Evolutionary Biology Lab

Department of Biological Sciences

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a Report by

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I am grateful to all my fellow mates who helped me in some way or the other and made this experience enjoyable.

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It is indeed with a great sense of pleasure and immense gratitude that I acknowledge the help of these individuals who made this working experience memorable.

Anushka Vaibhav Joshi

Declaration:

In my capacity as supervisor of the candidate's summer internship work, I clarify that the above statements by the candidate are true to the best of my knowledge.

Prof. N.G.Prasad

DBS IISER M

Introduction

About the Lab:

The Lab works in the broad area of evolutionary genetics. Here, the main interest is understanding the co-evolution of a species' males and females. The lab studies *Drosophila melanogaster* - a versatile model organism. The studies mainly include the following -

Sexual Conflict and Sexual Selection.
Evolutionary Ecology of Immunity.
Life-History Evolution.

Drosophila melanogaster (Common Fruit Fly):

O Genotype, O Phenotype,
This kiss had better last her;
He's off to see his other love,
Drosophila melanogaster.

from Love on the Fly
by Dani S. Grady

Drosophila melanogaster is a species of fly (the taxonomic order Diptera) in the family Drosophilidae. The species is often referred to as the **fruit fly.**

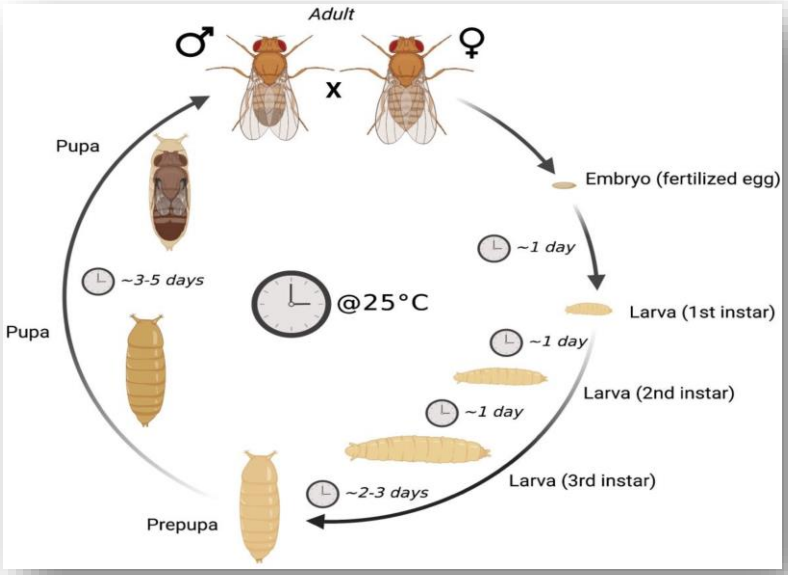
D. melanogaster continues to be widely used for biological research in genetics, physiology, microbial pathogenesis, and life history evolution.

Life Cycle:

Drosophila is a typical holometabolous insect, i.e., its life can be divided into four stages -
i) Embryo
ii)Larva
iii)Pupa
iv)Adult
there being a complete metamorphosis of the body form from larva to pupa.
In the laboratory, D. melanogaster is usually cultured at *25*°C.

The generation time is roughly ten days from fertilised egg to eclosed adult, and the maximum life span ranges from 60 to 80 days depending on the culture conditions.

Females lay roughly 100 embryos daily, and embryogenesis lasts only 24 h. The first instar larva begins to feed immediately on the surface of the medium. The second instar larvae burrow into the medium, and when the third instar larva is mature, it leaves the culture medium and wanders up the walls of the flask, searching for a place to pupariate for 24–48 h. Finally, the adult emerges between 9 and 10 d after egg fertilisation.



Source: Google images

The* Drosophila *Genome:

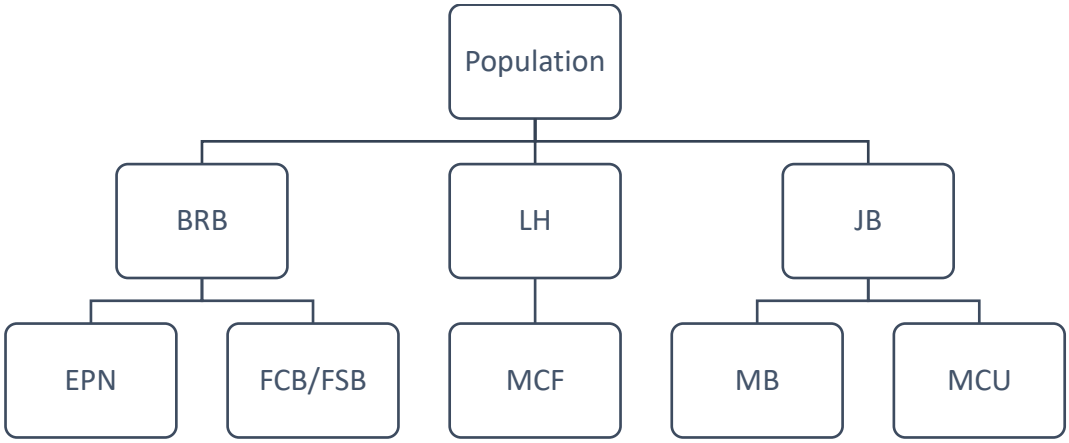
Drosophila has four pairs of chromosomes: X/Y, II, III, and IV, with most of the gene content located on chromosomes X, II, and III.

Working with Drosophila:

Flies are generally maintained in vials and bottles containing fly food. They can also be kept in plexiglass cages if required.

Female flies do not mate within the first 8–12 h after emergence as adults from the pupae. Thus, using this time window, flies can be collected, and females can be separated from the males and kept separately until needed. Males can be collected anytime, with the best mating efficiency between 3 and 10 d.
CO_2 is generally used to anaesthetise flies instead of the traditional ether because it is safer, easier, and avoids over-anesthetisation of the animals.

Populations in the Lab



1)BRB (Blue Ridge Baseline):

BRB is a large outbred Drosophila melanogaster population established from 19 iso-female lines. The iso-female lines were founded by 19 females caught in the wild from Blue Ridge, USA.

Five replicates of the BRB population (BRB_{1-5}) were maintained on a 14-day discrete generation cycle at 25^0C on a standard banana-yeast jaggery food.

The flies were kept at 12 hours of alternate light-dark cycle under 50-60% humidity conditions. BRB populations were maintained under these conditions for 35 generations with N_e of 2800 individuals.

i)EPN:

The setup includes 12 populations divided into three regimes:

a) E_{1-4} populations: Selected for increased post-infection survival. In each generation, 200 males and 200 females per population are infected with Enterococcus faecalis.

b) P_{1-4} populations: Controls for infection procedure. In each generation, 100 males and 100 females per population undergo sham infection.

c) N_{1-4} populations: Uninfected controls. In each generation, 100 males and 100 females per population are subjected to CO_2 anaesthesia.

All populations follow a 16-day generation cycle with infections on day 12. Flies are maintained on a standard banana-jaggery yeast medium in an incubator at 25^0C, 12:12 hours light: dark cycle, and 50% relative humidity.

ii)FCB/FSB:

After 35 generations, two populations were established from each BRB population: FSB (Freeze Shock resistant, cold stress resistant) and FCB (Controls).

Populations with the same BRB origin were denoted with subscripts, e.g., FSB_{-1} and FCB_{-1} from BRB_{-1}, forming Block-1. Five FSB (FSB_{1-5}) and FCB (FCB_{1-5}) populations were obtained. FSB and FCB populations were maintained on a 13-day discrete selection regime at 25^0C, 50-60% RH, and a 12:12 light: dark cycle, with an effective population size (Ne) of around 1400 individuals.

2)LH (Larry Harshman):

The LH population was founded in 1991 by Dr Larry Harshman using 400 wild inseminated females captured from an orchard near Modesto in California, US.

It follows a 14-day generation cycle on a cornmeal-molasses diet at 25^0C, 50% humidity, and 12:12 light: dark cycle. The population comprises 60 vials with about 150 eggs each. On the 12th day, flies are divided into six groups and sorted into vials containing 16 males and 16 females. After two days of interaction, females lay eggs for 18 hours, starting the next generation with 150 eggs per vial in 8-10 ml food.

i)MCF:

Three replicate populations (C_{1-3}) derived from LHst base populations were maintained for five generations under equal sex ratios. Each replicate gave rise to male-biased (M_{1-3}), control(C_{1-3}) and female-biased (F_{1-3}) regimes. Populations follow a 14-day cycle at 25^0C, with 60-80% relative humidity and a 12:12 hour light: dark cycle. Adult flies are collected as young virgins, mature in 8-10 hours, and are combined by sex in food vials. Flies interact for 48 hours before being transferred to oviposition vials. The next generation starts with an egg density of 150 per vial.

3)JB:

JB populations are maintained in incubators on a 21-day generation cycle at 25^0C, about 90% relative humidity, and constant light. They are fed a banana-jaggery diet with a larval density of 60-80 larvae per 8-dram vial with 6 ml of food.

Each population has approximately 1800 breeding adults housed in Plexiglas cages with abundant food in Petri plates. Eggs are collected by providing fresh Petri plates with food for 18 hours. Collected eggs are placed in 40 vials at a density of 60-80 eggs per vial. Flies are transferred to new food vials on the 12th, 14th, and 16th day after egg collection, and on the 18th day, hatched flies are gathered into Plexiglas cages with yeast-acetic acid paste. Eggs for the next generation are collected three days later.

i)MB:

The MB population (MB_{1-4}) is maintained on a 21-day cycle with standard cornmeal-charcoal food. Eggs are collected from 12-day-old females and transferred to glass vials (60-80 eggs/vial); 40 such vials are collected for each replicate. Then all the vials are incubated at 25^0C, 90% RH and constant light.

Flies emerge on the 9th day, and on the 12^{th} day, adult flies are transferred to Plexiglass cages containing a Petri plate of cornmeal-charcoal food and a piece of wet absorbent cotton for maintaining high RH levels. On the 18^{th} day post egg collection, a fresh food plate supplemented with *ad libitum* live yeast paste is provided in each cage, and after 48 hours, eggs are collected to start a new generation.

ii)MCU:

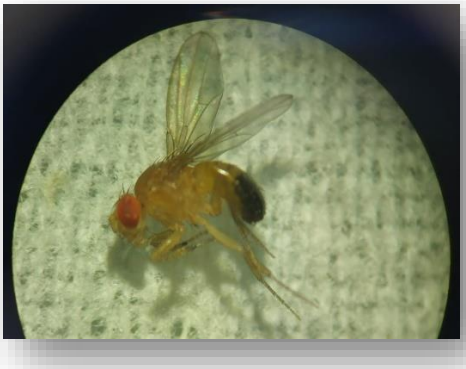
After 15 generations, MCU populations were derived from MB populations. Like MB populations, MCU populations follow a 21-day cycle at 25^0C, 90% RH, and constant light.

In MCU populations, eggs are kept in glass vials containing 1.5 ml of cornmeal-charcoal food (800 eggs/vial) for each replicate, with 24 vials collected and incubated in standard laboratory conditions. Pre-adult mortality is very high in this regime due to limited food and high density, resulting in approximately 100-120 adults per vial. To prevent overcrowding in vials, eclosed adults are transferred daily to Plexiglass cages with food and moist cotton from the 8th to the 18^{th}-day post egg collection. Every other day, fresh food plates are provided in the cages. On the 18th-day post egg collection, a new food plate supplemented with *ad libitum* live yeast paste is provided in each cage. After 48 hours, the flies are allowed to lay eggs for 18 hours. These eggs are collected to start the next generation.

Methods and Techniques learned

1. Egg collection: Required number of eggs are collected by observing the food under a microscope.
2. Cage preparation: Plexiglass cages are prepared for dumping of populations.
3. Dumping of Population: Populations are transferred in the respective plexiglass cages from the vials/ bottles.
4. Food Cook: Food for Drosophila melanogaster is prepared.
   a) Charcoal-Cornmeal.
   b) Cornmeal-Molasses.
   c) Banana-Jaggery.
5. Vial Plugging: Mouths of the vials are sealed with cotton plugs to avoid the escape of flies.
6. Egg counting: Eggs laid by the flies are counted under the microscope.
7. Flipping the flies in a new vial: The flies are transferred from one vial to another.
8. Sorting: Male and Female flies are differentiated.

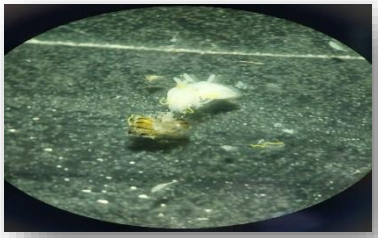
   a) Adult: i) Male flies have sex combs to hold the female while mating.
   ii) Males have a blackened spot on the posterior side of the body.
   iii) Males are smaller in size.
   i) Females lack sex combs.
   ii) Females are evenly striped.
   iii) Females are larger in size.



   b) Larva: i) In males, circular, translucent disc gonads are visible under a microscope.
   ii) In females, these translucent discs are absent.

9. Literature.
    a) Knowledge of Drosophila.
    b) Two decades of life history evolution.
10. Molecular Techniques.
    a) RNA isolation.
    b) RT-PCR.
11. Immune Assays.
    a) Infection: Flies are infected by dipping a fine needle in the culture and then pricking the fly at a triangular structure present on the anterior side of the fly.
    b) Plasmatocyte counting:

    c) Basic Microscopy.
12. Cold Shock Treatment: Flies were given a cold shock at -5^0C. An ice slurry was prepared at -80^0C using ice and salt for the purpose
13. Heat Shock Treatment: Flies were subjected to a heat shock by placing them in a water bath.
14. Diet manipulation: The protein or carbohydrate content of fly food can be changed, and its effects on the flies are studied.
15. Ovaries dissection: Female flies are dissected, and the ovaries are observed.



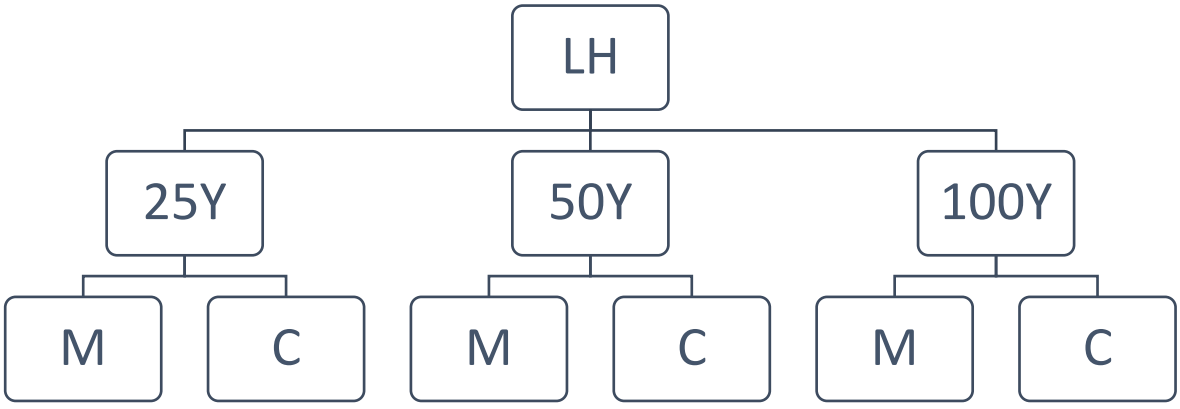
16. Testis dissection: Male flies are dissected, and the testis, seminal vesicles and accessory glands are observed.

Summer Project

Protein Manipulation to study Fecundity and Survivorship in Adult Drosophila melanogaster.

The experiment started on the 9th of June, the 3rd day after the eclosion. The experiment was conducted on two-day-old virgin flies. In the project that I worked on, we used the flies of the LH population. Flies were grown on standard cornmeal-molasses food.

Three treatments were made in the dilution of food, one with 100% yeast content (100 Y), the other with 50% yeast content (50Y) and the third one with 25% yeast content (25Y). Each Treatment was again divided into two more selections, the male-biased one (M) (3 males: 1 female) and the control (C) (1 male: 1 female). This was done to check the intensity of sexual conflict under altered protein concentration.



We were majorly working on two aspects,

1. We were interested in looking how the direct fitness of female under resource limitation (here protein). For that, we calculate

a. The number of progenies produced per female for eight days after combination.

b. Survivorship of female flies each day.

We also checked the survivorship of the *virgin* flies and observed how mating affects survivorship.

Experiment protocol-

a. The flies were flipped into fresh food vials every alternate day till their death. The vials were maintained inside an incubator. The eggs laid in the previous vials were allowed to transform into adult flies; the adult flies in each vial were counted after freezing at -20^0C to check the number of flies sired by each fly. First eight days, eggs were considered in all treatments in the data.

b. To check the survivorship of the flies, we examined the vials daily and noted if any female was dead. The day any female in a vial died was considered the life span of the flies in that vial. If any male was found dead, he was replaced by another male of the same age maintained in the same food treatment. Virgin male and female vials with food treatments were also maintained aside to keep a check.

Result:

1. We found the effect of treatment on fecundity. Protein restriction or a decrease in protein concentration causes a reduction in the number of progenies.

2. We also reported that the male-biased regime was more mate-harming than controlled. M-selection causes a decrease in females' survival and a lesser number of progenies.

3. With the **decrease** in nutrition (here protein), the mate-harming capacity is reduced, i.e. survival was higher in the 25 Y – M regime than in the 100 Y- M regime.

4. We also found a **trade-off** between reproduction and survivorship. In 100Y treatment, progenies were highest in early life but died early, while in 25Y, survivorship was higher as they laid fewer eggs.

Future implications:

The effects of protein dilution on male reproductive capacity still need to be addressed. We hypothesise that protein restriction may affect spermatogenesis and courtship behaviour. Quantification of mate harm will be further studied.

Limitation: Effects of male reproductive traits were not considered. The gradient of protein can also be decreased to 20% or 10 % Y. Higher concentrations of protein regime were not considered.