EFFECTS OF A HIGH SUGAR DIET AND LOW SUGAR DIET ON DROSOPHILA MELANOGASTER

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Aim

To study the effects of a high sugar diet and low sugar diet on *Drosophila melanogaster*

Introduction

1) Importance of drosophila melanogaster in today's world

Drosophila melanogaster or commonly known as fruit fly is the basis of modern genetics. It has been the basis of six Nobel prices till now:

- **1933** -Thomas Hunt Morgan used drosophila to uncover the role played by chromosomes in heredity
- **1946-** Hermann Joseph Muller used X-ray irradiation to increase mutation rates in fruit flies
- **1995** -Edward B Lewis, Christiane Nüsslein-Volhard, and Eric F Wieschaus used drosophila to understand genetic control of embryonic development
- **2004-** Richard Axel concentrated on odour receptors and the organisation of the olfactory system
- **2011 -**Jules A Hoffmann was given the award for his research on the activation of innate immunity
- **2017-** Jeffrey C Hall, Michael Rosbash and Michael W Young won the prize for uncovering the molecular mechanisms that control circadian rhythms

Fruit flies is used as a model organism in genetic research due to various reasons:

- Fruit flies, it transpires, have common features with humans to a remarkable degree (we share 60% of the same DNA). Today, scientists believe that about 75% of known human disease genes have a recognisable match in fruit flies. These include Down's, Alzheimer's, autism, diabetes and cancers of all types.
- The drosophila genome has only four pairs of chromosomes. Humans have 23. That means it is much easier to manipulate and study the function of genes and understand how they interact. In addition, fruit fly genes have very high mutation rates and that makes them very useful to study.
    - **Eg:** Institute of Cancer Research: "There is a biological process known as cell death which occurs in all living creatures – from worms to humans," said Professor Pascal Meier, head of the cell death and inflammation laboratory in the institute's breast cancer unit. "Essentially, it is a suicide mechanism that is fundamental to life, and key aspects were discovered first in fruit flies. When a cell becomes damaged, its death – a process known as apoptosis – is triggered and it is killed off, allowing organs to remain healthy." In cancers, however, cell deaths are not triggered, and this aids the process by which tumours spread. "We now know, from fruit fly research, that molecules known as inhibitors of apoptosis proteins (IAPs) are involved," said Meier. "They act to block cell deaths. In other words, if levels of IAPs are elevated, apoptosis is prevented and cancers spread."

- It has a relatively short life cycle of only 12 days

2) Morgan Detects an Unusual Pattern of Inheritance

- Morgan observed one day that instead of the typical red eyed fruit fly, the male, being observed had white eyes.
- He first performed a test cross between a white-eyed male fly and several purebred, red-eyed females to see whether white eyes might also occur in the next generation. The members of the resulting F_1 generation had all red eyes, but Morgan suspected that the white-eye trait was still present yet unexpressed in this hybrid generation, like a recessive trait would be. To test this idea, Morgan then crossed males (♂) and females (♀) from the F_1 generation to probe for a pattern of white eye reoccurrence. Upon doing so, he observed a 3:1 ratio of red eyes to white eyes in the F_2 generation. This result is very similar to those reported for breeding experiments for recessive traits, as first shown by Mendel. Strangely, however, all of Morgan's white-eyed F_2 flies were males , just like their grandfather—there were no white-eyed females at all. Correlation of a nonsexual trait with male or female identity had never been observed before.
- ***Expected Mendelian Ratios versus Morgan's Actual Results***

Cross	**Outcome**	
	Expected Phenotypes	**Observed Phenotypes**
P_1 Red ♀ × P_1 White ♂	F_1 = All Red	F_1 = All Red*
F_1 Red ♀ × F_1 Red ♂	75% Red ♀ and ♂ 25% White ♀ and ♂	50% Red ♀ 25% Red ♂ 25% White ♂

**Morgan did observe 3 white-eyed males in the F_1 generation. His original paper suggested that these white-eyed males were evidence of "further sporting."*

- Morgan was curious as to why female flies never had white eyes, and he considered several possible reasons for this phenomenon. One potential explanation was that white-eyed females never hatched, or that they died early in development. In other words, this hypothesis predicted that white eyes were lethal in female flies—therefore, among the progeny of a test cross of heterozygous (F_1) red-eyed females to white-eyed males, there should be no white-eyed females. Morgan conducted this very cross to see whether the results matched his predictions. Surprisingly, this cross yielded a 1:1:1:1 ratio of red-eyed females to white-eyed females to red-eyed males to white-eyed males. Based on these results, Morgan arrived at three conclusions:
  1) The appearance of white eyes in females shows that this trait is not lethal in females.
  2) All possible combinations of white eyes and sex are possible.
  3) The white-eye trait can be carried over to females when F_1 females are crossed with white-eyed males.

- Morgan knew of recent work by Nettie Stevens and E. B. Wilson that demonstrated that sex determination was related to the inheritance of an "accessory chromosome," more recently known as the X chromosome. He further recognized that the inheritance of the sex determination chromosomes in *Drosophila* seemed to follow closely with the inheritance of the white-eye phenotype.

Morgan's Test Crosses

- If eye colour is inherited along with the X chromosome, then it can be denoted as a linked trait by tagging the X chromosome with a symbol, as follows:

X^+ = Red-eye trait (wild type)
X^w = White-eye trait

- In his initial test cross aimed at exploring the precise relationship between eye colour and sex, Morgan bred white-eyed males (X^wY) with wild-type red-eyed females (X^+X^+). This cross yielded only red-eyed offspring.
- ***Morgan's First Test Cross***

		Male Gametes	
		X^w	Y
Female Gametes	X^+	X^+X^w	X^+Y
	X^+	X^+X^w	X^+Y

- Next, Morgan decided to cross two flies from the F_1 generation—specifically, a red-eyed female (X^+X^w) and a red-eyed male (X^+Y)—to test for a recessive pattern of inheritance.
- ***Morgan's Second Test Cross***

		Male Gametes	
		X^+	Y
Female Gametes	X^+	X^+X^+	X^+Y
	X^w	X^+X^w	X^wY

- As shown in the table, the offspring of this cross exhibited a 3:1 ratio of red eyes to white eyes, which indicated that white eyes were recessive. Moreover, all of the white-eyed F_2 offspring were male.
- Next, Morgan conducted a third cross to determine whether white eyes were lethal in female flies. Here, he bred red-eyed females (X^+X^w) with white-eyed males (X^wY).
- ***Morgan's Third Test Cross***

		Male Gametes	
		X^w	Y
Female Gametes	X^+	X^+X^w	X^+Y
	X^w	X^wX^w	X^wY

- This third cross revealed that white eyes were in fact not lethal in females, because it produced a 1:1:1:1 ratio of red-eyed females to white-eyed females to red-eyed males to white-eyed males.
- Finally, Morgan opted to conduct a fourth cross to determine whether the white-eye trait followed the inheritance of the X chromosome from maternal gametes to male offspring. This reciprocal F_1 cross was the most crucial part of this series of experiments, because Morgan could make some very concrete predictions if the trait was indeed sex-linked. Specifically, because the white-eyed trait appeared to be recessive, Morgan could predict that a white-eyed female would probably be homozygous recessive. Moreover, because males inherit their only X chromosome from their mother, the use of a white-eyed mother would mean that an X-linked white-eyed trait would be the only trait male flies could inherit from a homozygous mother. Thus, Morgan could predict that all male offspring resulting from a cross between a white-eyed female and a red-eyed male would be white eyed. Likewise, because female offspring inherit the only X chromosome that exists in the paternal gametes, all female offspring of this particular cross would carry the red-eye trait, and this trait would mask the recessive white-eye trait they inherited via the maternal gametes.
- To test these predictions, Morgan crossed a white-eyed female with a red-eyed male.
- ***Morgan's Fourth Test Cross***

		Male Gametes	
		X^+	Y
Female Gametes	X^w	X^+X^w	X^wY
	X^w	X^+X^w	X^wY

- Because this cross yielded all white-eyed males and all red-eyed females, Morgan could indeed conclude that the white-eye trait followed a sex-linked pattern of inheritance.

The Context of Morgan's Discovery

- Morgan's conclusion—that the white-eye trait followed patterns of sex chromosome inheritance—was at once very specific and very grand. A few years prior to these test crosses, Mendelian ideas of inheritance had been enthusiastically discussed by many researchers in the context of new findings about chromosomes. Indeed, after observing meiotic reductive divisions and correlating them to chromosome counts in male and female offspring, cytologists Walter Sutton, Nettie Stevens, and E. B. Wilson had all promoted the idea that sex was determined via chromosome-based inheritance. Morgan, however, had long resisted the idea that genes resided on chromosomes, because he did not approve of scientific data acquired by passive observation.
- Morgan set out to test the idea of inherited chromosomal factors using *Drosophila*. Because Morgan was particularly interested in experiments designed to test hypotheses, he turned to the fly system to maximize data acquisition over short periods of time. Soon after launching these experiments, Morgan saw his white-eyed fly peering back at him through his hand lens. Then, many crosses later, Morgan became convinced by his own empirical evidence that traits could in fact be passed on

in the same manner predicted by the inheritance of sex chromosomes. For his work with *Drosophila*, Morgan was awarded the Nobel Prize in 1933.

3) Classification

Kingdom - Animalia
Phylum – Arthropoda
- Hard exoskeleton
- Segmented with jointed appendages
- Bilateral symmetry
- Open circulatory system

Class – Insecta
- 3 pairs of jointed legs
- 2 pairs of wings in adults
- 3 body regions: head, thorax, abdomen

Subclass – Pterygota
Division – Endopterygota
Order – Diptera
- Membranous forewings
- Reduced hind legs as halters
- Enlarged thorax

Family – Drosophilidae
Genus – Drosophila
Species - melanogaster

Life cycle of fruit fly

- **Egg**
  The egg is ovoid, covered outside with a thin but strong envelope (chorion) from which project anteriorly two thin stalks whose terminal portions are each flattened into a spoon-like float. The latter serve as "water-wings" to prevent the egg from sinking and drowning in a semiliquid medium. At the anterior end of the egg is a minute pore (micropile) through which the spermatozoa enter the egg as it passes down the oviduct into the uterus. Although many sperm may enter the egg as it passes down the oviduct, only one fertilizes the female pronucleus and the others are soon absorbed in the developing embryonic tissue.

- **Larva**
  The larva is a white, segmented, worm-shaped burrower with black mouth parts (jaw hooks) in the narrower head region. For tracheal breathing it has a pair of spiracles (air intakes) at both the anterior and posterior ends. Since insect skin will not stretch, the young small larvae must periodically shed their skins (cuticle) in order to reach adult size. There are two such molts in *Drosophila* larval development that are accompanied by shedding of the mouth parts as well as the skins. During each period between molts, the larva is called an instar (there are three instars in total), i.e. the first instar is between hatching and the first molt. Both the size of the larva and the number of teeth on the dark coloured jaw hooks are an indication of which instar the larva has reached. After the second molt, the larva (now third instar) feed until ready to pupate. At this stage, the larva crawls out of the food medium onto a relatively dry place, ceases moving, and everts its anterior breathing spiracles.

- **Pupa**

Soon after everting its anterior spiracles, the larval body shortens and the cuticle becomes hardened and pigmented. A headless and wingless pre-pupa forms. This stage is followed by the formation of the pupa with everted head, wing pads, and legs. The puparium (outer case of the pupa) thus utilizes the cuticle of the third larval instar. The adult structures that seem to appear first during the pupal period have actually been present as small areas of dormant tissues as far back as the embryonic stage. These localized pre-adult tissues are called anlagen (or imaginal discs) and because of the ease in which they can be isolated have often been used in studies of developmental genetics. The main function of the pupa is to permit development of the anlagen to adult proportions.

- **Adult**

After forcing their way through the puparia, adults are light in colour and have elongated wings and abdomen. Within a few hours new adults darken.
Adults exhibit a typical insect anatomy, including compound eyes, three-part bodies (head, thorax, and abdomen), wings, and six jointed legs. The adult fruit fly is yellow-brown in colour and is 3mm long and 2mm wide. They have a rounded head with large red/white compact eyes and 2 antennae.

MALE ADULT

- Males have dark, rounded genitalia at the tip of their abdomen
- Male Drosophila are generally smaller than their female counterparts, and have a darker abdomen.
- The posterior segments of the Drosophila are almost completely pigmented in the males
- The males also have a unique characteristic on their forelegs; the sex comb. The sex comb is a small patch of bristles visible on the forelegs of the male and is perhaps the most reliable method of sexing the flies, but may be too time consuming in practice.

FEMALE ADULT

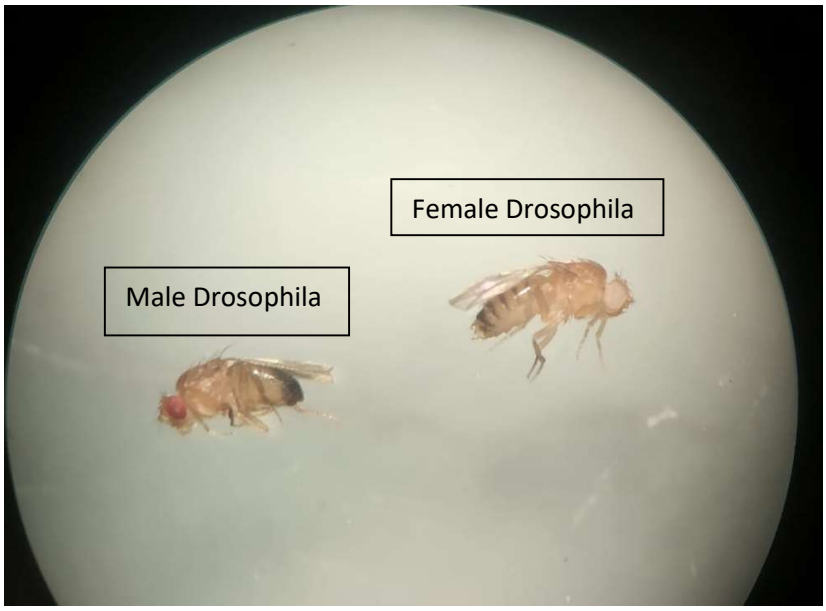
Female flies are unable to mate for several hours after they have eclosed as adults from their pupal cases. The very young, newly eclosed, females can have their wings folded and the abdomen may not have inflated yet.

1) Mature Adult Female

- Females have light, pointed genitalia.
- The posterior segments of the Drosophila female are only pigmented in their posterior halves.
- Sex comb is absent.

2) Virgin Adult Female (denoted by the symbol ☿)

- Dark green spot seen in abdomen (the meconium, waste products remaining from pupation).
- Distended, large abdomen
- Very pale

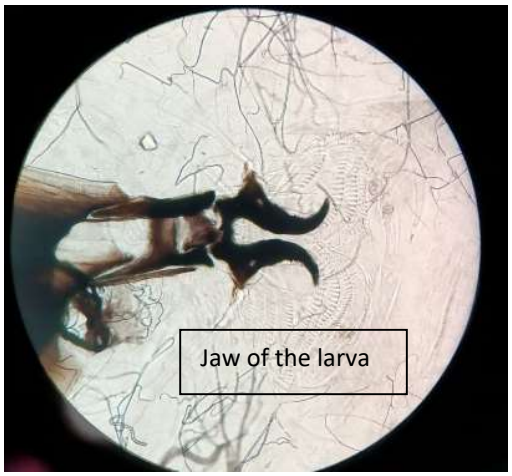


VIRGIN FEMALE

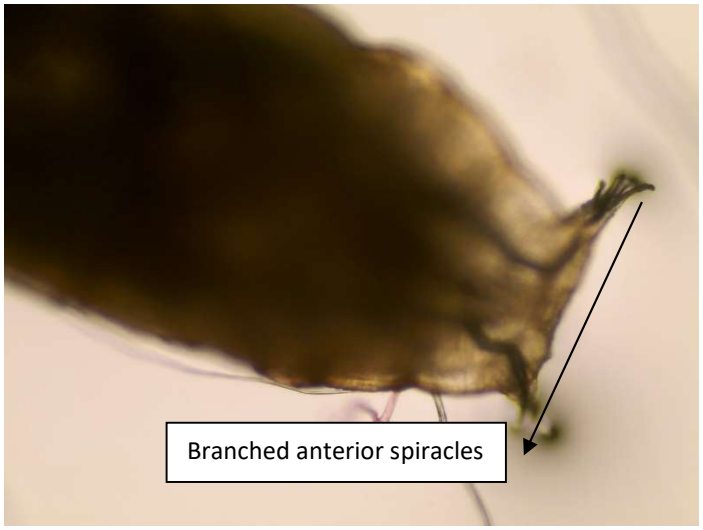

Sex
comb

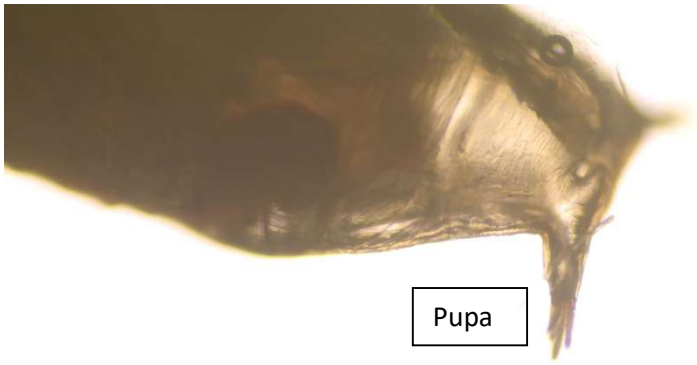

Egg


Labellum

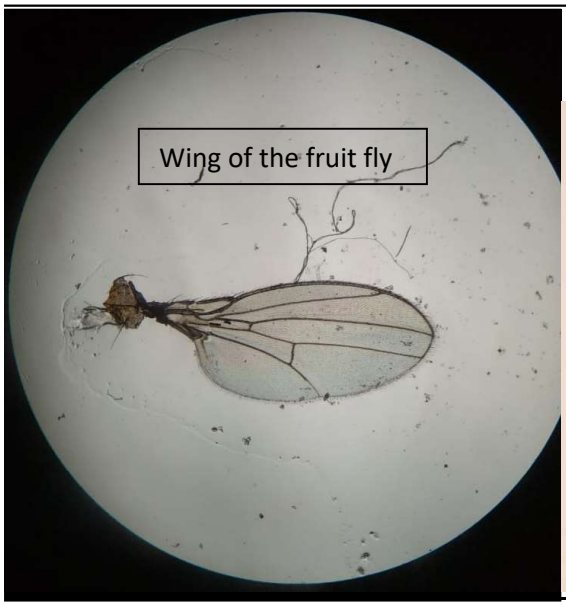

Jaw of the larva


Head of the fly

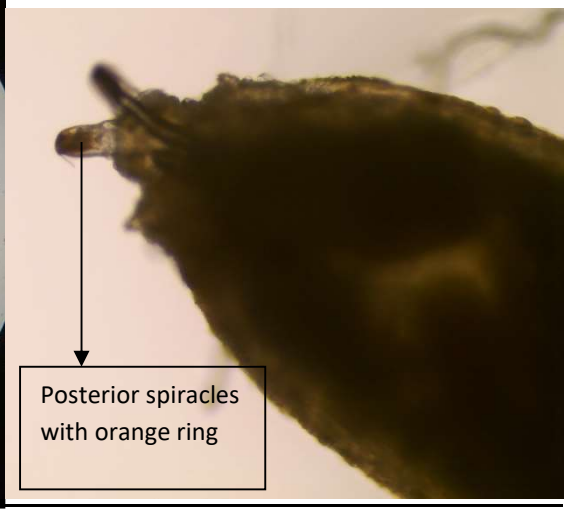

Branched anterior spiracles



Pupa



Wing of the fruit fly



Posterior spiracles with orange ring

Normal diet (nd) preparation

INGREDIENTS

- Water – 180 ml
- Agar-Agar – 1.25 g (helps solidify the food a bit so the flies don't sink)
- Corn/Maize Flour – 8.5 g
- Sugar – 7.5 g
- Yeast – 3 g
- Nipagin – 0.5 g (anti-fungal)
- Propionic Acid – 0.5 ml (anti-bacterial)
- Beakers
- Test-tubes
- Stirrers
- Micro- pipette

PREPARATION

- Divide the water into three beakers of volume 90 ml, 45 ml and 45 ml.
- Add all the dry ingredients to 45 ml of water except agar-agar.
- Till that time add the agar-agar to the 90 ml water and boil till 30% of the liquid evaporates.
- Now add the 45 ml solution from the second step to the agar-agar solution. Use the other 45 ml to rinse out all the remaining particle in the first 45 ml beaker. Cook it slowly till everything is mixed properly.
- Pour the ready diet into the beaker and then allow it to cool at room temperature. Once it has cooled for 5-7 minutes add the propionic acid using the micro- pipette and stir.
- Pour this diet into test tubes and allow it to cool.

High sugar diet (hsd) and its effects

- Instead of 7.5 g gram of sugar, add 10 g of sugar
- The method of preparation is the same as that of the normal diet.

When we observe the third larval instars who are feeding on the high sugar diet, we see:

- The larva is slightly more in width.
- The larva is also slightly longer than the larvae of the normal diet.
- It was also observed that when the larvae were introduced to chloroform and formulin, the larva of high sugar diet was alive for longer than the one of normal diet.

Low sugar diet (lsd) and its effects

- Instead of 7.5 g of sugar, 5 g of sugar needs to be added
- The method of preparation is the same as that of normal diet.

When we observe the third larval instars who are feeding on the low sugar diet, we see:

- The larva is slightly less in width.
- The larva is also shorter than the larvae of the normal diet.
- It was also observed that when the larvae were introduced to chloroform and formulin, the larva of low sugar diet was alive for longer than the one of normal diet.


Hsd
Nd
Lsd

Conclusion

Hence we see that the larval stages of drosophila melanogaster are affected by the change in the amount of sugar in their diet.

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