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Phototactic choice between two lights of various intensity in flies

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Abstract. Under the conditions of double choice in a Y-maze, proportion of flies gathering in its arm depended on its relative illumination, approximately so that (1) at, or below the lower critical relative intensity limit, it assumed a constant minimum value; (2) at, or above the species-specific upper critical limit, the proportion of choice assumed a constant maximum value; whereas (3) between these illumination limits it roughly depended linearly on the logarithm of relative intensity of the light. (However, variations of behavioural responsiveness of flies towards white lights with different intensities, underlying their choice in the maze, suggest that the dependence of proportion of choice on relative light intensity has presumably not a zigzag but a sigmoid-shape course). The slope of the graph of linear proportionality of choice to the logarithm of light intensity differed in the housefly and the blowflies, similarly as did the extreme values of the proportion of choice beyond the critical intensity limits. The proportion of choice depended on a type of the maze (being different in a Y- and a T-maze), but not on its size, and it did not depend either on adaptation of flies to light or darkness prior to the experiment, or on the age of flies.

Key words: phototaxis, light preference, housefly, blowflies

INTRODUCTION

Imagines of flies belong to photopositive insects. The photopositiveness is expressed in their preference for the physiologically permissible brightest regions of environment. As we have proved (Chmurzyński 1984), such a preference can be achieved due to photokineses: flies slow down their locomotor movement in more illuminated zones, which is called low (Fraenkel and Gunn 1961) or inverse (Kennedy 1945) orthokinesis; at the same time, flies tend to change randomly the direction of their movement when passing to an area with lower illumination, i.e., they exhibit low or inverse klinokinesis. In case of directional light acting in concert with the gradient of illumination, this mechanism can be supported by the locomotor orthokinetic movement, called by Richard (1960) the basic kinesis, oriented by positive phototropotaxis in a sense of Kühn (1919, cf. Jander 1970). Such a movement, driven by basic kinesis and oriented by taxis, called by Richard (1960) *le comportement taxique*, was termed by the author (Chmurzyński 1966) elasis1. In this case phototropo-elasis is concerned. The latter mechanism allows the photopositive insects to choose between two lights in a T- or a Y-maze (Bertholf 1931, 1932, Cameron 1938). As it has been known for a long time (Fraenkel and Gunn 1961), a photopositive insect exposed to simultaneous influence of two white lights, orients its body phototropotactically according to the rule of parallelogram. Thus, provided that the structure of a maze is appropriately fitted to the relative intensities of both lights illuminating its arms, and assuming deviation of responsiveness among individuals, one can expect that a great majority of insects introduced into the maze should gather at the brighter arm. It is, however, not the case: a proportion (P_{exp}) of insects which collect at a given light (called also in the paper the proportion of choice) only correlates with the logarithm of relative intensity within wide limits of relative illumination (Bertholf 1931). What is more, this is always a statistical process, and not all the insects make the choice[2]. In my pilot tests with flies (Chmurzyński 1969), I obtained an inversed Z-shaped dependence between the proportion of houseflies gathering in the maze arm and the illumination of the latter in relation to the other arm, which poses three following questions:

1. whether such a dependence is general for various flies, especially belonging to a different family, of Calliphoridae,
2. whether the curve discovered in the case of housefly should not rather be of sigmoidal character, and
3. whether the choice depends on the type (shape, dimensions) of maze.

This paper gives the results of experiments designed for the elucidation of these questions, presented in a preliminary form in my habilitatio thesis (Chmurzyński 1973).

METHODS

The experiments were performed, for the sake of comparison, on the housefly, *Musca domestica* L. from Muscidae, and on two species of blowflies, Calliphoridae, viz. *Calliphora erythrocephala* Meig. and *Protophormia terrae-novae* R.D. Insects were taken from a sexmixed[3] laboratory culture, in which they were kept in a chamber with artificial day-night regime, *L:D* = 16:8 h, steady temperature of 25°C, and relative humidity (RH) of 60-70%. The houseflies belonged to a laboratory strain, whereas the blowflies orginated from wild populations. Not starved 4-12-day old houseflies and 3-28-day old blowflies were used for testing. They were taken in a darkened cage (*A*, in Fig. 1*A*) to the darkened laboratory with temperature of 23-24°C and RH of 47-67%, at about 10 a.m. There, the cage was connected with an opaque wooden Y-maze *(B)*,

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[1] Some confusion has been caused by Jander (1970), who used this term for the "distance orientation" during translatory movement, which was followed by some German-speaking ethologists (Tembrock 1978, as Schöne 1980).

[2] Even in this case when one of the arms is dark, not all the insects enter the bright one (Brown and Hall 1936, Chmurzyński 1969).

[3] According to Cameron (1938), the phototactic reactions differ in both sexes.

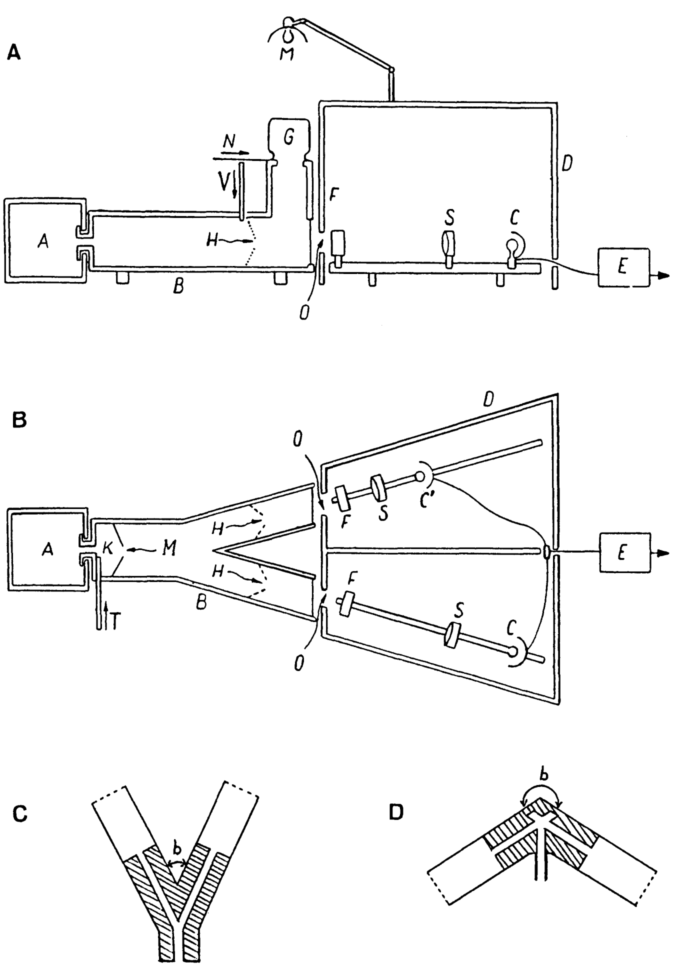

Fig. 1. Apparatus. Equipment with large Y-maze: *A*, lateral view; *B*, top view. Legend: *A*, cage with flies; *B*, Y-shaped maze; *C*, lamp, and *S*, lens emitting an almost parallel light beams, and mounted on an optical bench in a box, painted matt-black on the inside; current stabilized by a constant-voltage regulator *E*; *F*, heat filter. *C*, small Y- maze; *D*, T-maze top view (horizontal section). Further explanations in the text.

its 40 cm long arms diverging at the angle of 30^{0}, their width being 10 cm, and their height equalling 15 cm, similarly as the width of the "porch" *(K)*. The latter was separated from the gallery by two opaque screens divided by a vertical slit *(M)*, 3 cm wide, which enabled dozens of flies a quick entering to the gallery.

After 1 hour of adaptation to darkness, the opaque entrance shutter *(T* in Fig. 1*B)* was pushed aside, thus enabling a portion of some 2-8 dozens of flies to enter the maze, which was illuminated by two lamps *(C, C')* with 50W, 12V frosted bulbs with a colour temperature of 3100 K. The lamps were screened in a box *(D)* with circular windows *(O)*, 5 cm in diameter. The light entered into the arms through the panes closing the distant ends of the maze. Wire-netting funnels with 3 cm openings *(H)* prevented the flies attracted to the arms from returning to the gallery. The illumination, measured in the windows with a Czechoslovak MDLx photometer, was changed to desired intensities by sliding the lamps along optical benches between 7 and 28,800 lux. Except for the cases with one arm completely dark, absolute illuminations of the arms (in lux) are described as e_o (the standard light), and *e* (the variable one); analyses are made with the use of their relative intensities ($I = e/e_o$).

After each 10 min run, the opaque shutter T, and both vertical ones *(V* in Fig. 1*A)* were drawn and the *C* and *C'* lights were switched off with simultaneous switching on of lamps *M* illuminating jars *G* mounted on the upper openings of the chimneys which were situated at the ends of the maze arms. Thus, phototactic passage of flies to the the jars was caused and, then, the jars closed with a piece of cardboard *(N)* were removed and replaced by empty ones. The collected flies were counted under transient CO_2 or N_2O narcosis and placed in a new cage, so that each fly was used only once for a given test. Then the test was resumed until all the flies made the choice between the two arms of the maze. In contradistinction to similar Cameron's (1938) experiments, inactive flies were not eliminated but, instead, all flies were forced to go all the way behind the funnels by gentle blowing of the air through the maze entrance. In the housefly, the entire procedure, from adapting the flies to darkness to the counting of the last flies caught, took up to 5 h; blowflies moved much quicker and three 10-min stages of testing were usually sufficient, and the procedure took 2.5 h on an average. Each pair of lights was tested on different days at least twice, in doubtful cases even 14 times each time with another group of flies, the mutual position of lights being changed. The results were counted in the form of proportions, i.e., the number of insects found in an arm *(n_1 or n_2)* was divided by the number of all the insects found in both arms ($N = n_1 + n_2$), viz.: $p=n_1/N$, $q=n_2/N$ (always $P_{\text{exp}} = n_i/N$).

Standard deviation of proportion (SD=σ) is counted from the formula $\sigma = \sqrt{pq/N}$ (one must not, of course, misinterpret this symbol *N* with the former one, used for showing the cardboard closure of the jars!)

To test the influence of the maze shape on results, two additional experiments were performed in a (swallow-shaped) T-maze of Zabłocka (1972) one with the housefly and another one with the blowfly, *Protophormia terrae-novae*. Apart from different dimensions (which occurred to be unimportant – see below, in "Subsidiary data", point 1a), the angle of divergence between the arms of this maze was 240°. A small Y-maze (Fig. 1*C* and *D*) was also used.

RESULTS

Basic data

Individual tests with different relative intensities of variable lights were designed so as to enable
1. reckoning of minimum and maximum values (P_{min} and P_{max}) of proportions of flies P_{exp} gathering in the arm with variable light (they are usually given with Standard Deviation, ±σ);
2. establishing of dependence of P_{exp} on relative intensity of variable light *(I)* between P_{min} and P_{max} and, especially
3. deciding whether its course goes along sigmoid or zigzag line, i.e., P_{exp} assumes at first a plateau P_{min} from 0 lux up to I_{min}, then shows linear proportionality up to I_{max}, and then again reaches the plateau of P_{max}.

Two groups of experiments were performed. They constituted the 1st series carried out in the large Y-maze with *Musca* and *Calliphora*, respectively. In the case of Musca, some results from pilot tests (Chmurzyński 1966) with standards of 147 and 225 lux[4] were included.

Altogether 59 runs with ca 4250 individuals of *Musca*, and 120 runs with 6500 individuals of *Calliphora* were performed. The results are summarized in Fig. 2.

The following conclusions can be drawn from the graphs:

1. Photoelatic choice of lights by the flies in a maze did not depend on absolute illuminations of its arms (e_0 of the standard light, and e of the variable one) but on their relative intensities ($I=e/e_0$); it did not depend on the sum of both lights (e_0+e) from (e_0+e)=14 lux up, either.

2. a) The choice of the light weaker than a certain critical relative intensity ($I_{\text{crit min}}$), or of the dark arm – amounted to a minimum value of P_{min}. If we neglect the actual values of P_{min} and P_{max} resulting from the grouping of data into series based upon certain levels of standard lights[5], one can see that the proportion of choice of the very bright arm starting from its maximum critical relative intensity ($I_{\text{crit max}}$) up to the highest absolute illumination attainable in our laboratory assumed a symmetrical plateau of P_{max}, where

$$P_{\text{max}} = 1 - P_{\text{min}} \qquad [1]$$

This value (equal to P_{max}) was also obtained in the lightened arm as opposed to the dark one, irrespectively of the absolute intensity of light and starting from e=1 lux.[6]

b) Between these critical *I* values, the proportion (P_{exp}) of both muscid and calliphorid flies depended roughly on the logarithm of its relative

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[4] The first standard was calculated using the photometric equivalent of illumination (Oleszyński 1957) so as to match the illumination used by Cameron (1938) in his tests with the housefly (146.6 lux). The other standard was derived from two requirements: to be a divisor of the highest value attainable in our laboratory (28,800 lux) divided by a whole number which is a power of 2 (similarly as 14,400 or 900 lux in other tests), and so that its two thirds would not be far from 147 lux (so derived the standard of 225 lux, and, consequently, 14 lux).

[5] E.g., 900 lux was assumed as relative intensity I=64 of the standard of 14 lux, whereas the same score could be put transposed under the standard of 900 lux as I=1/64.

[6] Our experimental conditions did not allow to obtain such dim light like in the work of Brown and Hall (1936) (i.e., down to 10^{-3} lux), to test variation of the so-called "rate" of photoelatic choice (*taux de réaction* according to Dufay 1964).

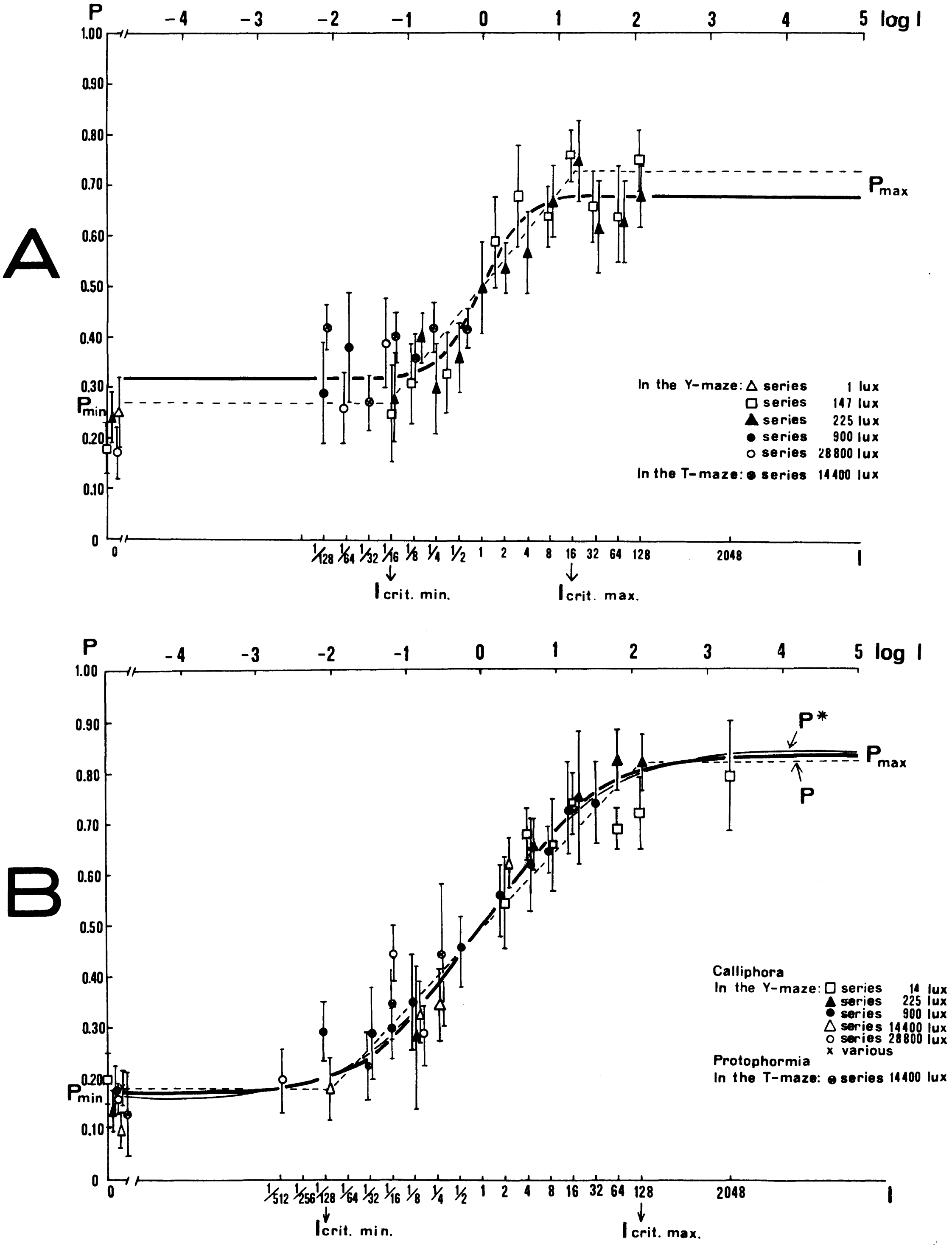

Fig. 2. Semilogarithmic diagrams of proportion of flies which aggregate in a maze at one of two white lights (marked with P at ordinate), depending on its relative intensity ($I{=}e/e_0$, where e and e_0 are absolute illuminations of the maze arms, in lux) – own results. On the average, each point corresponds to 4 tests with 300 different flies of both sexes. Confidence intervals for the level $P{=}0.001$ (i.e., $\pm 3.36\sigma$) are marked. Regression lines (dashed, P_{exp}) correspond to the Y-maze tests only. *A*, housefly, *Musca domestica*; *B*, Calliphorid fly. Boldface solid lines denote P_{theor} according to Bartoszyński's formula [4], whereas thin solid lines show its values (P^*_{theor}) calculated from the data rectified with use of δ^* (see Fig. 4).

light intensity (log$I$), as suggested in my previous paper (Chmurzyński 1969), i.e.,

$$P_{\mathrm{exp}} = k \log I + c \qquad [2]$$

Generally speaking, an approximated dependence of P_{exp} on log I was:

$$P_{\mathrm{exp}} = \begin{cases} P_{\mathrm{max}} & \text{for } I \geq I_{\mathrm{crit\ max}} \\ k \log I + c & \text{for } I_{\mathrm{crit\ max}} \geq I \geq I_{\mathrm{crit\ max}} \\ P_{\mathrm{min}} & \text{for } I \geq I_{\mathrm{crit\ min}} \end{cases} \qquad [3]$$

3. Proportionality of lights choice to their intensities depended on the taxon of the fly in two ways: the levels of minimum and maximum proportion *(*P_{max} and P_{min}*)* were more distant from each other in *Calliphora* than in *Musca*. In the first case, with illumination standards used in our experiments, P_{min}=0.17±0.004 was observed for relative intensities *(I)* equal to 1/128, 1/512, 1/2048, as well as for the dark arm (0 lux) irrespectively of intensity of the standard light. On the other hand, as many as P_{max}=0.77±0.012 of flies gathered at the brighter light with intensity *(I)* equal to 128 and 2048, as well as for 14 lux when opposed to the dark arm (0 lux). In *Musca*, the respective values were: P_{min}= 0.25±0.008 for I equal to 1/16, 1/64, 1/128, and 0 lux (dark arm), and P_{max}=0.70±0.008 for I equal to 16, 32, 64, 128, or for 1 lux opposed to the dark arm (0 lux).

As the standards were chosen arbitrarily, we may standardize our results taking only lower scores, i.e., calculating jointly P_{min} and 1-P_{max} data for $P_{\mathrm{total\ min}}$ value, and putting 1-$P_{\mathrm{total\ min}}$ for $P_{\mathrm{total\ max}}$. So doing, we obtain the following values:

$P_{\mathrm{total\ min}}$ = 0.27 (±0.005),
$P_{\mathrm{total\ max}}$ = 0.73 (±0.005) in *Musca*, and
$P_{\mathrm{total\ min}}$ = 0.18 (±0.005),
$P_{\mathrm{total\ max}}$ = 0.82 (±0.005) in *Calliphora*.

One sees from Fig. 2 that P_{exp} between $I_{\mathrm{crit\ min}}$ and $I_{\mathrm{crit\ max}}$ depends on log$I$ in both species in a different way. Respective equations of straight lines of P_{exp} (log$I$) values between these limits are:

for *Musca*

P_{exp} = 0.500 + 0.193 log$I$, with 0.186 error of the slope [2a]

and P_{exp} = 0.500 + 0.152 log$I$, with 0.055 error of the slope [2b]

for *Calliphora*

4. In each test group, the flies which emerged from pupae within a three days' period were used. Analysis of data from various runs, performed purposely with the use of insects of different age, did not show any significant influence of age on our results.

Subsidiary data

1. Two series of experiments were devoted to testing of the influence of the maze structure on the lights choice in flies.

a) To test the influence of the maze size on P_{max} and P_{min}, I performed an experiment with *Musca*, offering the flies the choice of the dark arm (0 lux) and the one illuminated with 14,400 lux. The tests were performed in a small Y-maze with arms 12 cm long, 1.5 cm wide (inside), diverging, however, at the same angle of 30° as in the standard maze; with its entrance porch being 2.5 cm wide (Fig. *1C*). The result (0.18±0.018 of flies selecting the dark arm *vs* 0.82±0.018, which went to the lightened one; *N*=432), similar to that obtained in the first series of "basic" experiments, in the standard maze with dark arm *vs* 28,800 lux of light intensity in the other arm shows the lack of influence of maze dimensions on P_{max} and P_{min} for the housefly.

b) The 2nd series of experiments was performed in a small T-maze of Zabłocka with almost the same dimensions as those of the previously used small Y-maze, but with the divergence angle between the arms amounting to 240° (which means that the lights could be seen by the entering flies with hind parts of their eyes, Fig. 1*D*). It consisted of two groups of experiments with standard lights of 14,400 lux intensity, carried out with *Musca domestica* and *Protophormia terrae-novae* (the latter belonging to the family of Calliphoridae). Six runs were performed with about 750 individuals of *Musca*, and 12 runs with 800 individuals of *Protophormia*. The results are presented in Fig. 2 (crossed circles). As it can be seen from Fig. 2, the difference in the divergence angle between the maze arms (*b*) exerted an influence on the choice of lights in *Musca*, making it closer to the chance level, whereas the results with *Protophormia* did not show such a dependence.

2. Several experiments were also performed to test the persistence of distribution of flies between lightened and dark arm in the Y-maze.

a) In the series 3, I carried out tests with *Musca* and *Calliphora,* where dark-adapted flies had to choose between the dark arm and the lightened one illuminated with the monochromatic light in the Y-maze. Results were as follows:

0.88±0.02 out of 335 houseflies chose the arm with blue (λ_{max}=478 nm) light (Experiment 14), similarly

0.82±0.03 out of 154 blowflies chose the same light (Experiment 15),

0.77±0.03 out of 204 blowflies chose the arm with yellow-green (λ_{max}=535 nm) light (Experiment 16),

0.81±0.03 out of 224 blowflies chose the arm with red (λ_{max}=695 nm) light (Experiment 17).

The results did not show any difference either between proportions of choice for different wavelengths or in comparison with the proportions for white light.

b) The series 4 of tests with another Calliphorid fly, *Protophormia terrae-novae* (which just was at hand) gave similar results in the T-maze:

0.78±0.03 out of 199 blue-bottle flies chose the arm with violet (λ_{max}=400nm) light (Experiment 18),

0.72±0.04 out of 108 flies chose the arm with orange (λ_{max}=650nm) light (Experiment 19),

0.80±0.05 out of 70 flies chose the arm with red (λ_{max}=675nm) light (Experiment 20).

c) In an additional series 5, I used the Y-maze and tested blowflies *(Calliphora erythrocephala)*, which prior to testing were adapted to coloured lights obtained with absorption filters, corresponding to the colours used in the tests themselves (Table I).

The results of this series did not show any difference either between tests or in comparison with experiments 15-17 performed without colour adap-

TABLE I

Series 5: choice of coloured light *vs* darkness in Y-maze after adaptation to coloured light

Calliphora erythrocephala

Experiment No	Adaptation colour	Test filter: λ_{max} (colour)	Total number	Number of flies gathering in dark arm	in lighted arm	(proportion ±σ)
21	Blue	478 nm (blue)	111	20	91	(0.82±0.04)
		535 nm (yellow-green)	103	21	82	(0.80±0.04)
		695 (red)	95	17	78	((0.82±0.04)
22	Yellow-green	478 nm (blue)	123	27	96	(0.78±0.04)
		535 nm (yellow-green)	113	23	90	(0.80±0.04)
		695 (red)	117	22	95	(0.81±0.04
23	Orange	478 nm (blue)	105	21	84	(0.80±0.04)
		535 nm (yellow-green)	110	21	89	(0.81±0.04
		695 (red)	157	35	122	(0.80±0.01)
		Total number of flies	1034	207	827	(0.80±0.01)

tation. They, in fact, agreed with $P_{\min}$ and $P_{\max}$ values obtained earlier, as well as with results of the next experiment 24.

In the latter, houseflies were tested in a Y-maze, where one arm was lightened with white light of 14,400 lux flickering 10 times per second, whereas the other arm was dark (0 lux). In two runs, the flies chose the intermittently lightened arm 279 times and the dark one 97 times, which gave the proportions of choice (±σ):

0.746±0.035 : 0.254±0.035

similar to that

0.773±0.025 : 0.226±0.025

found in the control with steady lights (318 : 93 in two runs, too).

To test the influence of dark adaptation on the results in the maze, I performed a complementary series 6 of tests, which at the same time were aimed at elucidation the mechanism of distribution of the flies between the arms under the conditions of a double choice in a Y-maze. The tests consisted of runs with the 0 : 14,400 lux choice by the groups of flies collected from both arms of the maze after their previous use in the same experiment (Table II). All

TABLE II

Series 6: choice of white light *vs* darkness in Y-maze depending on adaptation

Experiment No. Remarks	Illumination of lighted arm e_0 (lux)	Total number	Number of flies gathering in dark arm	in lighted arm	(proportion ±σ)
Group I: *Musca domestica*					
25 Non adapted	14,400	407	82	325	(0.80±0.02)
26 Flies which chose the lighted arm in exp. 25	14,400	248	48	200	(0.81±0.02
27 Flies which chose the dark arm in exp. 25	14,400	81	15	66	(0.81±0.04)
Control to 27 Other, dark adapted, flies	14,400	432	78	354	(0.82±0.02)
Total number of flies		1168	223	945	(0.81±0.01)
Group II: *Calliphora erythrocephala*					
28 Non adapted	14,400	230	16	214	(0.93±0.02)
29 Flies which chose the lighted arm in exp. 28	14,400	214	19	195	(0.91±0.01)
Total number of flies		444	35	409	(0.92±0.01)

the tests were carried out on the same day under the same laboratory conditions.

Houseflies and blowflies, not adapted to darkness and taken from the illuminated arm, chose the lights again in the same proportions as those adapted to darkness prior to the experiment, as well as individuals collected from the dark arm. Adaptation prior to tests either to light, or to darkness – did not show any influence on the behaviour of flies. This result was confirmed by subsequent series of tests devoted to the question of behaviour of flies in a maze.

Behaviour of flies in a maze

The series 7 of special tests was performed to reveal the dynamics of choice made by flies in a Y-maze. The experiments were designed to show whether proportions of choice between the lights depend on their relative intensity, time of testing, adaptation to darkness, or indicated a species specificity.

Experiment 30 consisted of five tests with standard light (e_o) of 146.6 lux opposed to variable lights (e) of 9, 18, 49, 225, and 2346 lux (i.e., $I = e/e_o$ equal to 1/16, 1/8, 1/3, 1.5 and 16, respectively). Scores obtained in each of two 15-min stages did not differ significantly from each other at a given light value, which is shown by small standard deviations (σ) of mean proportions of houseflies gathering in each arm at both 15-min stages (viz., 0.25±0.04, 0.36±0.03, 0.33±0.02, 0.63±0.02, 0.76±0.02). This can be visualized by results of Experiment 31, where 0.18±0.04 flies gathered in the dark arm opposed to the light arm with 28,800 lux in the first 15-min stage, and 0.21±0.03 in the second stage. A similar result (0.20±0.01 on average) was obtained in Experiment 32 with three 10-min stages in the same light conditions. Flies behaved similarly also in the three-stage Experiment 33. The mean values of choices at three stages for 146.6 lux of standard light opposed to the dark arm was 0.93±0.02. When the light arm had 18 lux (i.e., I=1/8) the mean proportion was 0.73±0.03; when 1176 lux of illumination (I=8) it was 0.64±0.02.

In three-stage Experiment 34 981 *Calliphora erythrocephala* flies chose equally often (0.16±0.01, on average) the dark arm opposed to the light arm with 28,800 lux intensity.

These results show that the scores of choice made by the flies in each of subsequent stages of any experiment (whether consisting of three 10-min stages, or two 15-min stages) did not differ significantly, irrespectively of relative intensities of used lights. Practically the same proportions of flies gather at respective lights, although the tested flies could be considered dark-adapted only in the first stage of the test. This result did not depend on the species of flies, similarly as did not the general trend that the majority of insects entered the arms at the beginning of testing and the number of flies making choice subsequently declined with time.

Having counted the per cents of flies which made the choice in three subsequent 10-min stages of experiment, pooling all the stages to 100% (in fact, a number of individuals which still remained in the porch of the maze was very small), I obtained the following data:
for *Musca domestica*

I – 49.6
II – 24.1
III – 26.2

and for *Calliphora erythrocephala*

I – 47.2
II – 26.9
III – 25.9

Similar results of pilot tests were already presented in Fig. 18 a of my earlier paper (Chmurzyński 1973).

DISCUSSION

Character and mechanism of the lights choice

The results directly answer the first question posed in the introduction. The character of the lights choice in a maze is basically the same in flies of various species from both families Muscidae and Calliphoridae, although the houseflies ignore changes of relative intensities of lights if difference between them is greater than 16 times only, whereas Calliphoridae discriminate relative intensity up to the product of standard intensity equalling 128; this choice of lights does not depend either on the age of flies, or their adaptation to light or darkness prior to testing; it depends, however, on the angle of divergence between the arms of a maze, making it closer to a chance level in a T-maze.

Two aspects may contribute to the answer to the second question considering the way of dependence between the preference of light and the logarithm of its relative intensity. It is known from my previous research (Chmurzyński 1984) that phototaxis in flies appeared to be polyphasic: there were both transient changes of its sign correlated with illumination, and transient states of phototactic neutrality. There are thus good reasons for the view that flies found in the dark arm of the maze (or the arm with relative illumination intensity below $I_{\min}$) belonged to those which did not make the phototactic choice. There is experimental support that phototactically negative individuals escaped from light, in other words, tried to return the porch. Houseflies counted after 60 min in a dark standard Y-maze showed specific distribution:23.8% out of 252 individuals gathered in the left arm, 29.0% in the right one, and 47.2% in a "trunk" of a maze. In *Calliphora*, relevant per cents of flies found in the arms were 17.2 and 14.0 (N=125); similar results were obtained in the T-maze (Fig. 1*D*): 25.4 and 21.3% for *Musca* (N=449), and 18.2 & 15.4% for *Calliphora* (N=292). These values well matched the level of $P_{\min}$ from Fig. 2.

Together with the experiment where these insects were tested for the second time, we have evidence here that the flies were temporarily differentiated according to their actual discriminating sensitivity to logarithm of relative intensities of lights. According to this interpretation, each insect, whose phototropoelatic incitement reached the threshold level, went to the brighter arm according to the resultant rule (Fraenkel and Gunn 1961). It may be assumed that the proportion of $a=2P_{\min}$ was constituted by individuals which showed undirected kinetic movements, and, consequently, randomly distributed in the maze arms. When the difference of intensity between the opposed lights lessened, more and more "other" flies entered the arms.

These are presumable aspects of the mechanism which are responsible for the phenomenon of linear dependence of choice on log$I$, seen in eqations [2] and [3]. In them, the parameter c=0.5 (see equations [2a] and [2b]) gives an equal probability of choice of both maze arms when equally illuminated (as in the case when $P_{\exp}=k \log I + c = 0.5$, where I=1, from whence log$I$=0, and hence c=0.5). On the other hand, $k \approx 0.19 = \mathrm{tg}\alpha$, where α is a slope of the line $P_{\exp}(I)$, which expresses general sensitivity of flies to the difference of relative intensity of light. Presumably the differentiation of individual sensitivity among flies agrees with normal distribution. Hence, from statistical reasons, it is no longer possible to support the zigzag (dashed) line in Fig. 2. Instead, a model of "sigmoid" line was proposed by my friend mathematician Robert Bartoszyński[7], who ascribed the formula:

$$P_{\text{theor}} = a/2 + (1-a)\phi_\sigma \log I = P_{\min} + (1 - 2P_{\min})\, \phi_\sigma \log I \qquad [4]$$

where $\phi_\sigma \log I$ is a cumulative function of normal distribution with standart deviation σ. Graphs of the function [4] corresponding to the data of our experiments in the Y-maze are shown with the boldface solid lines in Fig. 2.

An indirect support for such a sigmoid shape of the graph of the Bartoszyński's function can be seen in the results of Wehner and Schümperli (1969) who found the same dependence of choice of monochromatic lights by fruitfly on their intensity. An analogous curve was observed later by Daw and Pearlman (1970) in their studies on cats. It illustrated the dependence of double choice of colour

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[7] At that time, from the Institute of Mathematics, Polish Academy of Sciences, in Warsaw, recently: Department of Statistics, the Ohio State University, Columbus OH, USA.

lights on their variable relative intensity. They tested several individuals for the certain number of times and found that both sources of variation acted in concert, similarly as in Cameron's (1938) tests with flies, which basically gave analogous results to ours obtained with many individuals tested once in each test. In the light of these statements, it becomes clear that all the historical results (e.g. those of Bertholf 1931, 1932, Cameron 1938, Chmurzyński 1967, 1969, Dufay 1964) with logarithmic or even asymptotic dependence of P_{exp} on *(I)* have to be treated as invalid, possibly due to scarcity of data or influence of secondary factors not taken into consideration during experimenting.

There is no univocal answer to the third of our initial questions, whether a choice depends on a type (shape, dimensions) of the maze.

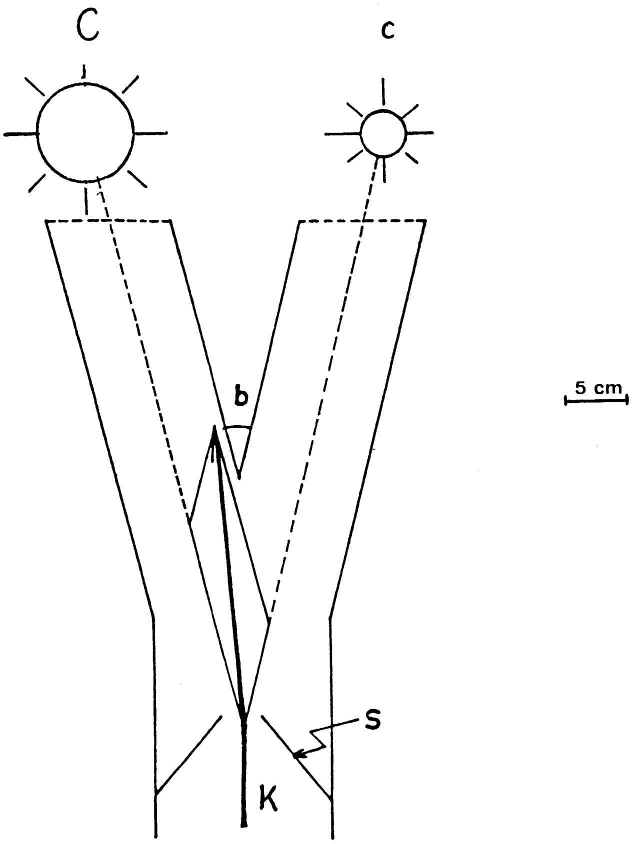

Fig. 3.Theoretical orientation of a positive phototropotactic insect in the large Y-maze under the influence of two light sources (*C* twice as strong as *c*), according to the resultant law. *S*, opaque screens; *K*, start point; *b*, angular divergence of the maze arms (in our experiments equal to 30° (cf. Fig. 1*B*).

First of all, it is *prima facie* not obvious where the *P (I)* dependence arises from.

As it is seen from Fig. 3, all the flies might theoretically gather in that arm of Y-maze with 30° divergence angle which is illuminated at least with light twice as strong as the light in the other arm, provided they would not make the choice at the right side of the 3 cm wide slit *(M* in Fig. 1*B)*, near the edge of right opaque screen *(S* in Fig. 3*)* from where a portion of them might get into right arm, even keeping the same vector of trajectory (thick arrow in Fig. 3). This Figure and Fig. 1*B* show that discrimination between the lights can be executed in various spots of the gallery, starting from the slit *(M)* between the screens walling it from the porch up to a place 15 cm distant from it, which results in differences of angle between the lights seen by various individuals. This illustrates possible sources of fluctuations which may result in deviations from theoretically expected values and cause a continuous character of changes. Nevertheless, the choice does not seem to depend largely on variation of positions of individual decision points in a maze. Results obtained in a small Y-maze with decision point defined with accuracy to 2.5 cm gave the same data as the standard maze. These findings exclude also suspicion based on the results of Ramachandra Rao (1947)[8] that mixed locomotion on wing and on foot might have been a source of variation in response of individuals in the large maze (in the small one, all the flies have to crawl).

As I showed (Chmurzyński 1984), a considerable variation of mean velocity of translatory basic [ortho-] kinesis in phototactic behaviour (photoelasis) of flies in conditions of photic gradient with directional illumination in a long transparent horizontal tube was observed. Proportions of individuals which, on their way from the darkness, crossed an arbitrary limit of 2,300 lux after 10, 20 and 30 min occurred to be decreasing analogically as mean fractions of flies which performed in the maze.

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[8] Difference of the phototaxis sign in normal mosquitoes and in the ones with amputated wings (the latters became photonegative) might have resulted from the role of wings as putative photoreceptors (Alexeyev 1969).

However, there is no correlation between this velocity and the phototactic choice.

There is an influence of the angle of divergence between the maze arms, too. In Calliphorid flies, the pilot test with the lights choice in a T-maze (with *Protophormia terrae-novae*) gave almost the same results as those (with *Calliphora erythrocephala*) in both kinds of the Y-maze, standard and small; this angle of divergence had observable influence on results in *Musca* (see Fig. 2). Proportions of insects choosing lights with different ratio of intensity (1:128, 1:16 and 1:4) in the T-maze were much closer to a chance level than in the Y-maze, which suggests both higher minimum value of proportion of choice ($P_{\min}$; which also means lower $P_{\max}$ value) and smaller inclination of $P_{\exp}(\log I)$ slope. This is a rather surprising result, since the works of Dolley and Wierda 1929, and Lüdtke 1935 showed the highest "turning effectivenes" just in hind ommatidia of the compound eyes of insects (also flies), which allowed to expect better performance of insects in the T-maze than in the Y-maze (in fact, the Zabłocka's T-maze purposely had a swallow-like shape to activate the hind ommatidia).

Lunar variability of flies reactiveness

If we match results of individual runs with the sigmoid regression line representing the Bartoszyński's model, one observes that the data obtained in various days show striking dispersion. For instance, mean proportion of blowflies which chose in 13 runs the arm with illumination of 28,800 lux as opposed to the dark arm (0 lux), amounted to 0.84±0.08. As it is known (Cameron 1938), sex exerts an influence on photic reactions of flies. I excluded it as a source of variation using randomly mixed populations (near to 1:1) of both sexes for experimenting. There was, however, no possibility in our laboratory to control temperature & humidity strictly, which varied between 22-24°C, and 47-67% RH, respectively. I may only hope that they were not strong enough to influence the results (cf. Alexeyev 1969).

Having, however, exact days of the tests, I could determine lunation *(L)* (the so-called "age" of the Moon), falling on each day of experimenting, that means, expressed in days, ^{d}, with their decimal fractions which elapsed to a given day from the last new moon phase [during the synodic month from $0^{d}00$ to $29^{d}53$]. Then, for separate runs, I counted the deviations δ of experimental data in the brighter (or equally illuminated) arm from theoretical P_{theor} values, i.e.,

$$\delta = P_{\exp} - P_{\text{theor}} \qquad [5]$$

and plotted them against the actual age of the moon. As the tests were randomly executed in various seasons during 5 years, it occured that they fell also on various days of synodic month, *L* (Fig. 4).

Preliminary analysis for only two equal intervals of the age of the moon $4^{d}4$ - $19^{d}1$, and $19^{d}2$ - $4^{d}3$ with the Fisher exact probability test showed statistically significant ($P \leq 0.01$) dependence of δ on *L* in *Calliphora*. Sinusoidal approximation of it, $\delta^* = f(\sin L)$,

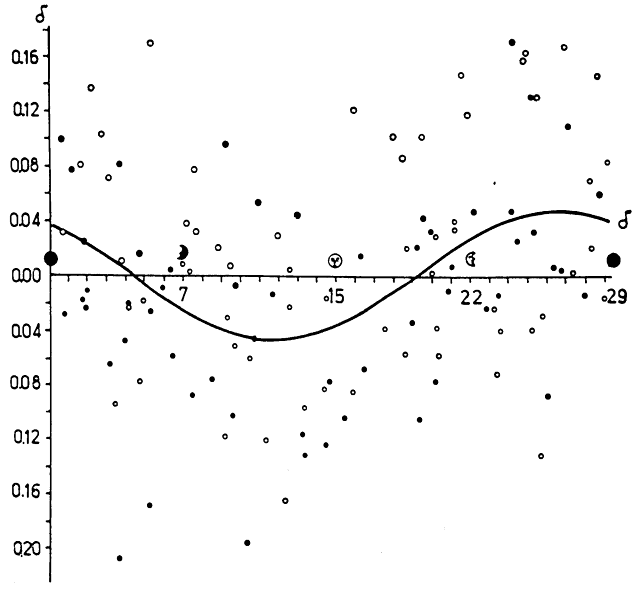

Fig. 4.Graph of the function δ*[6a] approximating the dependence of deviations on the moon age *(L)*, counted from the new moon - in blowfly, *Calliphora* (own results). The deviations in particular group tests with about 300 insects in Y-maze are marked by circles: full - when more flies than expected from the theoretical function [4] (fat solid line graph in Fig. 2*B*) gathered in the left arm of the maze, and open - when in the right arm.

obtained with the least squares method, is shown in Fig. 4. Its formulae are:

$$\delta^*=0.047\sin(12^{\circ}191/\mathrm{d}\ \times \mathrm{L} - 233^{\circ}547) \text{ for } \textit{Calliphora}, \text{ and} \quad [6a]$$

$$\delta^* = 0.016\sin(12^{\circ}191/\mathrm{d}\ \times \mathrm{L} - 99^{\circ}997) \text{ for } \textit{Musca}, \quad [6b]$$

where the dependence of δ on L was not significant [$19^{\circ}191$/d is a pulsation or angular velocity, i.e., growth of an angle per day (d) of synodic month, $360^{\circ}:29^{d}_{\cdot}5306$]. The sinusoidal model was used after Heckert (1961) for the sake of its simplicity, although a cycloid seemed to be better matching the δ data; such was the course of variations of phototaxis in a planarian, *Dugesia dorotocephala* (Brown and Park 1975), as well as in the seed weevil *Calandra granaria*, where minimum of photonegativism falls on culmination of the new moon (Birukow 1963, 1966). It is in concert with the fact that light-traps exert the lowest effect on many insect species at full moon (cf. Dufay 1964, 1965, Miller 1970, Richard 1958), although it was mostly interpreted as an inhibitory effect of moonlight. In our case, however, this could not play a role as the moon was not visible in the laboratory.

It is not possible to interpret unequivocally the mechanism of this rhythmicity of phototaxis. I have already excluded the simplest of them – the direct influence of moonlight on the insects.

A distinct extreme of the moon's effect on $0^{d}_{\cdot}0 \Leftrightarrow 29^{d}_{\cdot}5$ without an opposite one on $14^{d}_{\cdot}8$ would be expected if the hypothesis of screening (Mergentaler 1971), or, opposite, of focusing the corpuscular radiation of the sun (Presman 1968, Sollberger 1965) by the moon were taken into account. Also the conjecture of indirect influence of the moon on the insects through subtle geophysical rhythmic changes (Brown 1959, 1960, 1962, 1965) has to be abandoned, since all of the latter show synchronization with lunar synodic month with the tidal extremes on $0^{d}_{\cdot}0 \Leftrightarrow 29^{d}_{\cdot}5$ and $14^{d}_{\cdot}8$ of lunar synodic month.

If one was to base a search on the day of the maximum of the function [6a] of positive deviation δ^*, which falls in *Calliphora* on $26^{d}_{\cdot}5$ of the age of the moon [i.e., about 3 days before a new moon, $0^{d}_{\cdot}0 \Leftrightarrow 29^{d}_{\cdot}5$ (see Fig. 4)], we can say that this is the day when the moon culminates at the time of the onset of experimenting. Upper culmination of the moon (similarly as of the sun) suggests its gravitational influence. Although existence of responsiveness to gravity was proved in insects (viz. in cockchafer, Schneider 1964, Sollberger 1965), its role in flies would be consistent with our results only in the case that the moon's gravity would merely sensitize them to perform lights choice, i.e., in other words, it would change the percentage of insects which make the choice, thus lowering the P_{min} value and rising P_{max} at maximum of the function [4], whereas at the minimum of the curve, when the moon is in its lower culmination, exerting an opposite influence (as it actually was the case).

An idea of positive attraction of the moon's gravity has, however, to be abandoned. In all my tests the maze was so oriented that the lights were situated almost on the west (exactly: 5° from west towards south). If moon's gravitation exerted a positive attraction on flies, one should expect also changing the course of lights choice. Keeping in mind that celestial bodies (as, for instance, the sun or the moon) culminate on the south, the P_{exp} scores should show systematical deviations (δ) from mean values depending on that, whether a given light (e.g., *C*) was situated at the southern arm of the maze (on the moon's side), or at the northern one. This means, that in such a case full and open circles in Fig. 4 should be diversely distributed near extremes of the function [4], i.e., around $26^{d}_{\cdot}5$ and $11^{d}_{\cdot}9$ of the age of the moon. This, however, is not the case. What is more, gravitational influence might rather suggest tidal rhythms, with extremes on $0^{d}_{\cdot}0 \Leftrightarrow 29^{d}_{\cdot}5$ and $14^{d}_{\cdot}8$ (cf., e.g. Rybka 1957).

Paradoxically enough, caution prompts the simplest explanation that the maximum of the function [4] only accidentally coincides with the situation that on these days the moon culminated during the tests with the flies. Such a phenomenon might occur only when the lunar synodic rhythmicity of phototactic deviations (δ) in flies had a genetic base of an internal clock. This would lead (Aschoff 1981) to two observed facts. One is the phase shift of a freerunning circamonthly rhythm in blowflies kept for a few generations without access to a proper pacemaker (presumably a view of the moon). As already stated, a maximum of the rhythm, assumed on $0\overset{d}{.}0 \Leftrightarrow 29\overset{d}{.}5$, shifted to the observed position on $26\overset{d}{.}5$. The other expected fact would be a desynchronisation of the rhythm in a population of a laboratory strain of insects reared for many generations without access to a pacemaker. This was observed in *Musca* (although lack of clear lunar rhythm in housefly might have also been the effect of the fact that experimenting with them was much more stretched in time than with *Calliphora*).

Making use of the function [6a] as an approximation of lunar rhythmicity of deviations in photoelatic reactions of blowflies under the condition of double choice of lights in the Y-maze, I introduced their theoretical δ^* values as rectifications of actually observed mean P_{exp} values, and then, calculated again theoretical P^*_{theor} values of function [4]. Its graph is added with thin solid line to Fig. 2. Very small difference between both lines suggests that majority of observed deviations presumably had a non systematic character as far as the lights intensities are concerned.

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