

Brändle, E. and E. Hadorn. Zoologisches Institut der Universität, Zürich, Switzerland. Cell numbers in adult corpora allata of *Drosophila melanogaster*.

adults. In each of the 12 sex-age groups, 4-12 counts were made. No significant differences between sexes or age-groups were found. The numbers varied between 12 and 22. Mean:  $15.31 \pm 0.189$ .

*Corpora allata* were fixed in alcohol-acetic acid and stained in toto with gentian-violet. Numbers of cells were determined for glands of inseminated females, virgin females and males at 1, 2, 4 and 8 days after hatching of the

Ditman, W. F. Purdue University. Observation of *D. melanogaster* behavior.

The following observations were made while developing an apparatus and an experimental procedure for testing depth perception preference in *D. melanogaster*. General testing

procedure: Flies were released into the apparatus, 50 at a time from a clean, half-pint culture bottle. They were given 30 seconds to respond to the visual stimuli. Then the trap doors of the collecting chambers were closed and the flies were removed with an aspirator and counted. For details of the apparatus and procedure see: An Improved Method for Determining Visual Depth Preference in Large Numbers of *D. melanogaster*, this newsletter.

#### Experiment One: Effects of Ether on Flying Behavior

Flies used were wild stock (CS-200), kindly supplied by Dr. A. B. Burdick. The apparatus was specifically designed to admit light only through the ceiling. Yet, despite their positive phototropism and negative geotropism, less than 50 percent of the flies could be induced to ascend the inside (27") of the box to the ceiling. During preliminary work on depth preferences, the flies were etherized for counting out lots of 50.

A review of the preliminary data collected suggested that etherizing affected the flying behavior of *D. melanogaster*. In a test of this hypothesis, 400 flies were divided into groups of 50. Counting for this and all subsequent experiments was done by letting flies crawl singly through a narrow tube into half-pint bottles. The eight bottles were then arranged into an ABBA ABBA order in which all "B"s were etherized very lightly. The flies were tested in the same counterbalanced order six hours later.

Table 1

<u>Group</u>	<u>No. Released</u>	<u>No. Flew</u>	<u>% Flew</u>	<u>X<sup>2</sup> Obs.</u>
Etherized	197+	86	45.6	10.62**
Un-Etherized	206+	158	76.7	

+No. deviates from 200 because of miscount in dividing flies into groups of 50 each.

\*\*significant at the .01 level of probability.

The Chi Square value of 10.62 is significant at the .01 level of probability, indicating a difference in the behavior of etherized and un-etherized flies.

Conclusion: Flies that have been etherized even lightly and then given adequate time to recover show a significantly lower propensity for flying when given the opportunity than un-etherized flies. This phenomenon may be readily observed by placing an un-etherized group of flies into a clean dry culture bottle and an etherized group into a second bottle. After the flies have had time to recover the un-etherized flies will be observed crawling about their bottle with greater vigor than those in the other group. If the lids are removed more of the un-etherized flies will escape.

Curiously, it was noted that at certain times the drawing of the hand down along the side of the culture bottle stimulated the flies to rush up the inside of their bottle. When the hand was drawn up the sides of the bottle, the flies would circle the inside of their bottle. This phenomenon is most pronounced when flies are used which previously had been lightly etherized. Flies that had been heavily etherized were sluggish and did not respond. Un-etherized flies, on the other hand, were so responsive that almost any stimulus caused

them to rush up the side of their bottle. This may be a photokinetic effect but the circling behavior when the hand moves up seems unusual.

#### Experiment Two: Effects of Flight Distance on Flying Behavior and Depth Preference

Flies used in this experiment were wild *D. melanogaster* collected at a local fruit stand. Two main problems developed during the course of this experiment: (1) how to get more flies to fly and thus exhibit a preference, and (2) how to reduce the incidence of random choices and thereby achieve a better index of preference for one or the other of the two visual ceilings if a preference does exist. It is assumed that if there is no preference equal numbers will fly to the two stimulus ceilings. If the flies do have a preference the proportion of flies on the preferred slide should reflect the strength of that preference.

Since the distance of flight was hypothesized to be an important variable, a short flight distance (3"), and a long flight distance (49") were compared to the standard (27") by altering the height of the flight shaft accordingly. All other factors in this comparison were held constant.

Table 2

<u>Flight Distance</u>	<u>No. Released</u>	<u>No. Flew</u>	<u>% Flew</u>	<u>No. High</u>	<u>% High</u>	<u>X<sup>2</sup> Obs. (P = .5)</u>
3"	500	418	80.4	206	49.5	.09
27"	339+	284	83.7	175	61.6	15.33**
49"	500	322	64.4	182	56.5	5.48*

+should have been 350, difference due to miscount in dividing flies into groups of 50 each.

\*significant at the .05 level of probability.

\*\*significant at the .01 level of probability.

In Table 2 all observed response proportions were tested against the null hypothesis  $P = .5$  to each of the two stimuli.

Under the 3" flight distance conditions the flies apparently distributed themselves in a random fashion. On the other hand, over a flight distance of 27" flies clearly appeared to prefer the high side. Less preference for the high side was observed when the flight distance was 49". The results for the short (3") flight distance seem relevant to the work of Lewontin (1959) who studied the negative phototropic response of *D. pseudoobscura*. He found that this tropism only occurred in his apparatus, a bell jar, when the flies walked rather than flew. When they flew their choices appeared random. Fifty percent went to the dark side in the two choice situation. He hypothesized that this difference in behavior was a function of activity level. Another hypothesis would be that the distance from the neck of the bell jar to the choice point (located in the plane formed by the joining of the sides of the bottle and its shoulders) was too short. The flying *D. pseudoobscura* did not have time to perceive and act upon the differential stimuli. The results reported above for depth perception in *D. melanogaster* when a similar apparatus was used makes the latter hypothesis attractive.

#### Experiment Three: Effects of Illumination and of Perceived Depth

Flies used in this experiment were wild *D. melanogaster* collected at a local fruit stand.

Data in Table 2 indicate that approximately 60% of the flies that had not been etherized prefer the high ceiling when given a 27" flight distance. Preliminary studies had established that difference of illumination between the two ceilings had no effect upon response proportions except when these differences were very extreme. To rule out the possibility that the flies were responding to stimuli other than depth, flies were tested under two conditions: (1) no light, and (2) no depth difference between ceilings.

In the first case lights in the apparatus were turned out and all testing was done at night in a dark room.

In the second case the high ceiling was brought to the same level as the low ceiling and illumination of the two ceilings was made equal. The results are presented in Table 3.

Table 3

Condition	No.	No.	%	No.	%	$\chi^2$ obs.	
	Released	Flew	Flew	High	High	P = .5	P = .6
No light	400	126	30.2	68	54.0	.79	1.91
No depth	400	227	59.2	115	50.7	.04	8.25**

\*\*significantly different from chance at the .01 level of probability

The results of testing without any light and thus no visual stimuli are equivocal because only 30.2% flew. Of the 126 that flew without phototropic stimulation 54% went to the high side. A Chi Square test shows that this is not significantly different from chance (P = .5) nor significantly different from the expected response (P = .6).

The results of testing with no depth stimuli are obvious. Of the 227 that flew, 50.7% went to the former high ceiling. 50.7% is not significantly different from 50% but is significantly different from 60% at the .01 level using a Chi Square test. These results indicate that the depth related stimuli are the only ones operative in the previously observed 60% selection of the high ceiling.

#### Experiment Four: Strain Differences in the Depth Perception Preference of *D. melanogaster*

Behavior Genetics is a new field. As yet, no classical problem area of Psychology has been submitted to classical genetic investigation. To do so is the ultimate goal of this research. Having established the existence of depth perception preferences in Experiments Two and Three an attempt was made to discover strain differences. Data have already been obtained on wild fruit flies collected at a local fruit stand (see Table 2). Additional inbred strains: CS-200, negative geotropic and positive geotropic strains were tested. The latter two were developed by Erlenmeyer-Kimling, Hirsch and Weiss (1962). They were obtained for this study through the kindness of Dr. J. Hirsch. These flies had been selected for a specific geotropic response for 65 generations, after which selection was relaxed. They are relatively heterogeneous and were used here 149 and 150 generations from the beginning of selection. The comparison of the four strains is shown in Table 4. All of the flies were tested under the same conditions: the stimulus fields were of equal illumination; there was a 14" depth difference between High and Low ceiling; no ether was used; the flight distance was 27" and, as always, they were released in groups of 50.

Table 4

GROUP	No.	No.	%	No.	%	$\chi^2$ obs.	
	Released	Flew	Flew	High	High	P = .5	P = .6
CS-200	500	307	61.4	183	59.6	11.34**	.02
Neg. geotropic	589+	435	73.9	239	54.9	4.25*	4.64*
Pos. geotropic	697+	540	77.4	330	61.1	26.67**	.27
Wild	339+	284	83.7	175	61.6	15.34**	.37

+No. deviates from multiple of 50 because of initial miscount

\*significant at .05 level

\*\*significant at .01 level

A number of interesting facts emerge from the comparison of the above data. The highly inbred CS-200 flies flew least frequently while the wild flies flew most frequently. The negative and positive geotropic strains were intermediate.

Other data not reported here have indicated that wild *D. melanogaster* would demonstrate a 60% preference for the high ceiling. Chance performance would dictate a 50% preference for the high ceiling. Therefore, Chi Square tests were performed to test two hypothesis: (1) that observed depth preference was random (P = .5), and (2) that the observed depth preference was not random but rather equal to a 60% preference for the high ceiling. As can be seen in Table 4, the percent of flies in each of the four groups that preferred the high side is significantly different from 50% at the .05 level. All except the negative geotropic strain were not significantly different from 60%. In fact, the 59.6%, 61.1% and 61.6%

observed are quite similar and extremely close to 60%. The 54.9% of the negative geotropic strain is unique. Apparently this group had a weaker preference for the high ceiling than did the other groups, yet this strain has been selectively bred for negative geotropism. Since they do not exhibit the strongest preference for the high ceiling, negative geotropism and depth perception preferences are probably not correlated.

Summary: An apparatus has been successfully tested which will allow *D. melanogaster* to exhibit a preference based on depth perception. The preference exhibited was for the higher of two visual ceilings in a box. Strain differences relative to the strength of the preference were observed. A strain of *D. melanogaster* selected for negative geotropism showed a weaker preference for the high ceiling than did any other strain tested. This latter observation suggests that depth perception preference for a high ceiling is independent of negative geotropism.

References: Erlenmeyer-Kimling, Loise, Hirsch, J. & Weiss, Jane M. Studies in behavior genetics: III. Selection and hybridization analyses of individual differences in sign of geotaxis. *J. comp. physiol. Psychol.*, 1962, 55, 722 - 731.

Lewontin, R. C. On the anomalous response of *Drosophila pseudoobscura* to light. *Amer. Naturalist*, 1959, 93, 321 - 328.

Jost, P. University of Oregon, Eugene.  
Segregation in males with a normal or a  $sc^4-sc^8$  X chromosome and an attached-4.

The segregation behavior of an X chromosome deficient for a considerable portion of the basal heterochromatin ( $sc^4-sc^8$ ) shows a high frequency of primary non-disjunction (Gershenson, *J. Genet.* 28:297; Sandler and

Braver, *Genetics* 51:573; Peacock, *Genetics* 51:573). Peacock confirmed cytologically that a high frequency of univalents occur in meiosis. From Gershenson's conclusion that in  $sc^4-sc^8$  YY males pairing was predominantly of the YY type, with the X unpaired, it can be reasoned that in a  $sc^4-sc^8$  Y male the presence of a third unpaired chromosome that has exhibited a tendency to segregate non-randomly from the Y (in the female) might exhibit a non-random segregation. E. H. Grell (unpublished, cited by R. F. Grell, *Genetics* 50:151) and Jost (unpublished) have confirmed that an attached-4 chromosome does tend to show a non-random segregation in XX Y 44 females. The present experiment was designed to evaluate the behavior of an attached-4 (44) in  $sc^4-sc^8$  Y 44 males.

Homozygous  $y w; AF, c_i ey^R/gv l sv^n$  (no free 4) females were crossed with two types of R males, both carrying the  $sc^8 \cdot Y$ , which is marked with  $y$ , and an attached-4,  $AF, c_i ey^R/c_i ey^R$  (no free 4). The X chromosomes used were a normal X, marked with  $y w^a$ , and  $y sc^4 f v cv sc^8$ . All disjunctional and non-disjunctional classes of both parents were phenotypically distinguishable and diplo-4. An assumption of random segregation of the attached-4 with respect to the sex chromosomes was used to calculate the expected values shown below:

	$y w^a/sc^8 \cdot Y$		$sc^4-sc^8/sc^8 \cdot Y$	
	Observed	Expected	Observed	Expected
Y, 44 non-disjunction	417	424	701	741
X, 44 non-disjunction	445	457	961	921

The frequency of recovered gametic types is consistent with those reported by other authors, with the exception that X and Y were recovered with approximately equal frequency. This is in contrast to the data of both Sandler and Braver and of Peacock, whose results showed that the X was recovered on the order of twice as frequently as the Y. The data, without considering the attached-4, are shown below:

Paternal genotype	Sperm types				Total F <sub>1</sub>	Proportion of X-bearing gametes		
	X	Y	XY	0		Of Total	In dis-junctional classes	In nondis-junctional classes
$y w^a/sc^8 \cdot Y$	2828 (0.554)	2276 (0.446)	1	3	5108	0.554	0.554	---
$y sc^4 f v cv / sc^8/sc^8 \cdot Y$	1360 (0.395)	1258 (0.365)	188 (0.055)	637 (0.185)	3442	0.450	0.520	0.228