participation of but a single class of neural elements in the determination of the response;<sup>10, 12</sup> for other vertebrates the visual duplexity phenomenon seems to be definitely correlated with a second, different proportionality factor in the variation function for the exclusively cone range of the flicker

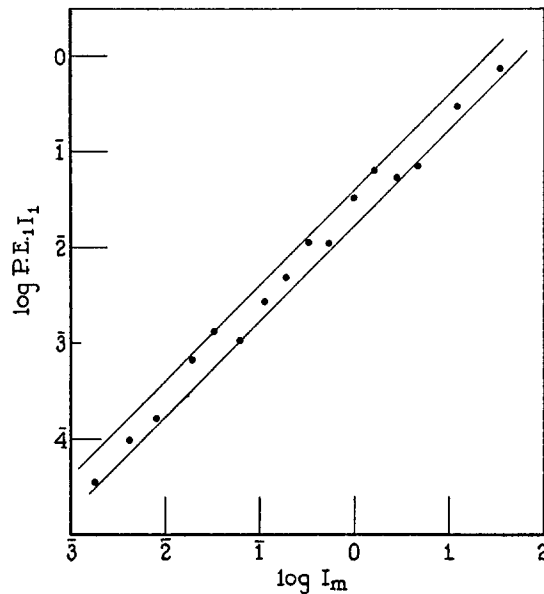


FIG. 1. The variation of mean critical intensities ( $I_1$ ) for response to flicker in *Asellus*, as a function of the average critical intensity. The slope of the band = 1, and  $P.E. I_1$  is consequently directly proportional to  $I_m$ . Data in Table I.

response contour.<sup>2</sup> The proportionality factor for *Asellus* is the same as for *Anax*.<sup>13</sup>

#### IV

The relation between  $I_m$  and  $F$  is shown graphically in Fig. 2. The curve drawn is a calculated probability integral,

<sup>11</sup> Crozier, 1935; 1935-36.

<sup>12</sup> Crozier, Wolf, and Zerrahn-Wolf, 1938a.

<sup>13</sup> Crozier, Wolf, and Zerrahn-Wolf, 1937-38c, e.

$$F = KF_{max} \int_{-\infty}^{\log I} \exp\left[\left(-\log \frac{I}{I_0}\right)^2 / \sigma_{\log I}^2\right] d \log I, \quad (1)$$

where  $I_0$  is the intensity at the inflection point ( $\log I_0 = \bar{1}.295$ ). The graph differs strikingly from those obtained for *Apis*, *Anax*, and the like; as we have already indicated, the curves of  $\log I_m$  vs.  $F$  for the latter are definitely asymmetrical. On the basis of expectation derived from the shape and structure of the eye of *Asellus* we were led to believe that the curve for the latter would not be skewed. The excellent description of the data by a log probability integral is

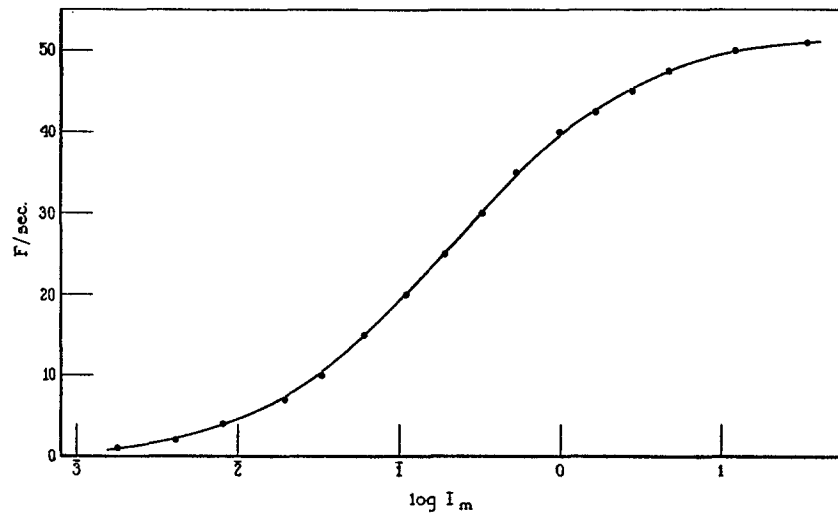


FIG. 2.  $\log I_m$  vs.  $F$  for *Asellus*. Data in Table I. The curve is a calculated probability integral (cf. Fig. 3).

indicated even better by Fig. 3 than in Fig. 2. In Fig. 3 the extent of the distortion of the flicker response contour for arthropods with large convex eyes is apparent.

The *Asellus* curve is clearly of the same nature as the uncomplicated curve given by the cone-retina turtle *Pseudemys*.<sup>14</sup> That it is in each case so well described by a log probability integral is of importance for the theory of the flicker recognition function. The interpretation of this function, of course, cannot in any case be based merely upon

<sup>14</sup> Crozier, Wolf, and Zerrahn-Wolf, 1938a, b; 1938-39.

the results of descriptive curve fitting. But in any event until very recently there were not available uncomplicated flicker response contours sufficiently complete in range to provide suitable material for such tests. The curve for *Pseudemys*<sup>14</sup> and that for *Asellus* are free

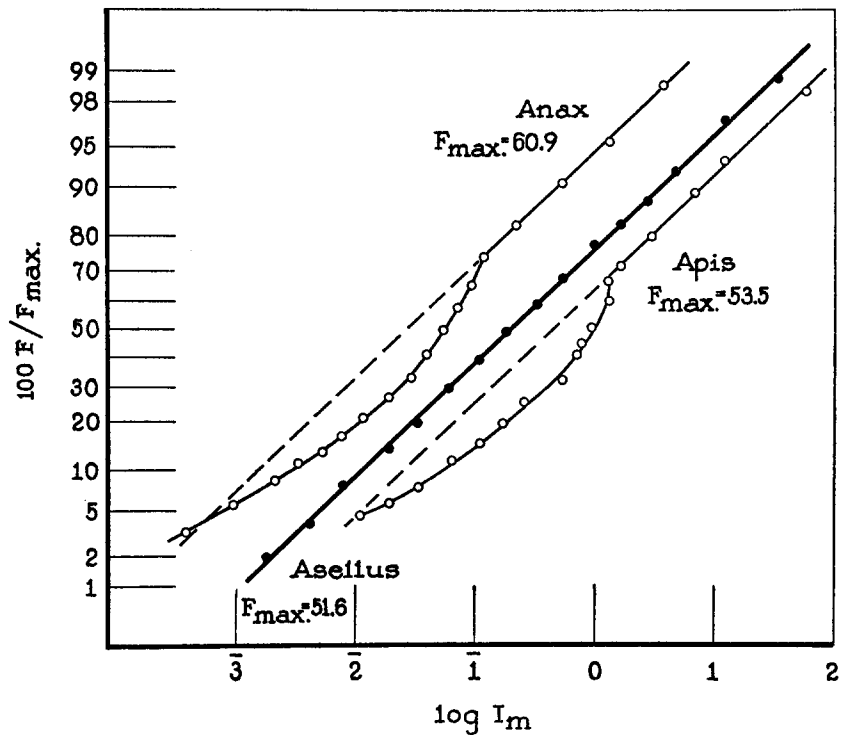


FIG. 3. The flicker response contours ( $F - \log I_m$ ) for *Apis*, *Anax*, and *Asellus*, on a probability grid (with  $F$  as a percentage of the respective  $F_{max}$ . in each case). The asymmetrical character of the graphs for bee and dragon fly nymph is discussed in the text. The slopes of the three probability integrals (straight lines) are sensibly identical, indicating that  $\sigma'_{\log I}$  is the same for these 3 arthropods; see text.

from structural complications. Each is decidedly well fitted by a probability integral in  $\log I$ . They cannot be adequately fitted by the logistic

$$F = F_{max.}/(1 + e^{-s \log q I}) \quad (2)$$



Fig. 4 shows that a maximum  $F$  may be assumed (about 4 per cent higher than that used for Figs. 2 and 3), which will give an apparently

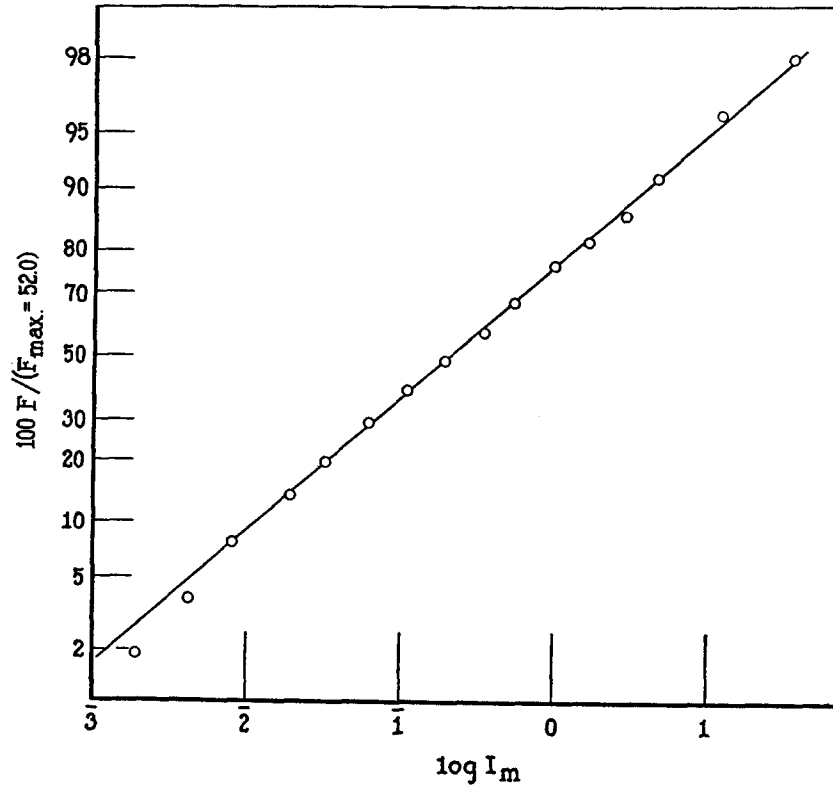


FIG. 4. The data of Table I and Fig. 2 on a logistic grid. The maximum of  $F$  has been chosen to give nearest approach to rectilinearity. The lower portion of the curve departs widely. Data from experiments on the turtle *Pseudemys*<sup>10</sup> show the same type of departure, and in each case calculation shows that the data on this grid really follow a sigmoid course; this could not be decided with certainty if the upper portion of the curve ( $F = 10$  to 50) were the only portion available; see text.

reasonable fit to the upper 80 per cent of the flash frequency range of the data. The fit decidedly fails, however, for the lower end.

Equation (2) is of interest because it is identical with the equation proposed by Hecht<sup>15</sup> for the flicker curve on the basis that  $F$  is pro-

<sup>15</sup> Hecht, 1937; Hecht, Schlaer, and Smith 1935; Hecht and Smith, 1935-36.

portional to the concentration of photoproducts in the retinal photosensory state:

$$pKI = (F)^n / (F_{max} - F)^m, \quad (3)$$

with  $m = n$ ;  $p$  is the proportion of the flash cycle time occupied by light of intensity  $I$ . If (3) is to be used, (2) can be adequate only when  $m = n = 1/z$ . In this event the slope  $d \log F / d \log I$  approaches  $1/z$  as a limit for very low values of  $F$ . If the data do not extend below  $F = 10$  or so, it is impossible to secure an unequivocal graphical adjustment of equation (3) with small integral values of  $m$  and  $n$  (*i.e.*, 1 or 2). For the two instances (*Pseudemys*; *Asellus*) in which data are reasonably complete this is impossible in any case, since the lower fifth of the curve departs too widely; for the *upper part* a fairly close fit could be gotten with  $m = n = 1$  for *Asellus* and  $= 2$  for *Pseudemys*, but calculation from the data shows that the departures are systematic and significant. Equation (3) also gives erroneous predictions as to the effects obtained by altering temperature<sup>16</sup> and  $p$  the fractional light time in the flash cycle.<sup>17</sup> There is accordingly no real basis whatever for the use of equation (3).

In using the probability integral for purposes of formulation,<sup>18</sup> however, we find a satisfactory fit over the whole range of the data in uncomplicated instances<sup>14</sup> (Fig. 2, *etc.*). A number of analytical advantages also arise in a simple way in dealing with the duplex (or even triplex<sup>8</sup>) flicker contours for typical vertebrates.<sup>2</sup> The basis for decision in the choice of a descriptive analytical function must, of course, be found in the properties of the parameters of the function. For the probability integral these parameters are (1) the asymptotic maximum ordinate ( $F_{max}$ ), (2) the abscissa of the inflection point ( $\log I_o$ ), and (3) the standard deviation of the first derivative of the curve ( $\sigma_{\log I}$ ). For convenience<sup>18</sup> we write  $\tau'$  for  $\sigma'_{\log I}$ , where the  $\sigma'$  refers to the parameter when  $F_{max}$  is taken as 100 per cent. The changes in the three parameters which result from alterations of retinal area,<sup>19</sup> temperature,<sup>9</sup> and proportion of light time in the flash

<sup>16</sup> Crozier, Wolf, and Zerrahn-Wolf, 1936-37b, c; 1938b.

<sup>17</sup> Crozier, Wolf, and Zerrahn-Wolf, 1937-38d, e.

<sup>18</sup> Crozier, 1937.

<sup>19</sup> Crozier, Wolf, and Zerrahn-Wolf, 1937-38c.

cycle<sup>18</sup> show that each of these quantities may be influenced independently of the other two, and in a quantitatively significant way in each case. The behavior of these constants, particularly of  $F_{max}$ , and of  $\sigma_{\log I}$ , in interspecific breeding experiments with fishes<sup>20</sup> shows that a definite physical basis must be presumed for each of them in the neural organization of the animal.

The data on several arthropods are of interest for this analysis. The curious and unexpected fact emerges that the value of  $\sigma'_{\log I}$  is nearly or even quite the same for *Apis*, *Anax*, and *Asellus*. This is easily judged by the parallel graphs in Fig. 3. It is to be recalled that  $\sigma'$  is experimentally an invariant, since neither change of temperature, of area, nor of  $t_L/t_D$  appears to affect it detectably.<sup>16, 17</sup> On the other hand it appears to be a characteristic, specific, constant, among vertebrates, since it behaves in a unitary manner in inheritance.<sup>20</sup> It must therefore be determined by the organization of the animal.

The values of  $\log I_o$  and of  $F_{max}$  are specific for each of the three arthropods tested, but  $\sigma'$  is the same for all. Thus (all at  $t^0 = 21.5$ ,  $t_L/t_D = 1$ ) the values of  $F_{max}$  are: *Anax*, 61.0; *Apis*, 53.5; *Asellus*, 51.3; of  $\log I_o$ , in the same order,  $\bar{2}.45$ ;  $\bar{1}.67$ ;  $\bar{1}.30$ . It must be concluded that some fundamental feature of neural organization is common to the three. It need not be supposed that the retinal structure supplies this common element. It may well be that the study of other arthropods will show divergent values of  $\sigma'$ . Otherwise one is left with the problem of accounting for the fact that the relative spread of the frequency distribution of intensity thresholds for the elements of effect concerned in the reaction to flicker is identical in bee, dragon fly nymph, and isopod.

## V

## SUMMARY

The flicker response contour for the isopod *Asellus* is a simple probability integral ( $F - \log I$ ) over the whole determinable range ( $F = 1$  to 51). This contrasts with the "distorted" asymmetrical curves obtained with *Apis*, *Anax*, and other arthropods with large

<sup>20</sup> Crozier, Wolf, and Zerrahn-Wolf, 1937; 1937-38a; 1938c; and further experiments about to be published.

convex eyes. The explanation of the distortion as due to mechanical conditions affecting photoreception is therefore confirmed, as the structure of the *Asellus* eye does not make such a factor likely to be expected for this case.

The *Asellus* curve agrees with the only other available complete and uncomplicated flicker response contour (from *Pseudemys*, turtle with rod-free retina), in showing the superiority of the probability integral formulation as compared with certain others which have been suggested.

It is noted as a curious and probably important fact that the relative dispersion of the intensity thresholds ( $\sigma'_{log I}$ ) for the elements implicated in determining the flicker contour appears to be identical in bee, dragon fly nymph, and isopod. Other relevant information derived from similar experiments with vertebrates shows that this quantity is specifically determined by the organization of the animal. The nature of the common feature of neural organization in three such diverse arthropods, as contrasted with the diversity seen within one class of vertebrates (*e.g.*, teleosts), remains to be discovered.

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