**How does predator hunting-mode affect the life history of *Drosophila melanogaster*?**

*Hypothesis*: Given that both spiders and mantids are adult predators of fruit flies, we would expect the evolution of similar life history traits between the two predation treatments. In fruit flies, there is an age at which copulation latency is least (because female receptivity is maximum). This age is approximately 3 days. In predated environments, because adults have a greater chance of dying, we should see a shift in this peak age.

**Copulation latency**

Get rid of maternal effects – to do so, let flies lay eggs in bottles, collect 100 adults and place in a fresh bottle. Let them lay eggs for 24 hours and remove adults. Reuse adults to make another set of bottles (stagger bottles in such a way that every 2 days you have a bottle that just started eclosing). Collect another 100 individuals from the original F1 bottle and 3 – 4 days after bottle B was seeded, place 100 F1 flies to make bottle C. Reuse these F1 adults to make bottle D 2 days later. This way development time and age will not be confounded.

Use F2 adults in all experiments. Collect virgin males and virgin females everyday for 14 days.

Age bins :

a) 1- 3

b) 4-7

c) 8- 11

4) 12-15

New age bins:

a) 0-1

b) 2-4

c) 4-6

First 3 days of collection goes into the last age bin. Next 2 days into age bin . Subsequent 4 days into age bin b. Last 2 days in age bin a. Because you will know exactly what age bin you are collecting form, make sure you lump the flies form each bin together and put them in 1 vial. Only collect two males and two females per day. Remember that you will ultimately use only one fly per age bin per replicate for observations.

Pre- mating

On day 5, randomly pick three females (from the two age bins you have) and put them in a fresh vial with three (previously collected) non-virgin males. Do the same with three randomly collected virgin males (by placing them with non-virgin females).

Place one male and one female fly of the same age bin in a vial and record the start time. Put female flies in first and give them a minimum of 5 mins to acclimatize. Then introduce the male. For 36 vials, this will take about 40 mins. When copulation occurs, record the time again. If copulation does not occur after 3-4 hours, stop the assay and record as female as non-receptive.

Things to track:

1) courtship start time

2) copulation start time

3) copulation duration

Make datasheets beforehand.

Sample size: 10 pairs per age bin per replicate. This makes it 10\*3\*12 = 360 observations. Do complete blocks.

Note from Abhijna May 2016: We did each trial for two days. First day (with virgins) we measured copulation, courtship, etc (these are data you can interpret). After the experiment was done, we left the male in there for 4 hours longer. Then we took out all the males and left the flies in the jungle overnight (in their vials). The next day we introduced another male. Once every 20 mins, we scanned all the vials and made a note of the ones that were copulating. I believe we were trying to measure receptivity to a new male in older non-virgins.

So each trial should have two files - a and b.

The rows that have an x all the way through are flies that died or escaped (when we were adding or removing the male).