GENETICS AND EVOLUTION

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Biology Lab III

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Atul Singh Arora: *Genetics and Evolution,* Biology Lab III, *Every honest researcher I know admits he's just a professional amateur. He's doing whatever he's doing for the first time. That makes him an amateur. He has sense enough to know that he's going to have a lot of trouble, so that makes him a professional.*

— Charles F. Kettering (1876-1958) (Holder of 186 patents)

ACKNOWLEDGEMENTS

I express my sincere gratitude to our instructors, Dr. N. G. Prasad and Dr. Rajesh Ramachandran, for not only describing the experiments and their science in depth, but also for teaching us the art of observing, that of performing experiments, so we can appreciate the intellect behind the procedures, rather than mindlessly following them.

I also thank Vivek Sagar (MS11017) for his contribution to this report as my lab-partner, who made the task of performing experiments immensely comfortable and productive at the same time.

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INTRODUCTORY SESSION

1.1 OBJECTIVE

To learn the art of observation and thereby analyse the morphological features of the wild type and mutant Drosophila melanogaster.

1.2 THEORY

1.2.1 *Morphology*

Morphology is the study of form and structure of organisms.

Drosophila melanogaster is a small fly. It has two red coloured compound eyes, made up of 700-800 hexagonal units. It has two translucent wings, and a pair of halteres. It has a hairy body. It also has a pair of antennas. The abdomen is striped with visible differences between males and females.

1.2.2 *Life Cycle*

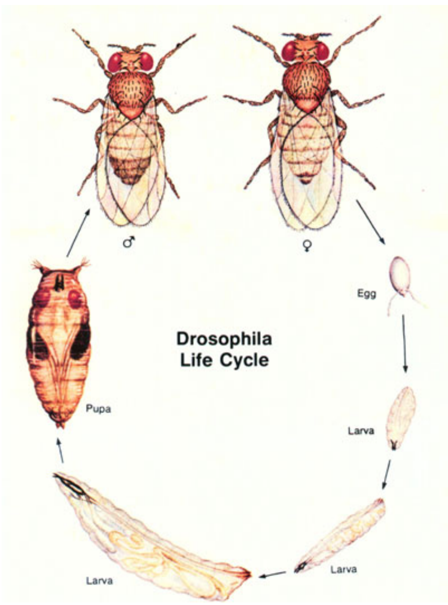


Figure 1: Drosophila Life Cycle [?]

1. Embryo: The first stage is from the fertilization of the egg, till the embryo hatches (under ideal conditions (at 25 °C), takes 12-

16 hours), inside the mother. The insect starts as a single cell, and then develops into the larval form before it hatches.

2. Larva: The second stage is from birth until the larva pupates. This stage is generally worm-like. It grows for about 4 days while *molting* twice (into 2nd- and 3rd-instar larvae), at about 24 and 48 h after hatching. They feed on the micro-organisms that decompose the fruit, as well as on the sugar of the fruit itself, during this period.

3. Pupa: this is the third stage, from pupation till eclosion. This stage is marked by reduced movement and often sealed with a cocoon. The metamorphosis takes about 4 days.

4. Imago: In this stage, the holometabolous insects are adults and usually have wings and functioning reproductive organs.

Holometabolism: This term is used to describe the specific kind of insect development which includes all four life stages.

Holometabolic development gives the offspring a very unique advantage of not being forced to compete with the adults since they inhabit different ecological niches due to the morphological differences in the different stages of their life cycle.

1.2.3 *Difference between Males and Females*

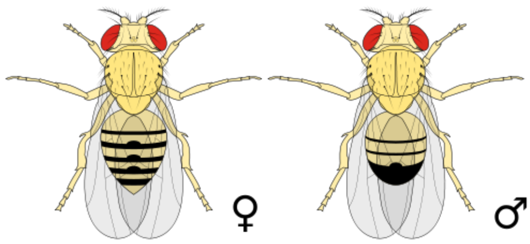


Figure 2: Male and Female Drosophila

1. Females have a shorter life compared to males.

2. On an average, females are larger than males (although not necessarily individually true)

3. Males have a larger portion of their back black compared to females. However, this distinction is not very clear until they're mature.

4. Males have sex-comb which is the most reliable distinguishing feature amongst males and females. It is present in the first leg.

1.3 EXPERIMENT

We were divided into groups of three and each group was given a vial containing over 30 Drosophila melanogaster. Our objective was to analyse them under a stereo microscope and study their morphology within 45 minutes.

My team consisted of Vivek Sagar (MS11017), Biplob Nandy (MS11004) and I (MS11003). The issue at hand was to focus a moving organism with the microscope. We came up with the following solution.

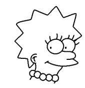
a. Locate a cylindrical object with a small diameter.

b. Use the object to push the cotton away from the walls of the container, while gradually moving into the container.

c. Continue the process till a fly gets into the gap between cotton and the vial wall, upon occurrence of which, release the cotton to firmly hold the fly.

d. This ensures that the fly has very restricted movements and is still alive.

This was followed by a discussion about the observations, after which we were told how to put the flies to "sleep", or more precisely, anaesthetising them. The method was straight forward. It involved the use of ether, which inhibits neurological pathways in drosophila. The protocol followed was:



After the flies recover their conciousness, their behaviour ceases to be normal.

a. Locate a funnel. At the terminal part of its conical region, attach a cotton ring.

b. Add a few drops of the anaesthesia to the cotton.

c. Now take an empty vial and place the funnel on its mouth, covering it completely.

d. Locate the vial which contains the drosophila desired to be anaesthetised.

e. Remove the cotton plug and instantly place the mouth of the vial on the funnel

f. The drosophila will fall through the funnel into the empty vial, unconscious.

g. Remove the funnel after a suitable duration.

One such unconscious fly's front leg was taken and focussed under a high power microscope and observed.

And lastly, Mutants were setup for viewing under stereo microscopes and we were asked to observe them.

1.4 OBSERVATIONS

1.4.1 *Coarse Focus*

1. Flies were of different size.
2. There were 3 pairs of legs.
3. All of them had Red coloured eyes.
4. All had their abdomen striped with Yellow Brown and Black
5. In most, there were 5 stripes.

1.4.2 *Fine Focus*

Observations of a particular fly

1. 2 hair like protrusions from the head were observed. Most likely they were antennas.
2. There were only 2 pairs of wings.
3. Back colour was Yellowish Brown
4. The body was shiny and globular.

Observations of a different fly

1. Hair like projections were visible on all three legs.
2. Abdomen was white in colour.
3. Halteres were observed.

Observations of yet another fly

1. Most of the body had black coloured hair, including the face.
2. Legs had a hook like structure
3. It seemed to be releasing a black shiny liquid
4. Lines in the wings were distinctly visible (later told to be veins)
5. Hexagonal eyes were visible. Could see the hexagonal elements.
6. Could see a slightly darker circle in the eye (later told to be sensory nerves)

Non-microscopic Observations

1. The flies try to run *away* from gravity.
2. The flies run *towards* light.

1.4.3 *High Power Microscope*

The sex comb was explicitly visible in the front leg.

1.4.4 *Mutants*

1. Barred eyes
2. Eye Colour
   a) White
   b) Orange
   c) Brown
3. Curly Wings
4. Gray and Yellow Body

1.5 ACKNOWLEDGEMENTS

I thank both my team members, Biplob Nandy (MS11004) and Vivek Sagar (MS11017) for their contribution to the performance of the experiment.

2 UNDERSTANDING GENETICS USING DROSOPHILA MELANOGASTER

2.1 OBJECTIVE

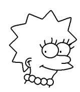
To understand how the genes for eye colour are inherited in Drosophila, by setting up various crosses of the pure breeds which have only.

2.2 MATERIAL REQUIRED

1. Virgin Drosophila (Wild type, Scarlet and Red eyed)
2. Wild type
3. Scarlet
4. White
5. Vials containing food
6. Empty Vials
7. Cotton plugs
8. Ice box and Ice pack
9. Paint Brush
10. Binocular Microscope
11. Needles
12. Tissue Paper
13. Yeast as extra food
14. Ether as anaesthesia
15. Funnel

2.3 IDEA

The wild type (the "normal") of drosophila has Red eye colour. We also have a bunch of mutants. In this experiment we'll use White and Scarlet eye coloured mutants. We take their pure breeds and do the crosses as listed in Table 1. For the actual experiment, we use 2 females and 2 males for each type. Also, we do the same cross thrice to be moderately rigorous.



Pure Breed: When bread amongst themselves, they yield the same phenotype

Red (wild type) Male	White Female
Red (wild type) Female	White Male
Red (wild type) Male	Scarlet Female
Red (wild type) Female	Scarlet Male

Table 1: F1 Crosses

We then observe their phenotype (eye colour) in the generation obtained, lets call them F1. We then note that in F1, for a given sex, there's only one phenotype. We count them. Then we self them and again observe their phenotype. (although this time its more painful, since we've to observe males females and their phenotypes, so no running away from the microscope for amatures like us!)

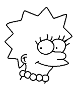
The objective is to investigate the mechanism by which genes are passed from one generation to another and since this experiment got Morgen the Nobel, therefore its rather obvious that there's more to it than the typical mono-hybrid cross.

2.4 PROCEDURE ESSENTIALS

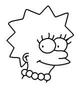
There is not much significance of re-writing the detailed sequential procedure. The repetitive modules, interesting, unexpected steps have been listed here.

1. When labels were put on the vials that were intended to kept in the freezer, they were taped. Sounds trivial but was very essential.

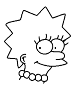
2. The method of setting up a cross:
   a) Took the vials, dried them and cooled them using ice.
   b) Then transferred the flies into these vials, put the cotton plug swiftly and put it back for cooling (for retaining the low temperature)
   c) When the flies become inactive, put them on an icepack with a tissue paper on top, which doesn't have air bubbles.
   d) Then selected the required males and females with the desired phenotypes, by observing them under a microscope if the need be, and transferred them into a new food vial, and plugged the cotton.
   e) Kept the food vial horizontal until the flies start moving and then restored the vials to a vertical position.
   f) Labelled the vial accordingly and stored it at 25^0C, in an incubator and waited for about 36 hours.



We dry them cause else the moisture condenses and that's not good for the flies

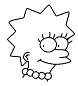


The air bubbles will be at a higher temperature!



To prevent the unconscious flies from getting stuck on the vials

g) Checked if there were enough flies. If not, waited for another 12 hours. Then the parent flies were discarded in a soap solution.



To prevent overcrowding of eggs.

h) Periodically, the new flies were transferred into new vials.

3. We used an improvised method for keeping the flies anaesthetized as listed below:

How does overcrowding make a difference here

   a) Took a petridish and filled it with crushed ice to the brim, without closing the lid.
   b) Using an aluminium foil, covered the surface of the petridish.
   c) Put a tissue paper on it

2.5 OBSERVATIONS AND ANALYSIS

Observations in this experiment are restricted to the number of flies of specific eye colour and sex, in a given generation. As described earlier, F1 is the first generation and F2 is the second generation. For various crosses, the observations have been summarized as below:

1. Cross 1: Scarlet Male with Red Female
   a) F1: Table 2
   b) F2: Table 3
2. Cross 2: Scarlet Male with Red Female
   a) F1: Table 4
   b) F2: Table 5
3. Cross 3: White Male with Red Female
   a) F1: Table 6
   b) F2: Table 7 [1]
4. Cross 4: Red Male with White Female
   a) F1: Table 8
   b) F2: Table 9

For analysing the data, we used what's known as a χ^2 test. The mathematical analysis has been appended, in accordance to which, our experiment confirmed the expected hypothesis[2]. For Cross 1 and 2, with parents as Scarlet and Red, the hypothesis that Scarlet Eye colour is a typical single locus, recessive trait was verified. Quantitatively, Cross 1 was found to have $\chi^2 = 0.0$, which was rather co-incidental , and for Cross 2, χ^2 was found to be 0.31 which is less than 3.148, the value

Don't think we performed the experiment really well!

1 total's excluding Vial 1

2 However, we did reject data collected from one vial in Cross 3, as it had been contaminated

F1		RED MALE	RED FEMALE
	VIAL 1	57	63
	VIAL 2	32	27
	VIAL 3	28	40
	TOTAL	117	130

Table 2: Cross 1: F1 Crosses (Scarlet Male with Red Female)

F2	RED MALE	RED FEMALE	SCARLET MALE	SCARLET FEMALE
VIAL 1	41	55	18	12
VIAL 2	45	58	21	14
VIAL 3	42	59	9	26
TOTAL	Red	300	Scarlet	100

Table 3: Cross 1: F2 Crosses (Red (F1) Male with Red (F1) Female)

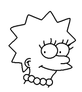
corresponding to 5% and single degree of freedom.
Now for the more interesting ones, Cross 3 and Cross 4, were subjected to two tests. First we assumed a null hypothesis similar to that of the first case, viz. White Eye colour is a typical single locus, recessive trait. For this, Cross 3 yielded $\chi^2 > 12.9$. Cross 4 produced results distinctly in contrast with the null hypothesis:

1. A significant number of White Female flies were observed which according to the hypothesis should *not* be observed at all

2. *No* Red Females were observed, which according to the hypothesis should constitute *half* the progeny.

This confirmed that the inheritance of White Eye colour can't be explained by the hypothesis.

The other null hypothesis was that the White Eye colour trait is recessive and sits on the X chromosome. According to this hypothesis, χ^2 was found to be 0.601 and 1.91 for Cross 3 and 4 respectively. Since both these numbers were found to be less than 3.841, the value corresponding to, again 5% and single degree of freedom, the null hypothesis satisfactorily explains inheritance of the White Eye colour.



Degree of freedom in this case was $(n-1)(n-1) = 1$, *for* $n = 2$

2.6 DISCUSSION

So far so good, but here's the catch; if there's a locus for eye colour on the autosome, *and* there's a locus for eye colour on the X chromosome, what happens when you attempt to cross a Scarlet with a White?

We could perform a experiments and find out. Others who've already performed and analysed them, explain the phenomenon as the

F1		RED MALE	RED FEMALE
	VIAL 1	47	47
	VIAL 2	45	56
	VIAL 3	68	73
	TOTAL	160	176

Table 4: Cross 2: F1 Crosses (Red Male with Scarlet Female)

F2	RED MALE	RED FEMALE	SCARLET MALE	SCARLET FEMALE
VIAL 1	23	50	13	14
VIAL 2	42	36	19	13
VIAL 3	24	27	5	9
TOTAL	Red	202	Scarlet	73

Table 5: Cross 2: F2 Crosses (Red (F1) Male with Red (F1) Female)

F1		RED MALE	RED FEMALE
	VIAL 1	96	74
	VIAL 2	76	102
	VIAL 3	98	72
	TOTAL	117	130

Table 6: C: F1 Crosses (White Male with Red Female)

F2	WHITE MALE	WHITE FEMALE	RED MALE	RED FEMALE
VIAL 1	11	16	7	16
VIAL 2	28	60	18	0
VIAL 3	11	34	22	0
TOTAL	39	94	40	0

Table 7: C: F2 Crosses (Red (F1) Male with Red (F1) Female)

F1		WHITE MALE	RED FEMALE
	VIAL 1	85	70
	VIAL 2	62	68
	VIAL 3	54	75
	TOTAL	201	213

Table 8: Cross 4: F1 Crosses (Red Male with White Female)

F2	RED MALE	RED FEMALE	WHITE MALE	WHITE FEMALE
VIAL 1	15	22	11	21
VIAL 2	17	14	12	12
VIAL 3	24	34	39	37
TOTAL	Red	300	White	100

Table 9: Cross 4: F2 Crosses (Red (F1) Male with Red (F1) Female)

following: the eye colour, is controlled by a two pathways. When both pathways are functional, the wild type, Red eye colour is obtained. When both are non-functional, then White eye colour is obtained. The other two cases result in Brown and Scarlet, as is given in Figure 3.

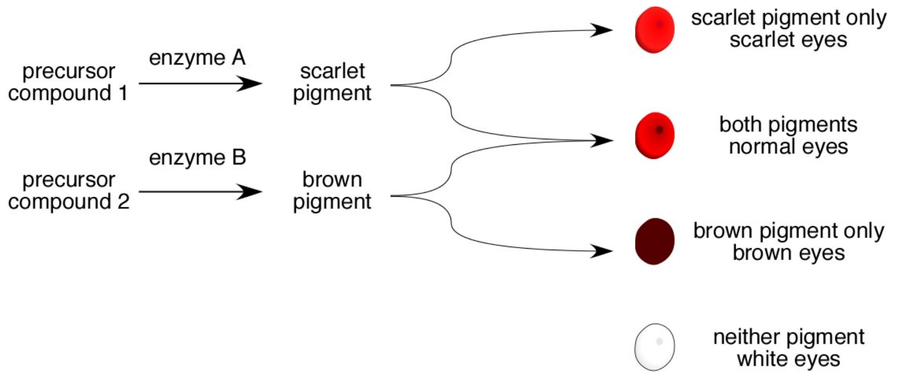


Figure 3: Drosophila eye colour

2.7 REFERENCE

http://www.indiana.edu/~oso/lessons/Genetics/bw_st.html

2.8 ACKNOWLEDGEMENT

I would like to sincerely thank Mr. Biplob Nandy, who helped us perform the experiment as a team member. I also acknowledge Vivek Sagar for his contribution to the project, as a team member. I thank our instructor, Dr. N. G. Prasad for his expert guidance and novel teaching methods.

Part II

THE SHOWCASE

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COLOPHON

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The style was inspired by Robert Bringhurst's seminal book on typography "*The Elements of Typographic Style*".

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https://github.com/toAtulArora/IISER_repo