MAZE BEHAVIOUR IN DROSOPHILA

SOME FACTORS AFFECTING MAZE BEHAVIOUR

OF

DROSOPHILA MELANOGASTER

by

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ABSTRACT

Previous experiments done by other workers indicated that associative learning in a complex maze could be demonstrated to exist for flies of some strains of Drosophila melanogaster. The experiments of this study gave similar results for the Canton S strain, however results indicative of learning, whether they are actually due to discrimination learning or pseudolearning, are only obtained for flies run through a maze with olfactory cues present in the path. The behaviour observed is shown to vary between the strains (Canton S and black) used.

PREFACE

This thesis describes studies done in the Department of Biology, McMaster University, from June 1976 to March 1977. The results from experiments described herein consist entirely of my own work. No similar thesis has, to my knowledge, been attempted or submitted at any other university.

I would like to sincerely thank Dr. S. F. H. Threlkeld, under whose benevolent supervision I conducted my research, for his advice, patience, encouragement and interest throughout the period during which this study developed and was finally conducted. I would also like to acknowledge the efforts of Mr. R. Gillies, who constructed the apparatus used, Ms. K. Hendriksen who rendered technical assistance in the lab, and Dr. R. A. Morton, who wrote the computer program used in the data analysis.

I am also grateful to the McMaster University Biology Department for financial support during my period of study.

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INTRODUCTION

The study of the biological bases of animal behaviour is one of the more interesting and active areas of inquiry found in science today. The physiology of behaviour has been studied since the beginning of the century, when Sherrington (1906) intruduced the science of neurophysiology, while the study of behaviour as an ecological factor has been undertaken by many zoologists and ethologists since the early works of Tinbergen (1952) and Lorenz (1958) and less systematic observations of naturalists since time immemorial. Behavioural psychology, thanks to such notable investigator as Pavlov (1927) and Skinner (1971) gave useful insights into the nature of behaviour, especially that type of behaviour commonly known as learning.

Much of the work with learning, however, has involved vertebrates or the higher animals in general, which are possessed of much more complicated nervous systems than simpler creatures, such as the insects. If behaviour could be studied in the neurally simpler insects in an analogous manner to the work done with higher animals, it may be possible to discover much about the innate bases of behaviour; and many studies of this type have been very successful in this respect, eg. Roeder (1963).

The genetics of behaviour has already been extensively studied in the fruit fly, Drosophila melanogaster, an organism whose genetic constitution has been well worked out over years of investigation. The work of Benzer et al (1973) with mozaic mapping and Suzuki (1974) with various mutants, have shown the existance of behavioural mutants in this species, and in many cases the genetics and physiological bases of the aberration have been well documented.

Since the genetic and physiological bases of simple behaviour have been studied so successfully in Drosophila, perhaps learning behaviour in this organism may also be investigated in a like manner with similar success.

The first step in any such investigation is to determine if learning is indeed part of the behavioural repertoire of Drosophila, and if it is, what types of learning behaviour is this organism capable of?

Ideally, the search for learning behaviour would be best accomplished by observing the animal in its natural state, to see if individuals exhibit behavioural adaptations to their environment over time, as is observed in the higher animals; however, the in situ observation of Drosophila in this manner would obviously be very difficult, due to the small size, high mobility and indistinguishability of individuals of this species.

Since the ethological approach to behaviour study is not too well suited to Drosophila research, investigators have attempted to adapt experimental systems from behaviour psychology for use in the study of learning behaviour in these and other insects; some of these attempts are described below.

One of the earliest studies of learning behaviour and its genetics in Drosophila was done by R. M. Murphey (1967), who claimed to have demonstrated instrumental conditioning, or learning, in Drosophila melanogaster. His experiments involved a T-maze, in which flies were trained to turn in a specific direction by rewarding them for a correct choice (reinforcement) or punishing them for an incorrect choice (instrumental conditioning). Replication of these experiments by Yeatman and Hirsch (1971), however, showed no evidence of learning whatsoever; and further attempts by these investigators to selectively breed for strains differing in learning ability (similar to earlier selective breeding experiments for other behaviours such as geo- and photo-taxis) gave no significant results over 10 generations for the type of learning behaviour sought.

Conditioned behaviour in Drosophila melanogaster was later investigated by Quinn, Harris and Benzer (1974), who managed to train flies to avoid a particular odor or colour of light by exposing them to the stimulus simultaneously with an electric shock, so that in subsequent tests, the flies avoided the odour or colour which they had associated with the shock. Similar results were obtained by Spatz, Emmons and Reichert (1974), who observed associative learning in D. melanogaster in a system which had the flies move along a training alley, in which they passed through two zones, each lit with a different colour of light; in one of the zones the flies were given an electrical shock as they passed through, and it was observed that flies which had been shocked in a particular zone of colour tended to turn in a direction away from that colour when they encountered a free choice T-junction at the end of the maze, which had each arm lit by one of the colours of the two training zones. Presumably the flies act in this way because they associate the shock with a particular colour, and they subsequently avoid that colour.

Both of the above conditioning studies involved aversive training which tests for the ability to learn to avoid an unpleasant stimulus (shock) and/or a normally innocuous stimulus associated with it; this is a type of learning commonly observed in nature in other animals and it is known to be adaptive, for example birds learn to avoid poisonous butterflies by associating the appearance of the butterfly with the unpleasant after-effects of a previous ingestion of one.

Another type of conditioning involves rewarding the subject for a desired response (the "correct" response),. thus forming an association in the subject between the correct response and the reward, with the reward acting as a reinforcement for the desired behaviour. Since Drosophila is a small insect, with perceptual mechanisms different from those of higher animals, it is difficult to know just how an aversive stimulus, such as a shock, really does affect the overall behaviour of the organism. Possibly, until more is known, the best systems for studying learning behaviour in these animals would be the least disruptive systems - those with little potential for radically altering the physiological/behavioural responses of the subject due to after-effects of intense stimuli.

Experiments have been done using simple association systems, such as those of Threlkeld et al. (1976), involving a T-maze, through which starved D. melanogaster were run for three trials, each time with a food source located at the end of the same arm of the free-choice turn. It was found that when these "experienced" flies were run in a clean food-free maze, they reached the previous food-source area faster than they did in the first trial, and faster than flies which were not experienced. This could indicate associative learning, with the flies associating one direction of turning with a reward (food). Strain differences in this behaviour were observed, indicating a genetic basis for the quantity studied.

An experiment involving a different behaviour pattern was done by D. A. Hay (1975), and it is his system which my own experiments are patterned after. Hay's experiments involved running flies in groups of 200 (100 male, 100 female) through a multi-level maze which consisted of two separate maze paths, one left and one right of the starting point, leading away horizontally to a light source at the end of the maze. Flies entering the maze were free to turn in either direction at the start, but once they had entered a specific maze path, they were forced to turn in the same direction at six consecutive turn points as they advanced through the maze.

Once the flies had passed through the forced turns (over a span of 24 hours) they were once again confronted by a free-choice turn, which allowed them to go in either direction and on, to a collecting system.

The flies were observed to have a higher probability of turning in the direction of the forced turns at the final choice point, after traversing the forced turns, than they had at the first choice point at the start, and the degree to which the probability of turn direction changed was observed to vary significantly between strains.

Hay has interpreted these results to be evidence of associative maze-learning in D. melanogaster, with flies being more likely to continue a sequence of turns which they were forced to adopt for the preceding six forced turns, since they learn that this sequence of turns enables them to proceed through the maze towards the light, which acts as a phototaxic attractant and possibly an orientation cue for their turning. The uses of such behaviour by Drosophila in the wild are not apparent, but it would appear to be involved in some type of motion orientation system.

The experiments to be described herein used a system similar to that of Hay's. A multi-level maze was used and the type of learning behaviour looked for was similar to that which Hay has claimed to observe.

METHODS

Apparatus - experiments were done using two identically constructed plexiglass mazes. Each maze consisted of a 15.8 cm X 34.3 cm X 1.2 cm thick base plate with a 0.3 cm thick face plate being attached to this base plate with four screws, thus the face plate is easily removable to facilitate cleaning of it and the maze path, which was milled into the base plate. (see figure 1)

With the face plate in place, the actual maze path has a 0.4 cm square cross section area, and the path itself is laid out in the configuration shown in figure 1. Due to the milling process used to make the maze, the recessed part of the path (that portion within the base plate) has rough-textured surfaces as opposed to the surface of the face plate, which is smooth and transparent when clean.

The exits of the maze are each fitted with an adaptor section, a short length of plexiglass rod which has been machined to a tubular form. The adaptors were attached permanently over the exit holes with chloroform (a plexiglass solvent) and it is over these adaptors that the collection vial collars fit. (see figure 2) The collection vial collars were also machined from plexiglass tubing, and were made so that their inside diameter was of sufficient size to allow them to fit snugly over the exit adaptors. The collars were glued over a hole drilled in the bottom of the plastic collection vials with styrene cement, giving a complete collection system unit as shown in figure 2, with the flies leaving the exit, passing through the adaptor and into the collection vial, which is held in place with the collar.

The collection vials were each fitted with a sponge plug to keep the flies in and allow them air. Each maze was fitted with a suspension

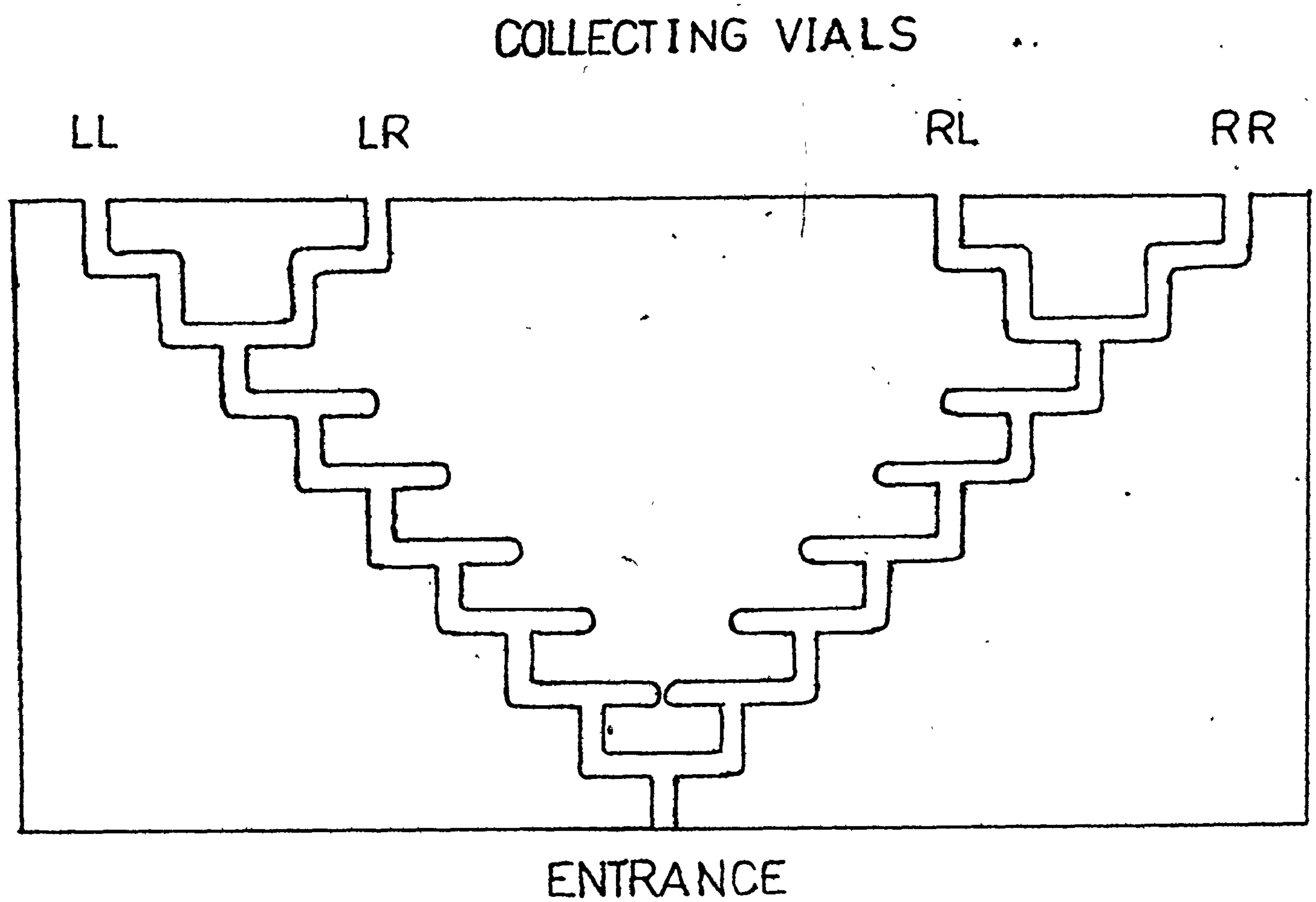


FIG. 1 - Maze Path

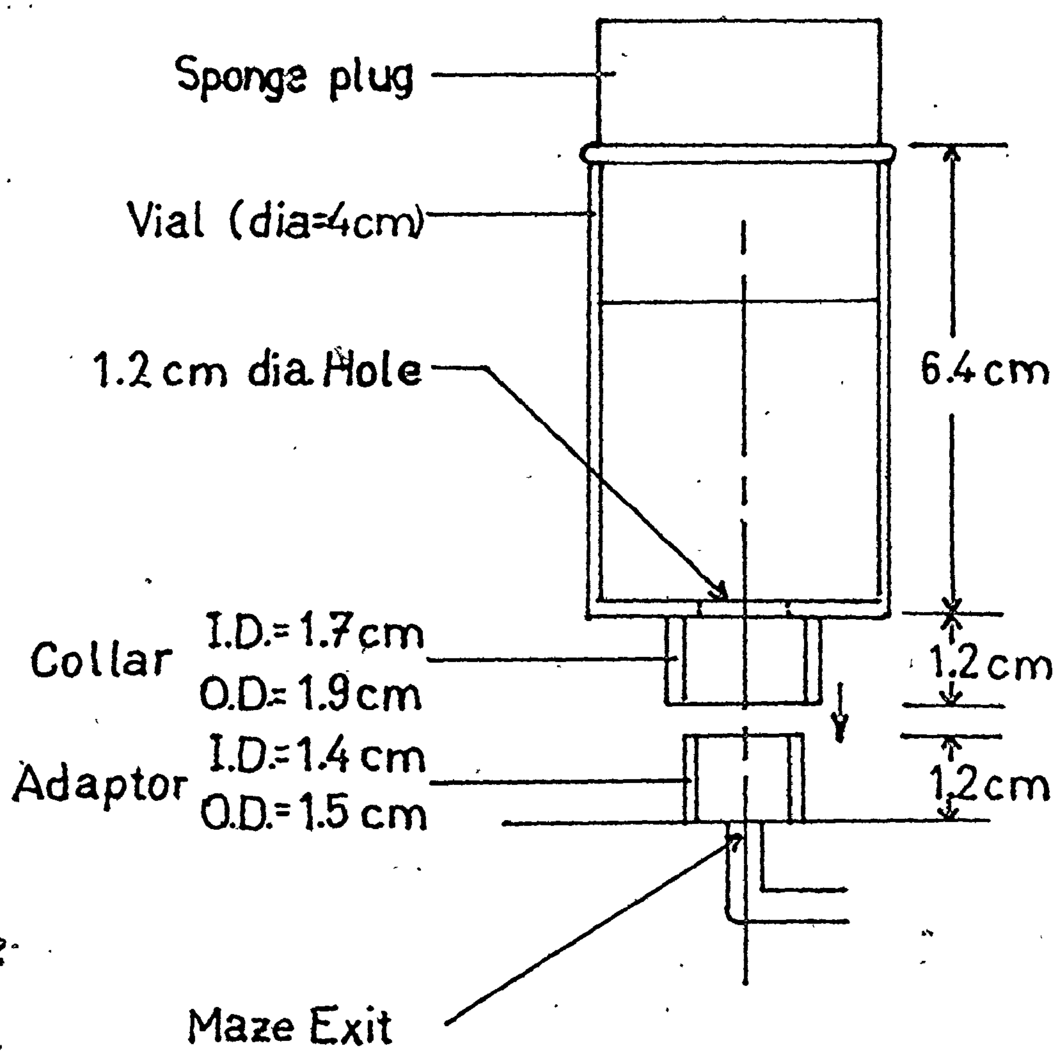


FIG. 2 - Collection System

Scale = 1:1

attachment at the back of the base plate, which was situated in the centre area to prevent its obscuring any part of the maze path.

The maze entrance was fitted with an adaptor of the same dimensions as the collection vial collars. The entrance adaptor was painted flat black inside and out, as were the holding chambers, the ends of which fitted into the entrance adaptor tightly enough to suspend them from it.

The holding chambers were constructed of various plastic components (vial lids and disposable syringes) in such a manner as to allow them to hold the flies in a light-free environment for several hours (volume of the holding area was 2ml as measured on the syringe barrel) and then, once a chamber is fitted into the entrance adaptor, the flies are released into the maze by simply rotating the assembly until the end of the holding area matches up to the hole leading to the maze, allowing the flies to leave. (see figure 3)

The holding area volume was kept small to crowd the flies and induce them to enter the maze. Before each run the chamber end, which was stoppered with a sponge plug to allow air in, was plugged airtight to cut off the airflow, adding another inducement to the flies to leave. The chambers and entrance adaptor were painted flat black, highlighting the maze entrance, which the flies moved towards due to positive phototaxis, negative geotaxis (the flies moved upwards to the entrance in the vertically mounted maze) and the crowding in the chamber.

The experiments were run in a light-tight 50cm X 56cm X 26cm plywood box, painted black inside and out. The light source, mounted inside the top of the box, was a 43 cm long fluorescent tube fixture. 10 cm of the centre of the tube was covered with black tape to give a split light source, with each arm of the final turns receiving approximately equal illumination.

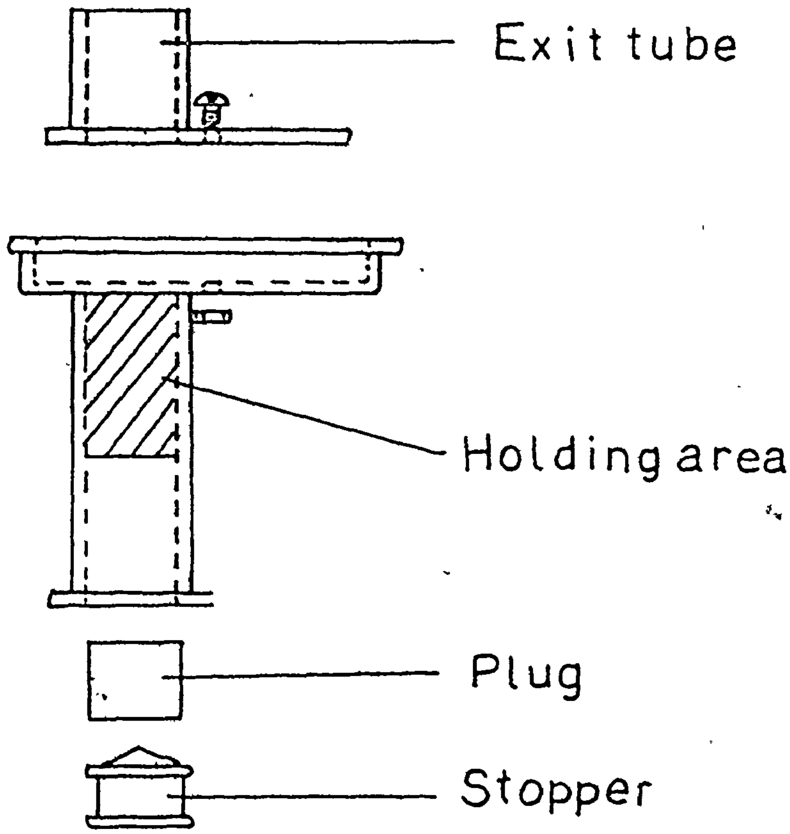
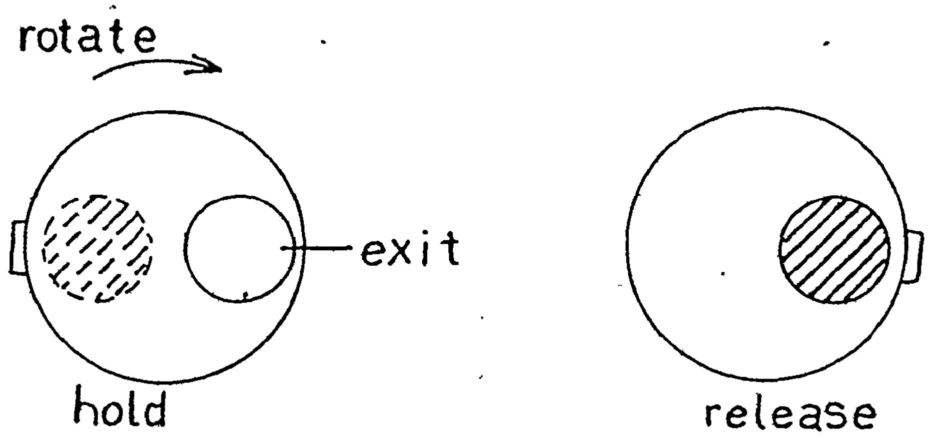


FIG.3 Holding Chamber (exploded view, 1:1 scale)



Positions

The front of the box opened via a hinged door, which was used when placing the maze inside and removing it from the box. The maze was suspended in the box via a 3-fingered holder, which grasped a piece of plexiglass rod mounted on the back of the maze with two standoff blocks. The box was also equipped with a simple heating device, used to maintain the inside temperature at a constant level, (this was employed to keep the box at 25 degrees C) the heat was supplied by a 40-watt incandescent bulb placed behind a black, light-tight aluminum shield, so that the bulb gave no light into the box.

General Procedure - the same basic procedure was followed for each maze run. The flies were divided into groups of 120, consisting of males and females in approximately equal proportions (60 ± 5 of each) all 3 to 5 days old, for each run. The elapsed time between removal from culture (small holding vials where the flies were kept for two days between removal from the stock culture bottles and running) and release into the maze was 3 to 3.5 hours, and all the flies spent at least 2 hours in a holding chamber immediately before their release. Immediately prior to the start of each run, the holding chamber containing the flies for the next run was blocked off at the air input (see Fig. 3) thus halting any air currents going through the maze. Immediately before the run, the chamber was attached to the maze, which had been mounted vertically in the box with the path leading upwards to the fluorescent light source which symmetrically illuminated the area of the maze. Next, the chamber was turned (rotated) to match the holding area exit to the maze entrance, releasing the flies into the maze, and the door to the light-tight box was closed for the duration of the run (5 min.)

The atmosphere inside the box was maintained as constant as possible at 25 degrees Celsius (plus or minus one degree) and 50 to 60 per cent relative humidity. The experimental runs were all done within the same time period each day, between 1100 hours and 1400 hours.

At the end of each run, the flies were anaesthesized with carbon dioxide, and the collection vials were scored according to the number of flies of each sex found in them at the end of the run. The collection vials are labelled in Fig. 1 and are referred to in the text according to the directions which the flies turned at the initial and final choice turns to reach the vial - eg. vial LL (for Left - Left) was reached by a fly which turned left at the maze entrance (initial choice turn) and turned left again at the final choice turn. The collection vials were cleaned (washed and dried with cellulose wipers) at the beginning of each day on which experiments were to be done, and when a different strain of lies were to be run, but they were otherwise unaltered, except for clearing of flies, from run to run. The sponge plugs atop each vial were the same for each run, and they were inverted when the strain of flies being run was changed, thus each surface of each plug was used specifically for one strain of flies (Canton S or black).

Some special points to remember concerning the effects of the experimental methods upon the behaviour of the flies are as follows:

i) the flies were dark adapted in the chambers before they entered the maze, which was mounted vertically with the light source at the top, thus the flies were expected to be motivated to move up the maze path due to positive phototaxis, negative geotaxis, and a desire to escape the crowded chamber. (Hay 1975)

ii) to maintain a consistent level of agitation of the flies, each chamber was tapped lightly on the table 6 times in rapid succession; this type of treatment is known to affect (intensify) positive phototaxis behaviour in some Drosophila (Lewontin 1959) hence it was important to control the mechanical stimulation which each fly received, to limit the effects of smaller perturbations incurred due to handling.

iii) the chambers were plugged at the ends immediately before their flies were run, thus there was little chance of anoxia occurring in the flies before they were released into the maze. The plugs were inserted to prevent flies from remaining at the bottom of the chamber, where the fresh air had been coming in previously, and also to prevent air currents being set up in the maze path, which may have altered the flies' behaviour.

iv) all flies run were approximately the same age (3 - 5 days), were starved for the same length of time (3 to 3.5 hours) and were run at the same approximate time each day (1100 to 1400 hours) hence they were all in approximately the same physiological state and at the same point in their diurnal behavioural rhythm (Hay 1972).

Strains of Flies Used - two strains of Drosophila melanogaster were used for these experiments, they were: (from Lindsley and Grell 1968)

1) black body - designated b, a spontaneous recessive mutant discovered by Morgan, located on chromosome 2 (map 48.5).
   Phenotype of homozygote: black pigment on body, tarsi, and wing veins.

2) Canton Special - designated Canton S or CS
   Phenotype of homozygote: wild type

Both strains showed approximately equal mobility through the clean maze in the experimental system used.

The flies were raised at 25 degrees Celsius in darkness on a cornmeal-molasses-agar medium made as follows: (from Strickberger 1962)

18 gms agar dissolved in 875 ml distilled H_2O plus 125 ml molasses

added to

125 ml cornmeal plus 50 ml Brewer's yeast plus 250 ml H_2O

this mixture was kept at a boil until the proper thickness was acheived, and then 7.5 ml of proprionic acid was added (mold retardant) and the medium was poured into culture bottles, in which the main breeding stocks were kept, and smaller holding vials, where the flies were kept for two days before running through the maze.

Data Analysis - testing for significant differences was done using contingency tables which were compared in a computer program via the G-test (Sokal and Rohlf, 1969) which calculates the value of the G statistic from the formula: (shown for a 2Xn table)

$$G = 2 \times \sum^{a} f_i \ln\left(\frac{f_i}{f_i'}\right) \text{ , where } a \ \#\text{classes}, \ f_i, f_i' \text{ data compared}$$

The formula for determining G is similar to the one used to determine the value of X^2 in the X^2 test:

$$X^2 = \sum^{a} \left(\frac{f_i - f_i'}{f_i'}\right)^2$$

however, the G-test more closely approximates a true Chi-square distribution compared to the X^2 test, and since the tests in this case were done on a computer, the added complication of obtaining logarithms had no effect upon calculation complexity or time, indeed, even if the tests had been done by hand or on a desk calculator the G-test would have been faster and simpler for the size of tables (up to 5X4) used.

The values of G obtained in each test were compared to a standard chi-spare distribution to give the p (probability) values given, and a p value less than 0.05 was considered to be significant.

Homogeneity tests were done for each class of runs using large contingency tables and the same G - test. The mean distributions and standard deviations of the data in the Total columns of the data tables (to follow) which are listed at the bottom of each data table were found by the following method: i) each number in each column was divided by the total number of flies which had completed its corresponding run, which was listed in the column headed TF at the extreme right side of each data table. ii) the mean and standard deviations of these proportions were then found in standard fashion.

The results of these calculations are listed under the appropriate headings (eg. results for the mean proportion of flies in each run which ended up in collection vial LL are listed under LL/TF). The means and standard deviations of the proportions, rather than the raw data, were calculated in order to correct for the differing activities (as represented by the number of flies finishing the maze in each run as listed in the TF columns) observed among the experimental groups of flies from run to run.

The mean proportion distributions were used for each class to indicate the basic pattern of behaviour observed for flies of each run class.

RESULTS

EXPERIMENTAL SERIES I - The Effects of Maze Status Upon the Final Turn Direction Preferance of Canton S Strain Flies.

Description: This series of experimental runs involved two different fly status conditions and two basic maze status conditions as follows:

Maze Status A - refers to a maze which was thouroughly scrubbed, rinsed and dried using sterile cotton swabs and water (the face plate being similarily cleaned with cellulose wipers) immediately before the run.

Maze Status B - refers to a maze which was cleared of flies from a previous run and aired but otherwise unaltered. The number of previous runs since the maze was last cleaned (i.e. at status A) is given in parentheses to denote the precise status of the maze, for example B(3) refers to a maze which has had three sets of flies run through it since the last cleaning.

Fly Status I - refers to flies being run in a maze for their first time.

Fly Status II - refers to flies being run in a maze for their second time, after a previous run in a maze of opposite status (maze status A is opposite to maze status B).

The combination of the strain name (eg. Canton S shortened to CS), fly status and maze status prior to the run gives the different run classes as follows: CS I - A, CS I - B(1) to CS I - B(4),

CS II - A and CS II - B

(eg. CS I - A refers to flies run for their first time in a status A maze, while CS II - A refers to flies being run in a status A maze after they have previously been through a status B maze)

Results - a) Raw Data and Homogeneity Tests: the results for the experimental runs of each class are given separately in the following set of tables (Tables 1 to 7). Each table lists the total number of flies (under Total) and the number of each sex (under Male and Female) found in the different collection vials at the end of each individual run. The column headings (LL,LR,RL,RR) refer to the collection vials (eg. LL stands for Left,Left) as previously described (page 12).

The extreme right hand column contains the total number of flies which completed the maze in each run. Runs in which less than 30 flies (25% of the starting number of 120) completed the maze were not counted. At the bottom of each column the overall totals of all runs of the class are printed.

Tests for homogeneity of the data were done for all the runs of each class by running them as a group in a contingency table to find the value of the G-statistic (page 14) which was then compared to a Chi-square distribution to find the probability (p) that the data constitute a homogeneous class, with results listed below each table. The results for male and female flies are recorded here for all classes, and although in all cases the data were homogeneous and no significant differences between sexes were observed, the small numbers being considered here may limit the validity of the testing, hence only the combined total data (under Total) homogeneity test results are listed.

In cases where the run set for a class was found to give a significant probability difference from homogeneity (p less than 0.05), some of the runs (those which appeared to deviate most obviously from the pattern of the others in the class - the mean distribution) were removed (marked by an *) until the set became homogeneous (p larger than

0.05), and the totals of the columns of this homogeneous set are given as the corrected totals. These corrected totals were then compared to the original overall totals in 2 X 4 contingency tables, and for all classes there was no significant difference between them, thus the overall totals will be carried forward for the inter- and intra- class comparisons to follow. (Overall Totals are listed in Table 8)

TABLE 1 - Results for Run Class CS I -

Male				Female				Total				TF # flies finished
LL	LR	RL	RR	LL	LR	RL	RR	LL	LR	RL	RR	
11	9	13	11	8	6	13	8	19	15	26	19	79
9	14	13	9	9	11	7	7	18	25	20	16	79
12	10	11	11	9	9	10	11	21	19	21	22	83
10	14	16	9	10	8	15	18	20	22	31	27	100
10	15	19	20	15	15	9	10	25	30	28	20	113
12	19	21	9	12	14	13	7	24	33	34	16	107
10	10	12	10	9	11	15	8	19	21	27	18	85
10	8	10	8	8	9	7	7	18	17	17	15	67
12	19	11	11	7	17	15	10	19	36	26	21	102
13	15	18	14	12	11	14	12	25	26	32	26	109
11	16	18	13	9	18	12	9	20	34	30	21	105
10	13	9	7	5	6	9	5	15	19	18	12	64
9	12	9	9	8	13	12	7	17	25	21	16	79
12	16	12	15	10	14	9	9	22	30	21	24	97
7	10	11	6	10	11	7	6	17	21	18	12	68
11	8	14	13	6	5	7	5	17	13	21	18	69
18	9	10	11	12	7	11	12	30	16	21	23	90
6	5	4	6	3	2	3	5	9	7	7	11	34
6	5	4	3	3	2	4	2	9	7	8	15	39
								365	416	426	362	1569

Homogeneity test: G is 44.8359, D.F. are 54, p is 0.80386

	$\frac{LL}{TF}$	$\frac{LR}{TF}$	$\frac{RL}{TF}$	$\frac{RR}{TF}$
Mean	0.2351	0.2594	0.2681	0.2328
Standard deviation	0.0325	0.0563	0.0379	0.0552

(see page 15)

TABLE 2 - <u>Results for Run Class CS I - B(1)</u>

Male				Female				Total				# flies (T
LL	LR	RL	RR	LL	LR	RL	RR	LL	LR	RL	RR	finished
9	7	9	19	7	5	11	15	16	12	20	30	78
8	5	3	12	6	5	4	9	14	10	7	21	52
13	15	7	10	8	11	11	11	21	26	18	21*	84
9	9	10	9	3	7	9	5	12	16	19	14*	61
9	2	4	6	8	2	4	9	17	4	8	15	44
7	6	6	7	6	6	4	5	13	12	10	12	47
22	3	9	13	14	3	8	13	36	7	17	26*	86
19	10	3	18	13	4	3	11	32	14	6	29*	81
15	11	8	15	15	9	6	11	30	20	14	26	90
10	12	4	8	9	11	3	6	19	23	7	14*	63
11	10	7	10	9	6	5	12	20	16	12	22	70
14	7	8	8	14	5	6	9	23	12	14	17	71
15	8	3	21	11	7	3	18	26	16	6	39	87
19	10	6	15	18	11	4	20	37	21	10	35	103
18	10	7	10	14	8	7	9	32	18	14	19	83
17	9	11	15	12	4	9	12	29	13	20	27	89
11	15	14	10	11	11	12	10	22	26	26	20*	94
21	8	4	18	16	5	5	12	37	13	9	30	89
11	10	11	20	10	8	8	15	21	18	19	35	93
21	10	9	15	15	11	7	10	36	21	16	25	98
17	9	11	13	14	5	9	16	31	14	20	29	94
				Overall Totals				506	333	285	492	
				Corrected Totals				387	220	199	382	

* - runs removed for corrected test table,

Homogeneity Tests:	G	D.F.	p	Table dimensions
All Runs	119.6200	60	0.00006	21 X 4
Corrected	57.0508	42	0.06561	15 X 4
Overall vs. Corrected Totals	2.8047	3	0.05750	4 X 2

	LL/TF	LR/TF	RL/TF	RR/TF
Mean	0.3123	0.2020	0.2006	0.3047
Standard Dev.	0.0680	0.0681	0.1291	0.0662

TABLE 3 - Results for Run Class CS I - B(2)

Male				Female				Total				TF # flies finished
LL	LR	RL	RR	LL	LR	RL	RR	LL	LR	RL	RR	
5	3	6	9	5	4	6	8	10	7	12	17	46
12	4	4	12	9	6	7	11	21	10	11	23	65
16	7	4	12	9	5	4	13	25	12	8	25	70
13	6	8	11	14	6	11	15	27	12	19	26	84
8	10	8	9	9	14	6	12	15	26	14	21*	76
9	13	11	16	7	8	7	12	16	21	18	28*	83
11	6	8	9	16	5	7	11	27	11	15	20	73
25	6	9	15	18	6	7	18	43	12	16	33	104
10	3	11	17	9	2	9	14	19	5	20	31	75
28	6	7	14	13	3	7	14	41	9	14	28	92
13	4	7	11	9	4	7	7	21	8	14	18	61
23	8	11	7	19	6	9	7	42	14	20	14	90
				Overall Totals				307	147	181	284	
				Corrected Totals				276	100	149	235	

* - removed for corrected homogeneity test table

Test between Corrected and Overall Totals: G is 3.30859, p is 0.34712

Homogeneity Tests: i) All runs: G is 71.7266, p is 0.00029 for 33 D. F.

ii) Corrected table: G is 38.5566, p is 0.07303 for 27 D. F.

	$\frac{LL}{TF}$	$\frac{LR}{TF}$	$\frac{RL}{TF}$	$\frac{RR}{TF}$
Mean	0.3252	0.1611	0.2001	0.3136
Standard Dev.	0.0936	0.0725	0.0462	0.0644

TABLE 4 - Results for Run Class CS I - B(3)

Male				Female				Total				TF # flies
LL	LR	RL	RR	LL	LR	RL	RR	LL	LR	RL	RR	finished
12	6	12	12	8	4	6	9	20	10	18	21	69
6	11	9	4	8	7	9	6	14	18	18	10*	60
12	3	2	10	8	4	4	7	20	7	6	17	50
10	9	14	13	9	10	11	12	19	19	25	25*	78
16	7	4	3	22	8	9	5	38	15	13	8*	74
9	7	8	9	10	4	6	10	19	11	14	19	63
12	6	5	16	8	2	2	14	28	8	7	17	59
15	6	4	17	19	3	2	14	34	9	6	31*	80
25	11	5	19	15	6	4	11	40	17	9	30	96
19	6	12	18	12	3	15	12	31	9	27	30	97
13	7	6	13	13	7	3	8	26	14	9	21	70
12	6	5	16	8	2	2	14	20	8	7	30	65
				Overall Totals				309	145	159	259	
				Corrected Totals				204	83	97	185	

* - removed for homogeneity test with corrected table

Test between Corrected and Overall Totals: G is 2.08594, p is 0.55844

Homogeneity Tests: i) All runs: G is 90.2383, p is 0.00001 for 33 D. F.

ii) Corrected Table: G is 32.2521, p is 0.05770 for 21 D. F.

	$\frac{LL}{TF}$	$\frac{LR}{TF}$	$\frac{RL}{TF}$	$\frac{RR}{TF}$
Mean	0.3581	0.1706	0.1834	0.3000
Standard Dev.	0.0895	0.0592	0.0883	0.0908

TABLE 4 - Results for Run Class CS I - B(3)

Male				Female				Total				TF # flies
LL	LR	RL	RR	LL	LR	RL	RR	LL	LR	RL	RR	finished
12	6	12	12	8	4	6	9	20	10	18	21	69
6	11	9	4	8	7	9	6	14	18	18	10*	60
12	3	2	10	8	4	4	7	20	7	6	17	50
10	9	14	13	9	10	11	12	19	19	25	25*	78
16	7	4	3	22	8	9	5	38	15	13	8*	74
9	7	8	9	10	4	6	10	19	11	14	19	63
12	6	5	16	8	2	2	14	28	8	7	17	59
15	6	4	17	19	3	2	14	34	9	6	31*	80
25	11	5	19	15	6	4	11	40	17	9	30	96
19	6	12	18	12	3	15	12	31	9	27	30	97
13	7	6	13	13	7	3	8	26	14	9	21	70
12	6	5	16	8	2	2	14	20	8	7	30	65
						Overall	Totals	309	145	159	259	
						Corrected	Totals	204	83	97	185	

* - removed for homogeneity test with corrected table

Test between Corrected and Overall Totals: G is 2.08594, p is 0.55844

Homogeneity Tests: i) All runs: G is 90.2383, p is 0.00001 for 33 D. F.

ii) Corrected Table: G is 32.2521, p is 0.05770 for 21 D. F.

	LL/TF	LR/TF	RL/TF	RR/TF
Mean	0.3581	0.1706	0.1834	0.3000
Standard Dev.	0.0895	0.0592	0.0883	0.0908

TABLE 6 - Results for Run Class CS II - A

Male				Female				Total				TF # flies
LL	LR	RL	RR	LL	LR	RL	RR	LL	LR	RL	RR	finished
6	9	4	6	5	7	4	9	11	16	8	15	50
7	5	6	5	7	4	7	8	14	9	13	13	49
9	8	14	7	8	7	13	6	17	15	27	13	72
13	16	12	11	10	11	7	13	23	27	19	24	93
9	6	11	8	11	4	8	6	20	10	19	14	63
10	11	5	7	8	13	6	11	18	24	11	18	71
13	9	10	12	7	10	11	10	20	19	21	22	82
12	24	8	13	9	20	7	12	21	44	15	25	105
13	10	12	7	11	10	10	10	24	20	22	17	83
7	10	13	9	9	12	11	9	16	22	24	18	82
				Overall Totals				184	206	179	181	

Homogeneity Tests: All runs: G is 39.2129, p is 0.06392 for 27 D. F.

	$\frac{LL}{TF}$	$\frac{LR}{TF}$	$\frac{RL}{TF}$	$\frac{RR}{TF}$
Mean	0.2488	0.2659	0.2418	0.2410
Standard Dev.	0.0395	0.0786	0.0750	0.0350

TABLE 7 - Results for Pun Class CS II - B(1)

Male				Female				Total				TF
LL	LR	RL	RR	LL	LR	RL	RR	LL	LR	RL	RR	# flies finished
11	3	5	6	9	2	4	6	20	5	9	12	46
17	3	8	10	16	4	11	9	33	7	19	19	78
12	6	6	14	11	8	4	11	23	14	10	25	72
19	7	8	13	15	7	6	12	34	14	14	25	87
20	7	5	21	14	7	6	13	34	14	11	34	93
5	2	2	9	6	5	3	7	11	7	5	16	39
12	7	4	15	11	6	6	11	23	13	10	26	72
5	3	5	12	4	3	5	8	9	6	8	20	43
							Overall ~~Totals~~	137	80	86	177	

Homogeneity Test: G is 22.4072, D.F. are 21, p is 0.3783

	LL/TF	LR/TF	RL/TF	RR/TF
Mean	0.3431	0.1505	0.1638	0.3426
Standard Dev.	0.0759	0.0365	0.0422	0.0765

TABLE 8 - Overall Totals of All Classes

CLASS	LL	LR	RL	RR	Left	Right	#runs in class
CS I - A	365	416	426	362	781	788	19
CS I - B(1)	506	333	285	492	839	777	21
CS I - B(2)	307	147	181	284	454	465	12
CS I - B(3)	309	145	159	259	454	418	12
CS I - B(4)	269	130	134	245	399	379	11
CS II - A	184	206	179	181	390	359	10
CS II - B(1)	187	80	86	177	267	263	8
Total CS I - B(1 to 4)	1391	755	759	1280	2146	2039	56

The Left and Right columns contain the data for number of flies turning either direction at the entrance choice turn, which were derived by adding LL plus LR and RL plus RR respectively. (thus only flies which completed the maze were recorded, in order to make further comparisons between the initial and final turn direction preferences valid.)

b) Inter-class Comparisons - these were done, using the totals for each class listed in Table 8, in order to find if any differences existed among the various run class results. Table 9 below lists the results of contingency table tests, with the classes compared listed in the left side column, and the probability that the totals compared constitute a homogeneous set are listed in the centre for each test. Significant differences (p less than 0.05) are marked with an *, and the dimensions of the contingency tables for each test are listed in the right side column (rows X columns).

TABLE 9 - Results of Inter-class Comparisons

Classes Compared	Probability	Table Dimensions
i) CS I - B(1 to 4)	0.08186	4 X 4
ii) CS I - A CS I - B(1 to 4) total	*0.0	2 X 4
iii) CS II - B(1) CS I - B(1 to 4) total	0.16092	2 X 4
iv) CS II - A CS I - A	0.58797	2 X 4

From the results of these comparisons, we see that there are no significant differences between runs done in a maze of status B (Table 9, i,iii), nor between runs done in a maze of status A (iv), however, there is a highly significant difference between runs done in a status A maze when compared to those done in a status B maze, (ii).

Further Comparisons - these comparisons were done in order to find the nature of the differences and similarities observed among the classes which were compared in Table 9.

The first test involves comparing the overall totals of each class (from Table 8) with a 1:1:1:1 distribution in 2 X 4 contingency tables. The 1:1:1:1 distributions were derived by summing all the totals for the class under consideration and dividing by four, eg. for class CS I - B(1) the quantities compared were:

Overall Totals	506	333	285	492	sum is 1616
1:1:1:1 Dist.	404	404	404	404	sum is 1616

The results of these tests are listed for each class in Table 10 following; significant differences are marked with an *.

TABLE 10 - Results of Comparisons of Class Totals vs. 1:1:1:1

Class Tested	Probability of LL:LR:RL:RR being the same as 1:1:1:1
CS I - A	0.23196
CS I - B(1)	*0.0
CS I - B(2)	*0.0
CS I - B(3)	*0.0
CS I - B(4)	*0.0
CS II - A	0.75409
CS II - B(1)	*0.0

From the results of these comparisons we see that the status B maze run classes all show a highly significant deviation from a 1:1:1:1 distribution at the collection vials while the status A maze runs do not.

Having established from Table 10 that the maze status B runs deviate significantly from a 1:1:1:1 distribution (the mean distribution is actually 1.8:1:1:1.8), the pattern of this deviation will now be investigated. A contingency table test done with the entrance turn direction data (Left and Right columns of Table 8) for all classes gave a p value of 0.11599 for homogeneity, indicating that turning behaviour at the entrance does not vary significantly among the classes. The mean ratio of left to right turning at the entrance is 1.042 : 1, thus if the flies continued to choose their direction of turning at the final choice point with the same probability, the distribution of flies in the collection vials at the end of each run would be about 1:1:1:1.

The maze status A run class totals do not deviate significantly from the expected 1:1:1:1 distribution, hence the probability of direction of turning of the flies did not change significantly between the entrance and the final choice point in these runs.

The maze status B run class totals do deviate significantly from the 1:1:1:1 distribution, indicating that the probability of direction of turning of the flies was different at the final choice point when compared to at the entrance. The symmetry of the collection vial distribution for classes CS I - B(1 to 4) is shown by the following comparisons using the combined totals (Table 8):

LL,LR vs. RL,RR (2 X 2 table), p is 0.0
LL,LR vs. RR,RL (2 X 2 table), p is 0.16577

thus the distribution is symmetrical with respect to the maze midline, with a significant turn direction preference towards the outer collection vials (LL,RR) occurring at each final choice point.

General Observations: some test runs (data not recorded) were done with the front door of the maze box open in order that the behaviour of the flies could be observed as they actually moved through the maze. Some of the observations made were as follows:

Status A Maze: both Canton S and black strain flies appeared to act the same in the clean maze. Flies left the chamber immediately upon release and travelled in single file, with flies seldom moving side by side in the same direction. Very little retracing or movement back down the maze was observed, with most flies tending to move rapidly up the maze, while a few stopped, usually in the blind alleys. Most (~ 60%) of the flies stayed mainly on the face plate and the surface of the maze path parallel to it. The bulk of the flies which finished the maze each run reached the collection vials in less than 3 minutes on the average, and once in a vial, the flies did not leave. By monitoring the blind alleys, it was seen that about the same number of flies entered each one during the course of each run.

Status B Maze: the black strain flies did not appear to act different in this type of maze, except some flies were observed to enter the maze briefly and then go back into the chamber. The Canton S showed slightly different behaviour from that observed in the Status A maze, in that their preference for walking on the face plate and the parallel maze path surface was more marked, with about 80% of the flies being on these surfaces at any one time; also, far fewer flies turned into the blind alleys, especially the upper ones, in these runs than in the Status A runs.

Preliminary Discussion: the interclass comparisons indicate that for the two basic maze status conditions: A (just cleaned maze), and B (maze previously occupied since last cleaning);

1) runs in a status B maze do not deviate significantly from each other and thus follow the same pattern regardless of whether only one or up to four groups of flies preceded the run through the maze.

2) results for status A maze runs and status B maze runs differ significantly from each other, indicating that the flies behave differently at the final choice point (turning behaviour at the entrance did not vary significantly) according to maze status.

3) the status of the flies, within the limits tested here, does not alter the dependancy of their behaviour (at the final choice point) upon maze status. Flies which were run in a status A maze first and then in a status B(1) maze (class CS II - B(1)) show results consistent with those obtained for first run maze status B flies (and vice versa for CS II - A flies).

The intraclass comparisons indicate that :

1) for flies run in a status A maze, no significant difference in turning behaviour occurs between the initial and final choice points.

2) for flies in a status B maze, there is a significant alteration in turn direction preference at the final choice points, with the direction of the initial turn and the subsequent forced turns being favoured over the opposite direction. (eg. flies which turned left at the start tend to turn left again at the final choice point.)

The results for the maze status B runs in these experiments are similar in pattern to those obtained by Hay (pg. 4 this thesis) which he interpreted to be an indication that the flies had learned to turn away from the blocked off ends of the forced turns while going through the maze, and continued to show this turn direction preference at the final choice point, giving the type of distribution (here approx. 1.8:1:1:1.8) observed.

If the status B maze run results are indeed due to learning by the flies, then why do the maze status A run results not show the same pattern? This question will be examined in the discussion of Experimental Series III, while Experimental Series II examines the maze behaviour of the black strain of <u>D. melanogaster</u> and compares it to the results for the Canton S strain.

EXPERIMENTAL SERIES II - The Effects of Strain Difference Upon the Maze Behaviour of Drosophila Melanogaster.

Description: this series of experimental maze runs was done with black strain flies in a manner similar to that previously described for the Canton S strain. The basic maze status conditions (A and B) were the same, and the three classes of runs, using similar notation, are: b I - A, b I - B(1) and b I - B(2) (eg. b I - A refers to flies of the black strain (shortened to b) run for their first time in a just cleaned maze).

Results: a) Raw Data and Homogeneity Tests - the results for each run class are given separately in Tables 11, 12 and 13 following. The homogeneity tests were done in the manner previously described for the Canton S strain results, and the overall totals for each class are listed in Table 14. (the overall and corrected totals for class b I - B(1) did not differ significantly, so the overall totals are carried forward to Table 14)

Some runs of class b I - B(3) were attempted, however the majority of these gave a total number of flies completing the maze of less than thirty, therefore only the results up to class b I - B(2) are listed and used here.

TABLE 11 - Results for Run Class b I - A

Male				Female				Total				TF # flies
LL	LR	RL	RR	LL	LR	RL	RR	LL	LR	RL	RR	finished
12	8	14	3	9	11	18	8	21	19	32	11	83
12	11	12	13	10	14	14	12	22	25	26	25	98
13	11	13	6	9	11	10	10	22	22	23	16	83
12	10	6	4	9	14	6	5	21	24	12	9	66
13	24	23	11	11	12	13	14	24	36	36	25	94
5	10	8	5	7	8	8	6	12	18	16	11	57
6	9	2	1	10	9	4	5	16	18	6	6	46
9	14	12	5	9	8	8	5	18	22	20	10	70
6	6	3	8	5	4	3	5	11	10	6	13	40
11	11	10	11	11	5	5	13	22	16	16	24	78
10	8	13	4	9	10	14	5	19	18	27	9	73
7	6	6	7	14	8	10	11	21	14	16	18	69
					Overall Totals			229	236	221	171	857

Homogeneity Test: G is 47.5625, D. F. are 33, p is 0.05199

	$\frac{LL}{TF}$	$\frac{LR}{TF}$	$\frac{RL}{TF}$	$\frac{RR}{TF}$
Mean	0.2711	0.2852	0.2622	0.2055
Standard Dev.	0.0383	0.0668	0.0869	0.0743

TABLE 12 - Results for Run Class b I - B(1)

Male LL	LR	RL	RR	Female LL	LR	RL	RR	Total LL	LR	RL	RR	TF # flie finishe
7	10	12	5	7	6	7	7	14	16	19	12	61
5	11	10	9	5	4	6	8	10	15	16	17	58
3	14	6	4	4	9	5	4	7	23	11	8*	49
11	10	9	13	4	5	6	15	15	15	15	28	73
15	10	6	8	8	6	6	10	23	16	12	18	69
8	6	7	6	9	8	4	6	17	14	11	12	54
14	7	11	18	17	7	11	13	31	14	22	31	98
11	11	6	4	8	7	6	7	19	18	12	11	60
7	9	2	13	3	8	3	9	10	17	5	22	54
12	11	10	14	9	12	14	18	21	23	24	32	100
3	9	2	10	6	3	0	8	9	12	2	18*	41
3	7	13	8	6	19	6	6	9	26	19	14*	68
9	8	5	10	11	8	7	9	20	16	12	19	67
7	4	9	10	11	5	9	10	18	9	18	20	65
7	8	2	4	7	4	4	4	14	12	6	6	38
				Overall Totals				237	246	204	268	
				Corrected Totals				212	185	172	228	

* - removed for homogeneity test with corrected table

Test Between Corrected and Overall Totals: G is 1.74219, p is 0.63205

Homogeneity Tests: i) All Runs: G is 83.5781, p is 0.00037 for 42 D. F.

ii) Corrected Table: G is 46.7598, p is 0.06065 for 33 D. F.

	LL	LR	RL	RR
Mean	0.2482	0.2695	0.2063	0.2761
Standard Dev.	0.0744	0.0845	0.0707	0.0886

TABLE 13 - Results for Run Class b I - B(2)

Male				Female				Total				TF # flies
LL	LR	RL	RR	LL	LR	RL	RR	LL	LR	RL	RR	finishe
7	6	9	10	2	3	7	9	9	9	16	19	53
8	7	11	6	3	4	9	7	11	11	20	13	55
12	17	8	7	11	11	8	8	23	28	16	15	82
7	9	6	6	6	5	3	7	13	14	9	13	49
7	3	3	4	7	2	5	8	14	5	8	12	39
8	6	4	6	5	6	4	6	13	12	8	12	45
9	7	8	12	10	5	4	13	19	12	12	25	68
8	9	7	10	10	7	8	9	18	16	15	19	68
7	5	4	4	6	4	1	3	13	9	5	7	34
7	8	9	11	4	4	5	7	11	12	14	18	55
12	8	7	10	13	8	6	10	25	16	13	20	74
6	11	11	6	5	7	7	9	11	18	18	15	62
8	7	7	8	5	5	7	9	13	12	14	17	56
6	3	5	7	7	3	6	5	13	6	11	12	50
						Overall Totals:		206	180	179	225	790

Homogeneity Test: G is 44.7285, D.F. are 39, p is 0.249.

	LL/TF	LR/TF	RL/TF	RR/TF
Mean	0.2544	0.2234	0.2259	0.2753
Standard Dev.	0.0634	0.0629	0.0601	0.0537

TABLE 14 - Overall Totals for black Strain Run Classes

Class	LL	LR	RL	RR	Left	Right	# runs in class
b I - A	229	236	221	171	465	392	12
b I - B(1)	237	246	204	268	483	472	15
b I - B(2)	206	180	179	225	386	404	14

b) Interclass Comparisons: A contingency table (2 X 4) test between the two maze status B classes (b I - B(1 and 2)) gave a p value of 0.535 , indicating that these results do not differ significantly from each other and they were combined for the next test. A contingency table (3 X 4) test done with the results from all three black strain classes gave a value of p of 0.001, showing a significant difference between the maze status A overall totals and those for runs in maze status B. From these comparisons, we see a maze status-dependant difference in collection vial distribution, as occurred in the Canton S strain.

c) Intraclass Comparisons: since the data for the initial turn direction choice (Left, Right in Table 14) form a homogeneous set (p is 0.120), with a mean left : right ratio of 1.04 : 1, the overall total distributions of the three black strain run classes were compared to a 1:1:1:1 distribution (as on page 26 for Canton S) to see if any differences occurred between turning direction probabilities at the initial and final choice points. The results of these comparisons were as follows:

b I - A , p is 0.088
b I - B(1) , p is 0.208
b I - B(2) , p is 0.296

None of these tests showed a significant deviation from a 1:1:1:1 ratio (the ratio which was expected if no change in turn choice behaviour occurred during the course of the run.)

d) Comparisons With Canton S Strain Results: a contingency table (10 X 2) test run with the initial choice point turn direction data (Left and Right columns, Tables 8 and 14) for all Canton S and black strain classes gave a p value of 0.116, indicating that the behaviour of the flies at the entrance of the maze is independant of maze status and fly strain. The mean ratio of left : right is 1.04 : 1, thus the flies show no preference for turning in a particular direction as they enter the maze.

A contingency table (4 X 2) test between totals for the maze status A runs of both strains (classes CS I - A and b I - A) gave a p value of 0.122 , indicating that there is no strain difference in turning behaviour at the final choice point for flies run in a status A maze.

A contingency table (4 X 6) test with the totals for maze status B runs of both strains gave a p value of 0.0, indicating a significant strain difference in final choice point turning behaviour in the status B maze, which was expected since the Canton S strain results differed significantly from a 1:1:1:1 distribution, while the black strain results did not.

Preliminary Discussion - from the inter- and intra- class comparisons done with the black strain results, we see that although there is a significant difference between the results for maze status A runs and maze status B runs, neither of these classes show a significant deviation from a 1:1:1:1 distribution at the collection vials, hence there is no indication of a significant change in the turn direction choice behaviour of the flies between the initial and the final choice points. Therefore, there is no evidence that learning by the black strain flies has taken place under either of the maze status conditions.

The comparisons between the results for the black and Canton S strains show no significant differences in initial choice point turning behaviour, with all classes showing no preference for either direction (left or right) at the start of the maze. Comparisons of the final choice point turning results, however, show that the Canton S maze status B results differ significantly from all other classes, in that they show a definite preference by the flies to turn towards the outer collection vial at the final choice point in each maze path. Thus the Canton S maze status B runs appear to be the only ones in which a pattern suggestive of associative learning was observed, with the results for these runs differing significantly from the results obtained for black strain flies run in the same status maze.

The nature of the pattern observed for the Canton S maze status B runs will be examined in Experimental Series III.

EXPERIMENTAL SERIES III - An Investigation Into the Nature of the Maze Status-Specific Behaviour of Canton S Strain Flies

Description: this series of experimental runs was designed in an attempt to find the reason for the observed differences in turning behaviour at the final choice point, when compared to that shown at the initial choice point, of flies of the Canton S strain which were run in a status B maze, as seen in Expt. Series I. Since a status B maze is one in which at least one group of flies has been run through since the last cleaning, it was thought that some chemical trace left by these flies may be responsible for the observed peculiarities of flies run after them in the status B maze.

In order to test this hypothesis, various changes were made in maze status and their effects observed. The first change involved taking a status B(1) maze (one group of flies run through since the last cleaning) and cleaning the final choice point (from the T-junction to exits) to give a status C maze. The results of runs done in the status C maze are listed below in Table 15.

TABLE 15 - Results for Run Class CS I - C

Total LL	LR	RL	RR	#flies (TF) finished
18	12	11	17	58
29	9	10	23	71
9	9	2	10	30
20	8	4	16	48
9	11	9	12	41
27	6	8	21	62
15	11	10	12	48
12	11	10	12	45
20	13	6	17	56
159	90	70	140	Overall Totals

Homogeneity test: G is 25.7422, D.F. are 24, p is 0.36859

	LL/TF	LR/TF	RL/TF	RR/TF
Mean	0.3363	0.2079	0.1519	0.3039
Stand. dev	0.0733	0.0663	0.0599	0.0314

The status C runs were done to see what effect, if any, the distribution of the hypothesized trace substance at the final choice point had upon the flies' behaviour. This distribution would be expected to be equal to the left or right on the average for a maze of status B(1), due to the observed tendency of the flies run previously in the clean maze (status A) to turn in either direction with approximately equal probability, as seen in the results from Expt. Series I. The distribution of trace substance would be expected to be uneven, with more left in the outer arms of the final choice points, for a maze of status B(2 to 4), since in the preceding runs the flies tended to show a significantly higher probability of turn-outwards at the final choice points. Since no significant differences were found among the results for runs in maze status B (1 to 4) in Expt. Series I, the differences in distribution of trace substances at the final choice point was not thought to be involved in determining direction preferences in the status B maze. This expected situation is shown by the results for the runs in the status C maze, which, when compared to the previously obtained results for status B maze runs in a contingency table (2 X 4) gave a p value of 0.576 , which indicates that the cleaning of the final choice point has no effect upon final choice point turn direction preference, and hence the distribution of trace substances in general at the final choice point has no significant effect upon behaviour of flies of the Canton S strain run in a status B maze.

Since there are no discernable effects upon behaviour due to trace substances left at the final choice points in the status B maze, further experiments were done to see if the distribution of these hypothesized substances in the lower maze path (between the entrance and the final choice point) had any effects upon behaviour of the flies.

If the amount of trace substances left in any part of the maze path is proportional to the number of flies which passed through it, then during the course of any experimental run, less trace substance will be left in the blind alleys, since not all of the flies will have gone into each of them, while the rest of the ascending maze path will contain more trace substance, since all of the flies which went through the maze had to follow this path. Thus we could have the formation of a chemical trace pathway, which marks out the correct path through the maze past the blind alleys, and the apparent learning behaviour of the Canton S strain flies in the status B maze may not be due to their association of visual cues in the maze, which should have occurred in the status A maze as well, but instead it may be the result of the flies tending to follow the path of greatest chemical trace concentration through the maze, and then continuing to follow the established turning pattern at the final choice point.

This hypothesis was tested in the runs done in the status D condition maze. Status D refers to a maze into which a group of 80 flies was put prior to the experimental run; the maze was left lying flat for at least 15 minutes, so that the flies would wander randomly through all parts of the maze path, and to ensure that the distribution of any chemical traces was equal in all parts of the maze path, the flies were repeatedly shaken into all of the forced turns and allowed to crawl back up them. These flies were then removed just before the experimental run.

The final choice points were also cleaned in the Status D maze, with the cleaning done before the pre-run group of flies were put in and the final choice points were blocked off to keep the pre-run group in the lower maze path .

Results - the results for the runs in the status D (class CS I - D), overall totals only, are listed in Table 16.

TABLE 16 - Results For Run Class CS I - D

LL	LR	RL	RR	# flies (TF) finished
10	12	13	18	53
20	33	22	21	96
25	28	12	16	81
14	11	16	14	55
10	13	12	14	51
22	15	22	19	78
101	112	97	104	Overall Totals

Homogeneity Test: G is 18.4062, D.F. are 15, p is 0.24382

	LL/TF	LR/TF	RL/TF	RR/TF
Mean	0.2397	0.2605	0.2385	0.2534
Stand. dev.	0.0495	0.0688	0.0509	0.0496

TABLE 17 - Results for Run Class CS I - E

LL	LR	RL	RR	# flies (TF) finished
32	18	10	20	80
23	19	12	19	73
33	15	10	13	71
18	7	4	22*	51
33	14	15	26	88
31	26	8	19*	84
38	11	21	25	95
28	6	5	25*	64
32	18	7	32	89
27	24	21	32*	104
295	158	113	233	

* runs removed for corrected table

Homogeneity Tests: i) All runs: G is 51.1133, p is 0.00425, 27 D. F.

ii) Corrected Table: G is 26.9873, p is 0.08425 for 18 D. F.

	LL/TF	LR/TF	RL/TF	RR/TF
Mean	0.3734	0.1945	0.1354	0.2967
Stand. dev.	0.0568	0.0675	0.0531	0.0771

When the overall totals for the status D maze runs are compared to the results for the status B maze in a contingency table (2 X 4), the value of p obtained is 0.0001, a highly significant difference. A similar comparison between the results of the status D and status A maze runs gives a p value of 0.530 .

These comparisons indicate that the distribution of trace substances in the maze path may be the factor determining the patterns of behaviour of flies run through it. By having flies go through all parts of the maze approximately equally before the run (status D), the behaviour of the group of flies run next is made similar to that observed for flies run through a freshly cleaned maze, and the apparent reason for this similarity in behaviour is that both the status A and status D maze conditions have any trace substances present equally distributed throughout the maze path. The fact that the status D maze would have much more trace substance in it than the status A maze does not appear to affect the behaviour being observed here.

The results for the status E maze runs are listed in Table 17. Status E refers to a maze which was prepared the same as a status D maze, and then the blind alleys in each maze path were cleaned (washed and dried with swabs) up to the point where they joined onto the intersections. The face plate was left untreated, since there was no way to mark out the sections to be cleaned on it without leaving extraneous cues for the flies.

Thus the status E maze would have trace substances in the blind alleys at a lower concentration and amount than in the maze main path. Comparisons of the totals for this class with those for the status D runs gave a p value of 0.0, as did comparisons between Status E and status A class totals (2 X 4 table). Comparisons with the combined results for classes CS I - B(1 to 4) gave a p value of 0.014, and the status E maze totals deviate significantly (p is 0.0) from a 1:1:1:1 distribution.

The pattern of the results (as shown by the mean distribution and comparison to 1:1:1:1) of the status E runs is similar to that seen for status B runs, and certainly much different from the pattern for the status A and status D results. This is a further indication that the distribution of trace substances in the maze is affecting the behaviour of flies running through it.

DISCUSSION

I) Treatment of Data: the data tables for each run class contain both the raw results for each experimental run in the class and the results of some simple statistical analyses done with these data. At the extreme right side of each table, in the column headed TF, are listed the numbers of flies which finished the maze in each individual run in the class. The number of flies completing the maze can be seen to vary considerably from run to run, which may indicate there was some variance in maze activity among different groups of flies and/or among individuals.

As previously mentioned (page 12 this thesis) the experimental system was designed to keep constant the various factors known to affect the flies' behaviour, however, there may still have been ambient changes in the maze and chamber microenvironments which could have led to differences in activity (eg. humidity in the maze path).

The variance in maze activity, and other behaviour to be considered later, may also be due to idiosyncratic differences among individuals, as observed in many animal behaviour experiments (Breland and Breland 1961) and, as noted in a case more relevant to this discussion, by Dethier (1974) in his experiments with blowflies, in which he observed a great deal of idiosyncrasy concerning the chemical response behavioural system of blow flies which had been prepered in an identical manner and which were genetically identical as were the Drosophila flies (within each strain) used in the experiments described in this thesis.

In order to obtain some idea of the pattern, if any, which the flies' behaviour in each run class was following, the data in the Total columns for each run were divided by the number of flies completing the run, thus expressing the number of flies which ended up in each collection vial as a proportion of the total number of flies which completed the maze. The mean and standard deviation of these proportions were found for each collection vial (LL to RR) in each class, with the results listed at the bottom of each data table under LL/TF etc.

The mean proportion distrubutions thus obtained for each class were used in the homogeneity tests. The first step in each homogeneity test involved running all of the data in the Total columns for that class in a contingency table test to find the values of the G statistic and p, the probability of the data constituting a homogeneous class. If a p value of less than 0.05 was obtained, the data were checked for runs which deviated obviously from the basic distribution pattern indicated by the mean proportion distribution for the class. The first runs removed were those which deviated asymmetrically from the basic pattern of the class - eg. for run class CS I - B(3) (Table 4 page 22) the basic pattern is one with more flies in the outer (LL, RR) than inner (LR, RL) collection vials, with approximately a 2:1:1:2 ratio of flies in the collection vials, and the second, fourth, and fifth runs in the table (marked by *) obviously deviate from this pattern.

If a contingency test of the data with the above described runs removed still gave a p value of less than 0.05, the "exagerrated pattern" runs were removed. These runs are the ones which show the same type of pattern as the basic class pattern - eg. for class CS I - B(3) more flies in the outer (LL, RR) collection vial than the inner (LR, RL) ones, but with an exagerrated concentration of flies in the outer vials - eg. run 8 in class CS I - B(3) has an approximately 4:1:1:4 collection vial distribution. The exagerrated pattern runs were removed one by one until a p value greater than 0.05 was obtained for contingency testing of the data in the "corrected table".

The totals for the data in the corrected tables thus obtained are given in the data tables for each class as the corrected totals. In all cases the corrected totals and the totals of all the runs in the class (overall totals in data tables) did not differ significantly; probably due to the fact that the percentage of runs removed was small (less than 25% in most cases).

For further tests of the data, the overall totals were used in all cases, but the runs which were removed to give the corrected homogeneity test tables were examined further to see how they were different from the rest of the runs in their respective classes. For the black strain, only the status B(1) maze run data (Run class b I-B(1), Table 12 page 35) showed any significantly aberrant runs, and these three removed runs show little apparent pattern among themselves, hence little may be said as to their origin.

For the Canton S strain run results in Experimental Series I, we see that these results indicate a high degree of homogeneity (p is 0.8) among the data for runs done in the status A maze (run class CS I-A, Table 1, page 19) and no runs had to be removed. Since the results for the four first run status B maze classes (run classes CS I-B(1) to CS I-B(4), Tables 2 - 6, pages 20 - 23) do not differ significantly from each other (page 27) they will be considered together. From a total of 56 status B runs in these four classes, 15 were removed for the corrected tables. Of these 15 runs, 4 showed the exaggerated class pattern previously described (runs 7, 8 in Table 2, 8 Table 4, 6 Table 5), 5 runs showed an asymmetrical distribution (runs 9 Table 2; 5, 6 Table 3; 5 Table 4; 5 Table 5), and 6 runs showed the same distribution pattern as observed for the status A run flies (runs 3, 4, 17 Table 2; 2, 5, Table 4; 3 Table 5).

The reasons for the occurance of the aberrant runs in the Canton S strain data and the behaviour which produced them may have been due to undetected changes in apparatus, or individual differences, as cited earlier in the discussion of the variation in the numbers of flies completing the maze each run, or the aberrant runs may have arisen due to variance in the factor which is responsible for the observed behaviour difference between flies run in the status A and status B mazes. This latter possibility will be discussed further once the class results have been interpreted in the section to follow.

II) Interpretation of Results - from the data derived from the experiments and the comparisons made with it in the Results section of this thesis the following facts emerge:

a) from Experimental Series I, the effects of maze status upon the behaviour of Canton S strain flies are as follows:

i) flies show the same behaviour at the initial choice turn regardless of whether the maze was freshly cleaned (status A maze) or had been exposed to flies since its last cleaning (status B mazes). The flies show an equal probability of turning left or right at the initial choice turn (page 29).

ii) flies show the same final choice point turning behaviour in a status B maze regardless of the number (up to 4) of groups of flies which had been run through the maze since its last cleaning (page 27).

iii) the final choice point turning behaviour observed for flies run under status A maze conditions differs significantly from that observed for flies run under status B maze conditions.

iv) the turn direction preference of the flies at the final choice points was not found to be significantly different from that which they displayed at the initial choice point for flies run in a status A maze. At both the initial and final choice points the ratio of the number of flies turning left and right did not deviate significantly from 1:1 (page 28).

v) flies run in a status B maze showed a significant alteration in turning behaviour at the final choice points compared to their behaviour at the initial choice point. At the final choice points the flies showed a preference for continued turning in the same direction they had been forced to turn in at the forced turns at each step during their ascent of the maze (page 28).

vi) the behaviour observed in the flies is dependant upon the maze status alone, within the limits tested for here (flies run twice). The same flies will show different behaviour when run under different maze status conditions (page 27).

b) from Experimental Series II which investigated the behaviour of the black strain flies under the same maze status conditions:

i) the turning behaviour at the initial choice point does not vary significantly with maze status for this strain (page 36), nor does it differ significantly from that observed for the Canton S strain (page 38).

ii) the turn choice behaviour of the flies was not found to vary significantly between the initial and final choice points for flies run either in status A or status B maze conditions, and while the totals obtained for the status B runs differ significantly from those obtained for the status A runs, this difference does not occur as a result of a change in turning behaviour at the final choice point as occurred in the Canton S status B maze run flies.

c) from Experimental Series III in which the maze status specific behaviour of the Canton S strain flies was investigated:

i) cleaning the final choice point in a status B maze (to give a status C maze) had no significant effect upon the final choice point turning behaviour of the flies, with the status B final collection vial distribution being preserved (pages 40, 41).

ii) flies run in a maze in which flies had previously travelled through all sections of the maze path approximately equally (status D) showed the same type of behaviour at the final choice point which was observed for flies run under status A maze conditions (page 44).

iii) flios run in a maze which was initially prepared as a status D maze and then had the blind alleys of the maze path cleaned showed a significant difference in turning behaviour between the initial and final choice points. (page 45)

III) Conclusions - the interpretations of the results of Experimental Series I lead to the conclusion that the Canton S flies behave differentl; when run in a status B maze than they do when they are run in a status A maze, due to an alteration in their turning behaviour which must occur at some point past the initial choice point during their trip up the maze.

Canton S flies which are run in any status maze have the same turning behaviour at the initial choice point, with no significant preference shown for turning in one direction (left or right) over the other. In the status A maze, the flies continue to show this type of turning behaviour at the final choice points, thus the status A maze conditions do not alter their behaviour during the course of the run, and the forced turns have no effect upon the flies as they pass through to alter the turn direction choice behaviour of the flies at the final choice points.

In the status B maze the flies show a definite and significant preference for turning, at the final choice points, in the same direction which they had been forced to turn in in their trip up the maze. This turn direction preference constitutes a large change in turning behaviour from that which the same flies displayed at the initial choice point, and the factors which caused this change must have taken effect after the flies had passed through the initial choice point, since the similarity of behaviour of flies at the initial choice point in both maze status conditions indicates that the flies behave the same as they enter the maze regardless of its status.

The questions which arise from these conclusions concern the possible reasons why the flies behaved differently in the status B maze at the final choice points than they behaved at the initial choice point or at the status A maze final choice points. Specifically, what factors present

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The questions which arise from these conclusions concern the possible reasons why the flies behaved differently in the status B maze at the final choice points than they behaved at the initial choice point or at the status A maze final choice points. Specifically, what factors present limits which could have been occurring here, does not affect the flies behaviour, although presumably a minimum amount is required before an effect is noted.

This may account for some of the aberrant runs (mentioned in the Treatment of Data section of the discussion) found in the status B run Canton S strain data, particularily those in the run class CS I-B(1) which showed a status A maze run type distribution, which may have occurred due to the previous group of flies leaving too little trace substance in the maze to affect the behaviour of those run later.

The effects of possible trace substances on the behaviour of Canton S strain flies in the maze were investigated in Experimental Series III. The first possibility explored was that the presence of traces at the final choice point was affecting the behaviour of status B maze run flies. The results for the status C maze runs show that there was no such effect, since the flies retained the Status B maze type behaviour even when the final choice point was thoroughly cleaned. This result was to be expected, since the behaviour observed at the initial choice turn was the same in both status A and status B maze runs, hence the potential presence of any trace substances did not affect turning behaviour at this point, so there was little reason to expect them to be responsible for the different behaviour observed at the final choice points.

From the facts which have emerged so far, it appears certain that, if some type of turning behaviour modification due to the effects of chemical traces is occurring in the Canton S flies run under Status B maze conditions, then this modification must be occurring in the maze path, between the initial and final choice points. The hypothesis to be tested here is one which predicts that a chemical trace pathway is set up in the maze path by the flies run in the status A maze which alters the behaviour of the flies in subsequent runs in the now status B maze. Specifically, there is more trace substance left in the main maze path (bypassing the blind alleys - see Fig. 4), and the flies use this trace pathway as a cue to allow them to discriminate between the possible alternates (turn directions left or right) at each turn point, eventually learning the turn sequence which allows them to continue up the maze.

The formation of this hypothesis was guided by three considerations:

i) the observation (as noted in General Observations page 30) that the flies enter the upper blind alleys less frequently when run in a status B maze compared to when they are run in a status A maze, hence they tend to stay in the main path, especially once they have passed the first two turn points. This indicates that the turning behaviour which they show at the final choice points is established earlier in the maze ascent, possibly due to discrimination learning which has led to proprioceptive control, a concept which will be discussed later.

ii) the observed difference in the degree of variability of the results from the Status A versus the status B maze runs for the Canton S strain. The average standard deviation (using the standard deviation as listed in the data tables and averaging over LL/TF to RR/TF) for run class CS I - A is 0.046 while that for classes CS I - B(1 to 4) is 0.078, with the average standard deviation being consistently higher for the status B first run classes (ranging from 0.069 to 0.082) than the status A first run class. Since the deviation due to individual differences in behaviour would be expected to be about the same for both class types there may be some other factor at work which is contributing to the increased variability of the flies' behaviour, and this factor may also be the one which is causing the previously noted differences in turning behaviour of flies run under status B maze conditions., If this factor is indeed some form of chemical trace, then the observed increase in variability in the presence of this trace substance in the status B maze may be due to individual variability in the behaviour of flies in response to it, and/or variability in the amount and pattern of distribution of the trace in the maze.

Since the variability of individual responses, while potentially very interesting, was beyond the scope of this study; and since the behaviour of the flies in the status B maze was not observed to vary significantly from the CS I - B(1) run class to the CS I - B(4) run class, the latter of which presumably had progressively higher amounts of trace substances present in the maze, the effects of possible changes in the pattern of distribution of trace substances were chosen for further experimental testing, as opposed to the effects of the overall amounts of such substances present in the maze. The experimental tests done were guided by the following consideration.

iii)since the turn choice behaviour of the Canton S flies run under status A maze conditions was found to be the same at the initial and final choice points, and they showed an equal probability of turning either left or right at these points, then it is reasonable to assume that they behaved in a similar manner at each turn choice point they encountered in the maze path. This being the case, then while ascending the status A maze, each fly had a probability of $\frac{1}{2}$ of turning into any particular blind alley, but whether it turned into any blind alleys or not, each fly which completed the maze had to follow the entire main path (Fig. 4), thus on the average all flies which completed the maze

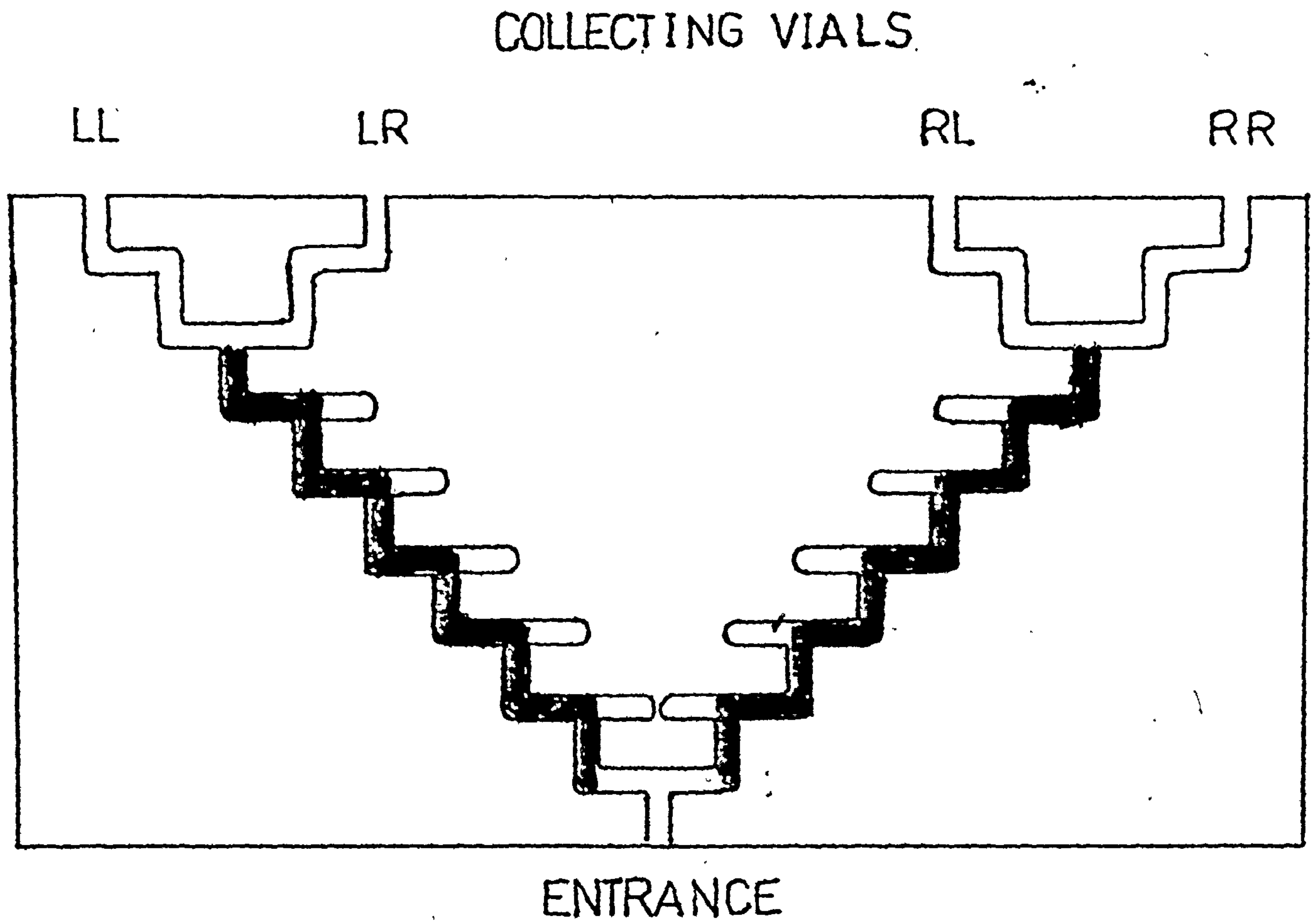

FIG.4 - Maze Path
(Main path marked in black)

traversed the main path, while half of them went into each blind alley. If we assume that the amount of trace substance left in any part of the maze is proportional to the number of flies which passed through it, then on the average there will be twice as much trace left in the main path as compared to the amount left in each blind alley, thus in subsequent status B maze runs, the maze path may be marked out as an olfactory highway, along which it would be possible to discriminate left from right at each turn point using olfactory cues.

Since the actual distribution of traces in the maze path would vary from run to run due to the observed variation in the status A maze run flies' behaviour, this may be the reason for the increase in the variance observed in the status B run results, if the trace distribution in the maze path had a specific effect upon the flies' behaviour.

In order to test whether the flies could actually have been using a trace pathway in the status B maze, experiments were done with the status D (page 42) and status E (page 44) maze conditions. The status D maze was prepared in a manner which ensured the approximately equal passage of flies through each part of the maze prior to the experimental run, thus there would have been no differential distribution of trace substances in the maze path, as was hypothesized to exist prior to the status B maze runs.

The results for the status D maze runs show that the Canton S strain flies behave similarily under these and status A maze conditions, with no change in turn choice behaviour occurring between the initial and final choice points. This fact indicates that the mere presence of trace substances in the maze is not sufficient to alter turning behaviour in the flies, implying that the distribution of these substances is the critical determinant of the behaviour change observed for flies run under status B maze conditions.

The final experimental test of this hypothesis was done with the status E maze runs. The status E maze was prepared the same as a status D maze and then the blind alleys were cleaned out (washed) in an an attempt to re-create the hypothetical status B maze trace pathway, with less trace substance in the blind alleys than in the main path. The results for the status E maze runs show a difference in turning behaviour at the final choice point from that seen at the initial choice point. The change in turning behaviour, while not identical to that observed for status B maze run flies, (page 45) is nevertheless of the same type, with the flies showing a clear and significant preference for continued turning in the direction of the forced turns, at the final choice points.

The difference in the results for the status B and status E maze runs may be due to the fact that the status E maze runs were done under conditions which were not identical to the status B maze conditions, since for the status E maze runs there was far less trace substance left in the blind alleys compared to the main path, with all of the trace in the blind alleys being on the face plate surface, which was not cleaned. The difference in trace substance distribution between the blind alleys and the main path would have been less in the status B maze, and the trace would have been distributed more evenly over all of the inner maze path surfaces of the blind alleys. The act of cleaning the blind alleys may also have disrupted the maze conditions to some degree, altering slightly the behaviour of the flies run through it in a unexpected fashion.

From the preceding discussion of the behaviour of the Canton S flies under various conditions in the maze, the conclusion emerges that, in order for the flies to change their turn choice behaviour from a type in which they have an equal probability of turning left or right to one in which they show a preference for turning in the direction of the forced turns which they have passed through on their way up the maze, they must be presented with a maze which contains traces left by flies run previously in the maze, and these traces must be distributed such that there is a different amount in the main path compared to the blind alleys, such that the two may be olfactorily distinguished.

The Canton S flies run in a status B maze were observed to move more frequently in the main path of the maze than in any other part (the blind alleys). This indicates that the distribution of traces in this path was affecting their behaviour. Experiments in which attempts were made to influence the flies' behaviour with a water soluble extract gave no progress, hence the exact nature of these trace substances remains unknown, although with both male and female flies present in each run, a likely candidate would be some type or types of sex pheromone.

There are two simple hypotheses which could be used to explain the behaviour observed for the Canton S strain flies run in status B maze conditions:

i) the flies move up the maze path and learn to use the differential trace substance distribution as a cue to discriminate between left and right, allowing them to associate these directions with blind alleys and the main path, and eventually they learn which direction to turn at each turn point to continue up the maze, with subsequent choices being oriented by the olfacory cues previously mentioned. After the flies have followed the "correct" turn sequence thus learned, their behaviour shifts from sensory, or exteroceptive, control to proprioceptive control, with the turning pattern being followed automatically; this explains the persistenc of the established turning pattern at the final choice points, since by the time the flies have reached these points, most of them are following this established sequence automatically. (ref. Mackintosh 1974, pg. 554)

ii) the flies follow the trace path, due to the attractant nature of the traces, with no actual discrimination learning occurring, just chemotaxis. By following the chemically marked out main path in this fashion, the flies may still have switched to proprioceptive control at the final choice point and thus they continued to follow the turn sequence established in the main path. This would produce results which may appear to indicate learning but are in fact due to pseudolearning, similar to that observed in lower organisms by Jensen (1964).

The resolution of the question of which, if either, of these hypotheses applies to the experiments of this thesis will require further experimentation, in which the distribution of trace substances within the maze will have to be varied to see if chemotaxis is affecting the flies' behaviour, and this type of experimentation will be better accomplished once the actual substances involved are isolated.

The experiments with the black strain flies (Expt. Series II) indicate that the status B maze behaviour observed for the Canton S flies has a genetic basis which is variable between different strains, since the mutant black strain has an entirely different status B maze behaviour pattern, with no significant turn direction preference shown at the final choice point. Neither strain shows any turn direction preference in the maze at the initial choice point nor at the status A maze final choice point, thus the only strain difference in maze behaviour occurs under status B maze conditions.

It appears, from the data and general observations (page 30) that the black strain flies do not follow the main path preferentially in the status B maze. A reason for this may be hinted at by the observed reluctance of the black strain flies to even enter a maze which has been previously occupied by flies of the same strain. (the mean number of flies completing each run dropped from 71.4 for status A maze runs to 56.4 for status B(2) maze runs, while most status B(3) runs attempted gave fewer than 30 flies completing the maze - page 33) This fact being known, we can see that the black strain flies might not be expected to show discrimination or pseudolearning due to chemotaxis in the status B maze, since the chemical traces left therein are unattractive to them and they may have been avoiding the main path. Another possibility is that the black flies are incapable of using the olfactory cues in discrimination learning in the status B maze.

Thus the observed strain differences in behaviour between the Canton S and black strain flies in the status B maze may be due to differing responses to traces left in the maze, differing discrimination learning ability, or other behavioural differences which are not immediately discernable. The observed similarity of the behaviour of the two strains under the status A maze conditions would tend to rule out such factors as maze activity and preparation artifacts as being responsible for the behavioural differences observed between the strains. This similarity in status A maze conditions also indicates that, for neither strain does discrimination learning using solely visual cues occur in the experimental system used here.

This elimination of the possiblity of discrimination learning using visual cues occurring for either strain in the maze used in these experiments is important when comparisons to previous learning experiments are made, particularily to those of Hay (1975), a discussion of which follows. As far as the experiments described in this thesis are concerned, the only type of learning which can be possibly said to have occurred is discrimination learning using olfactory and possibly visual cues, with the visual cues being insufficient by themselves to facilitate learning in the maze used.

IV) Comparisons to Previous Work

a) Hay's Experiments: as mentioned in the Introduction and elsewhere in this thesis, the experiments described herein are loosely based upon those of D. A. Hay (1975). There are, however, several important differences, as follows:

i) the strains used are different in both cases, with Hay using locally caught Australian strains, thus, although the flies used were the same age and were run in large groups of males and females together (200 flies/group in Hay's experiments) in both cases, strain differences in behaviour are certain to be observed within and between experiments.

ii) the maze used by Hay had a complex internal geometry, with flies taking as long as 45 minutes to move through one intersection, and the maze was mounted horizontally, with positive phototaxis towards a light mounted near the collection tubes being the only stimulus to flies moving slowly through the maze.

iii) Hay allowed 24 hours for each group of flies to move through the maze, as opposed to 5 minutes in the experiments of this thesis.

iv) Hay's maze had 6 forced turns with no blind alleys.

Hay observed a great deal of strain difference in maze behaviour in his experiments, and for several strains he obtained distributions of flies at the collection vials which had similar patterns to that observed for the Canton S strain status B maze runs in this thesis. Hay concluded that this distribution of flies favouring the outer collection vials "indicates that when presented with a choice, flies are more likely to continue the sequence of turns they were forced to adopt for the previous six turns. That is, they learn that this sequence of turns enables them to proceed through the maze towards the light, which presumably also acts as an orientation cue in their turning."

The associative learning hypothesized by Hay, although he does not name it precisely as such, is of the discrimination learning type, with the positive reinforcement for turning in the direction of the forced turn being continued progress up the maze, which allows the flies to respond to their taxic tendency to move towards the light. The flies explore the junctions as they move through the maze, eventually associating visual cues present at each turn point with a particular turn sequence which allows them to progress through the maze, and they continue to follow this sequence at the final choice point.

This conclusion was argued against by Bicker and Spatz (1976) who pointed out that similar collection vial distributions were obtained with a single forced turn maze for flies run in the light or dark, conditions under which associative learning of the type observed by Hay would not occur. Unfortunately, Bicker and Spatz used only one strain of Drosophila melanogaster, hence their results may be peculiar to this strain, as Hay points out in his rebuttal (Hay 1976).

Nevertheless, Bicker and Spatz point out that what appears at first to be learning behaviour may actually be "correcting behaviour" of the type noted by Dingle (1965) with various Heteroptera, where the insects, after being forced to turn in one direction, will proceed to turn in the opposite direction at the next opportunity. This type of behaviour may have occurred in Hay's experiments, for example, flies coming to the final choice point in the left hand maze path have just turned to their right (use Fig. 4 and imagine the maze lying horizontally), hence they might be expected to turn left at the choice point to correct for their previous turn.

This type of explanation, as well as others which hypothesize similar results due to stampede effects (Quinn, Harris, Benzer 1974), wall hugging, and centrifugal swing (Jensen 1959) (Murphey 1965, Walton 1968) cannot be causing the behaviour observed for the status B maze run Canton S flies in the experiments described in this thesis, since in all cases the factors involved would have been operating in the status A maze as well, where no deviations in behaviour were observed.

At first glance, the results from the status B maze Canton S strain runs described in my experiments and the results of Bicker and Spatz' experiments appear to indicate learning behaviour of the type described by Hay, however Bicker and Spatz' results show that Hay's results may be caused by pseudolearning which is a byproduct of correction behaviour. The status B maze run Canton S strain results in my experiments, however, cannot be explained as pseudolearning in the same manner, although pseudolearning as a result of chemotaxis cannot be ruled out.

If we accept the idea that learning is actually taking place in the experiments described in this thesis, then the results indicate that the Canton S strain flies show discrimination learning only when presented with olfactory as well as visual cues in the maze, as opposed to the strains used by Hay in his experiments, which, he assumes, learn using visual cues alone. My experiments indicate that there may in fact be more cues present in the maze than Hay and other maze workers suspect, and these cues may also be affecting the behaviour of flies run in their mazes, as we shall see in the following section which compares the maze experiments of this thesis with those of other workers.

b) Comparisons to Maze Experiments with Drosophila in General:

Comparisons of the experiments of this thesis to any other experiments with Drosophila in multi-step mazes must be strongly tempered by two considerations:

i) strain differences in behaviour, as we have seen previously, are abundant and large, hence differing results may be explained on this basis.

ii) the behaviour of Drosophila has been observed to vary greatly under different experimental conditions. A simple taxic behaviour such as phototaxis has been found to vary greatly depending upon the apparatus used to measure it, treatment, and condition of the flies (Benzer 1967, Markow and Scavarda 1977, Hadler 1964b) thus a behaviour like learning, which presumably involves a higher level of neural complexity since it depends upon memory and association, may be expected to vary as much as or more than taxic behaviour under different experimental conditions.

Given these restrictions, a basic statement can be made concerning comparisons of my experiments with those of Hirsch(1953) and Hadler (1964b which were involved with measuring phototaxis, and the further experiments of Hirsch (1959) measuring geotaxis of D. melanogaster as well as with those previously mentioned (Bicker & Spatz, and Hay). While the maze experiments of these workers allowed from 12 hours to over 24 hours for each experimental run, my experiments allowed 5 minutes per run, and thus may be termed "fast" maze experiments.

The use of the fast maze system allowed detection of the effects of transit of groups of flies through the maze upon the behaviour of those following afterwards (status A vs. status B runs). This maze status specific behaviour alteration was not tested for in the experiments of the other workers listed, since they cleaned their mazes before each run, but status-specific behaviour may still have been occurring in these experiments, since the first flies through their mazes may have altered the maze status sufficiently to affect the behaviour of flies following later in the hours-long experimental runs. This fact may have significant bearing upon the interpretations of the results of these "slow" maze experiments.

In conlusion, the fast maze type experiments with D. melanogaster as described in this thesis are useful in discovering aspects of behaviour which are not observable as discrete phenomena in other multi-step maze experiments done with this species. Specifically, the results indicate that for the potential learning situation used in these experiments, olfactory cues are necessary for the Canton S strain flies to either discriminate between alternatives in the maze path and learn a specific turn direction sequence, or show pseudolearning due to chemotaxis. In general, the results obtained here raise the question of the nature and possible existance of discrimination learning in Drosophila melanogaster, and what variables, experimental and genetic, affect this behaviour.

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