The Effect of Object Distance on Pattern Preferences in the Walking Fly, *Drosophila melanogaster*

In insects, visual localization of distances by means of stereopsis has only been proved for the estimation of catching distances in predatory insects such as Cicindelid beetles[1], mantids[2,3], and dragonfly larvae[4-6]. On the other hand, it has never been shown that binocular vision is used to assess long distances in behavioral performances others than catching prey. Although in the mantid, *Stagmatoptera biocellata,* binocular vision is indispensible to detect a reduction in long distances of objects moving towards the insect[7], no mechanism of depth perception could be found for objects presented at fixed distances. Furthermore, studies on a conspicuous frightening display of mantids against large and small species of bird predators[8], and some early observations on the mating behavior of the butterfly *Eumenis semele*[9], have revealed some – but not fully conclusive – evidence for binocular estimation of long distances. This situation, however, will change, since Burkhardt et al.[10] have just presented a theoretical analysis of binocular vision by compound eyes, and since Stavenga has determined the range of binocular vision in flies[11].

Notwithstanding stereopsis, some information about the distances of objects may be also obtained by other cues, e.g. *motion parallax.* If two objects are positioned at different distances and appear under identical angular dimensions as seen from the actual position of the animal, the experimental animal moving towards these objects may take advantage of the different angular velocities of the retinal images in order to discriminate between both objects. In the present communication, we present strong evidence that the pattern preference of running *Drosophila* flies does not only depend on the spatial frequency of vertical stripe patterns[12] but also on the distance of the patterns with regard to the fly's position (Figure).

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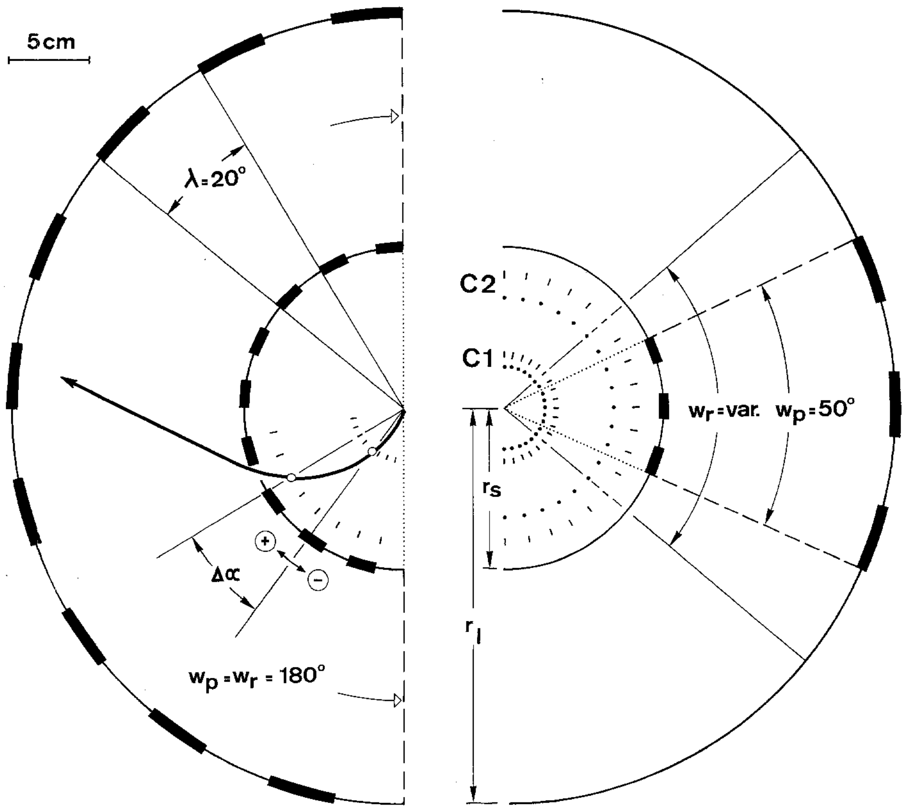

Schematic drawing of experimental set up as seen from above. Only the vertically striped halves of the small drum (r_s = 96 mm) and the large drum (r_l = 245 mm) are shown (left: 180°-wide patterns, right: 50°-wide patterns). A fly's running course is drawn on the lefthand side. C1, C2 circle 1 and circle 2 where the azimuth positions of the flies are recorded (*dashed,* small drum; *hatched,* large drum); $\Delta\alpha$ angular difference between the fly's positions at C1 and C2; λ spatial wavelength of vertically and horizontally striped patterns; r radius of the drum (object distance from the center of the drum); w_p width of the vertically and horizontally striped patterns; w_r width of the sector where the fly's positions are recorded ($50° < w_r < 110°$ in case of $w_p = 50°$; $w_r = 180°$ in case of $w_p = 180°$).

Choice frequencies of vertical stripes by walking flies (*Drosophila melanogaster*), when vertically and horizontally inclined stripes are simultaneously presented to the flies. Data are given for small (r = 96 mm) and large (r = 245 mm) object distances

r	w_p	w_r	C1			C2		
			h	$n(V)/n$	p	n	$n(V)/n$	p
96	50°	50°	326	0.47	$0.3 < p < 0.5$	270	0.56	> 0.05
		60°	349	0.48	$0.3 < p < 0.5$	293	0.56	> 0.05
		70°	372	0.48	$0.3 < p < 0.5$	330	0.53	$0.1 < p < 0.3$
		80°	387	0.47	$0.1 < p < 0.3$	355	0.52	$0.3 < p < 0.5$
		90°	396	0.46	$0.1 < p < 0.3$	383	0.51	$0.7 < p < 0.9$
		100°	407	0.46	$0.1 < p < 0.3$	394	0.50	> 0.9
		110°	424	0.45	< 0.1	415	0.50	> 0.9
	180°	180°	720	0.49	$0.3 < p < 0.5$	720	0.54	> 0.025
245	50°	50°	284	0.60	< 0.001	318	0.62	< 0.001
		60°	355	0.60	< 0.001	346	0.62	< 0.001
		70°	377	0.62	< 0.001	361	0.62	< 0.001
		80°	421	0.61	< 0.001	398	0.60	< 0.001
		90°	429	0.61	< 0.001	403	0.63	< 0.001
		100°	449	0.61	< 0.001	423	0.63	< 0.001
		110°	453	0.61	< 0.001	432	0.63	< 0.001
	180°	180°	378	0.72	< 0.001	378	0.71	< 0.001

The spatial wavelength of all stripe patterns is $\lambda = 20°$. n total number of flies tested. $n(V)$ number of flies preferring vertical stripes. p-values calculated by means of χ^2-tests. For explanation of C1 and C2, r, w_p, and w_r see Figure.

Methods. Wild-type flies, *Drosophila melanogaster*, stock Sevelen (5–6 days old, sex ratio 1:1) were de-winged under 4° anaesthesia 24 h before and dark-adapted 1 h before tested. In subsequent tests each fly was released in the center of the cylindrical drum, varying in diameter and by this in pattern distance. The patterns of vertically and horizontally inclined equidistant stripes presented at the periphery of the drum were either fixed on transparent ground-glass cylinders and illuminated from behind by Osram Halogene-beams, or they were attached to the solid walls of the drum and illuminated by a reflector lamp (Osram Concentra, 100 W, 2600–2700°K) located above the center of the drum. In the first group of experiments, the vertical as well as the horizontal stripes extended for azimuth angles of 180°; in the second group of experiments, the vertical or horizontal patterns extended for a width of 50°, the angle between the center of the patterns being 180°. Due to previous experiments[12], the pattern wavelength of $\lambda = 20°$, which caused maximum attractiveness of the vertical stripes, was used for both pattern distances: $r_1 = 96$ mm (small drum) and $r_2 = 245$ mm (large drum). The azimuth angles of the flies' running courses (1 per fly for each test situation) towards the periphery of the drum were individually recorded to the nearest 5° at 2 distances from the center of the drum (C1, C2). Further details on apparatus and testing procedures are given elsewhere[13].

Results. In both groups of experiments, the vertical stripes are highly significantly preferred in the large drum. The choice frequencies reach n (vertical)$/N = 0.72 \pm 0.03$ for the 180°-wide patterns, and 0.61 ± 0.02 for the 50°-wide patterns (Table). That preference, however, is lost when the small drum is used. In the Table, results are given for the 2 distances C1 and C2 of the walking flies from the releasing point in the center of the drum. This effect of pattern distance on the preference of vertical stripes is independent of pattern wavelength, pattern contrast, and overall light intensity. Furthermore, it is independent of the kind of illumination, i.e. if the drum is illuminated from behind or from above.

If the angular differences ($\Delta\alpha$) of the fly's positions at C1 and C2 are calculated, the same values are obtained for the small and the large drum: $\Delta\alpha = 0.4 \pm 31.3°$ and $-1.5 \pm 32.8°$ for the large and the small drum, respectively (180°-wide patterns). In a white drum where there are no stripes at all, the angular differences between C1 and C2 are much larger ($\Delta\alpha = -2.5 \pm 53.7°$; $p < 0.001$) meaning that more rotatory responses are performed by the flies when no stripes are available for visual course control. If, in the striped drum, the same radius of curvature were due to a fly's walking course irrespective of whether the diameter of the drum is small (s) or large (l), $\Delta\alpha$ should be larger in the latter case because of the difference in the radial distances (d) between C1 and C2 in both types of drums ($d_l = 55$ mm, $d_s = 35$ mm). As, however, the $\Delta\alpha$-values coincide in both sets of experiments, one may conclude that the flies' walking courses are more straight in the large than in the small drum[14].

Discussion. In very few cases, insects have been experimentally deprived of binocular vision. The choice frequencies of these monocular insects confronted with visual objects of different sizes and at different distances, only depend on the visual angles subtended by the objects[15]. In the desert locust *Schistocerca gregaria*, however, a visual scanning method[16,17] has been described by which the locust nymphs are able to use motion parallax as a cue for the estimation of distance. In general, if an

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[13] E. HORN and R. WEHNER, in press.

[14] Just recently K. G. GOETZ (in press) has mentioned that freely walking flies show the more rotatory responses the nearer they are to a stationary visual environment.

[15] The measured speed is nearly 5 times the average walking speed recorded for a single *Drosophila* fly by K. G. GOETZ, J. comp. Physiol. *85*, 235 (1973), p. 241.

[16] When locust nymphs while sitting are in the process of orientating to objects in the visual field they perform a lateral swaying movement of the anterior part of the body. During that movement described as *peering* head and body are not titled.

[17] G. K. WALLACE, J. exp. Biol. *36*, 512 (1959).

insect prefers objects of a given angular size, motion parallax may be one mechanism to determine distance and absolute size of the object.

Although, in our experiments on the walking fly, a highly significant effect of pattern distance could be proved, that effect is not necessarily to be explained by binocular or monocular mechanisms of distance measuring. According to previous results, the spontaneous preference of *Drosophila* flies towards vertically inclined stripes is due to the fact that vertical stripes facilitate any mechanism of locomotor course control[12]. That explanation coincides with the experimental data that the preference for vertical stripes fades when the wavelength approaches the resolution threshold at $\lambda = 2\,\Delta\varphi$ ($\Delta\varphi$ = divergence angle of neighbouring visual units), and that it is even reversed in the range of $\Delta\varphi < \lambda < 2\,\Delta\varphi$ (preference for horizontal stripes). The recent results support the hypothesis that this mechanism of visual course control also depends on the distance of the vertical stripe pattern with regard to the fly's position (provided that λ is identical in all cases): the smaller that distance becomes, the more the capacity of maintaining a straight course is lost. One difference caused by near and far stripes which coincide in angular dimensions consists in the different angular velocities of the vertical images, when the fly approaches the stripes (motion parallax). The velocity of image motion increases with decreasing distance between walking fly and stripe pattern. According to some cinematographic measurements, the de-winged flies are walking by a speed of about 25 $\mathrm{mm \cdot sec^{-1}}$ [15]. By this one can calculate the angular velocity of a contrast line moving laterally over the fly's eyes when the fly is approaching the center of a vertical stripe. When the fly is crossing the circle C1, these angular velocities are $0.5^\circ\ \mathrm{sec}^{-1}$ and $2.3^\circ\ \mathrm{sec}^{-1}$ in the large and small drum, respectively. The mechanism of locomotor course control, and thus the preference for vertical stripes, may well depend on those differences in the velocity of image motion and may have nothing at all to do with distance measuring[18].

Zusammenfassung. Fliegen bevorzugen sehwinkelkonstante Vertikal- gegenüber Horizontalstreifen bei grossen, nicht dagegen bei kleinen Musterentfernungen. Diese entfernungsabhängige Vertikalstreifenpräferenz wird als Folge eines Mechanismus zur optischen Kurskontrolle verstanden, der von Art und Geschwindigkeit der retinalen Bildverschiebung abhängt.

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Lysis of Zoospores of *Phytophthora palmivora* induced by Concanavalin A

Major changes occur on the surface of zoospores of *Phytophthora palmivora* undergoing encystment. Within 2 min, the wall-less zoospore secretes an amorphous cyst coat and elaborates de novo a microfibrillar cell wall made of β-glucans[1,2]. The unencysted zoospore binds concanavalin A (Con A) on its plasmalemma. This surface binding capacity increases greatly during encystment as new Con A receptor sites appear on the cell surface[3]. These new sites correspond to the amorphous coat material secreted by the so-called peripheral vesicles[4]. We believe that this Con A binding material is involved in cell adhesion and cyst wall formation.

In the course of studying the binding of Con A to zoospores, we noted a lethal action. Motile zoospores incubated with Con A at concentrations as low as, 25–50 µg/ml ceased swimming, agglutinated weakly and began lysing within a few minutes. By 30 min, essentially all zoospores had burst (Table I). The lytic action of Con A could be completely nullified by methyl-α-D-mannoside (0.166 *M*) if the mannoside was added within 3 min after exposing the motile zoospores to Con A (200 µg/ml). If the addition was made after 5 min, about half of the zoospores lysed while the rest encysted normally. After a 10 min exposure to Con A, the entire population lysed.

Table I. Effect of concanavalin A on zoospores of *P. palmivora*

Concanavalin A (µg/ml)	Concanavalin A	Concanavalin A plus methyl-α-D-mannoside
200	++++	–
100	++++	–
50	++++	–
25	+	–
12.5	–	–

(++++) = total lysis, (–) = no lysis. The zoospore suspension was incubated at room temperature and scored for lysis after 45–60 min under a light microscope. The incubation mixture contained: 0.7 ml motile zoospore suspension (2×10^6 cells/ml), 0.2 ml Con A at the appropriate concn in 0.05 *M* NaCl and either 0.1 ml of 4 m*M* sodium phosphate buffer, pH 7.2, in 0.05 *M* NaCl, or 0.1 ml of 0.8 *M* methyl-α-D-mannoside in the same buffer/salt.

Annulment of the lytic effect of Con A by methyl-α-D-mannoside indicated that Con A was operating as a lectin[5]. It could be argued that methyl-α-D-mannoside prevented lysis by affording osmotic protection to the cells. However, this was ruled out because mannitol (0.166 *M*) added in lieu of methyl-α-D-mannoside did not prevent Con A-induced lysis. At 0.32 *M* mannitol, the zoospores also lysed by the action of Con A, but many of the cells did not burst; they became irregular in shape, showed a granulated cytoplasmic appearance, and failed to germinate. In the presence of 0.32 *M* mannitol alone, the cells germinated normally.

In sharp contrast to zoospores, cysts (prepared by vigorous agitation of a zoospore suspension[1]) were not susceptible to Con A. They germinated normally in the

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