Biology 322 Fall 10 Wasp Genetics

This experiment will explore the genetic principles of complementation.

<u>You</u> will set up various crosses, and manage the life cycles of the model organism <u>Nasonia vitripenni</u> in order to address the following questions:

Wild-type eye color in *Nasonia vitripennis* is a deep reddish-brown. You have isolated two new mutations that result in scarlet eyes, st^M *and* st^N. Is either mutant strain a new allele of the st^{DR} gene, previously mapped to chromosome I? Or a mutation at another genetic locus that results in the same phenotype?

Determine the set of crosses that would best address these questions. You will be required to show meaningful results in the F1 and F2 generations. Be sure to read about sex determination and life cycles in wasps (below) before you design your experiments.

Phenotypes

Wild-type eye color: dark reddish-brown
Mutant eye color: scarlet *(st)*

Strains that we will be working with:

- st^{DR} st^{DR} is located on chromosome I
- st^M scarlet eyes
- st^N scarlet eyes
- st^+ wildtype eyes

Introduction to the wasp *Nasonia vitripennis*

Nasonia vitripennis is a solitary wasp which parasitizes dipteran pupae. This species is easily reared in the laboratory on *Sarcophaga* pupae at temperatures ranging from 15ºC to 30ºC. The generation time is similar to that of *Drosophila melanogaster:* ~ 10 days at 28ºC and one month at 18ºC . Male and female pupae can be held at 4º (no meaningful development). Eclosed males can be held at 4º if they have been fed honey water for 24h.

The adult female drills into the host puparium, feeds on host fluids and deposits her eggs. The larvae feed and pupate on the host pupae. Virgin females are typically collected by dissecting *Nasonia* pupae from the host pupae before the former have eclosed. If allowed to eclose inside the host pupae, the adults gnaw a hole through the puparium, emerge and mate. Males average 2 mm in length and females 2.5.

Handling Wasps

Steromicroscope
- use incident light rather than transmitted light
- use white stage plate

Rate of Wasp Development
- we have a 28^0 and 18^o incubators,
- pupae can be "held" at 4^o effectively stopping development
- hydrated, eclosed males can be held at 4^o for up to a week

The Tubes
- cap with cotton balls, make sure there are no "tunnels" or creases that might allow the wasp to escape
- store in plastic baskets, wrapped with "stockings"

Light
- females must be in continuous light after eclosing in order to lay eggs that will develop normally and not enter diapause
- this is not necessary for males

Mating Potentials
- female wasps start mating ~ 24 hr after eclosing
- males are at sexual "peak" ~ 24 hr after eclosing
- ideally, eclose, hydrate for one day, chill at 4^o

Moving Wasps
- chill your males for ease of handling (4^o). They wake up fast.
- tap the wasps to the bottom of the tube before removing cotton

Mating
- move one male wasp of the correct genotype into a tube with the correct female.
- ***Watch to ensure mating,*** if after ~ 5 minutes, no success, add a second male, etc.
- once mating has been accomplished, add two Sarcophaga pupae (careful not to break the pupae with your forceps).
- males will die off in a couple of days females will lay eggs in the pupae and will also suck pupal juices

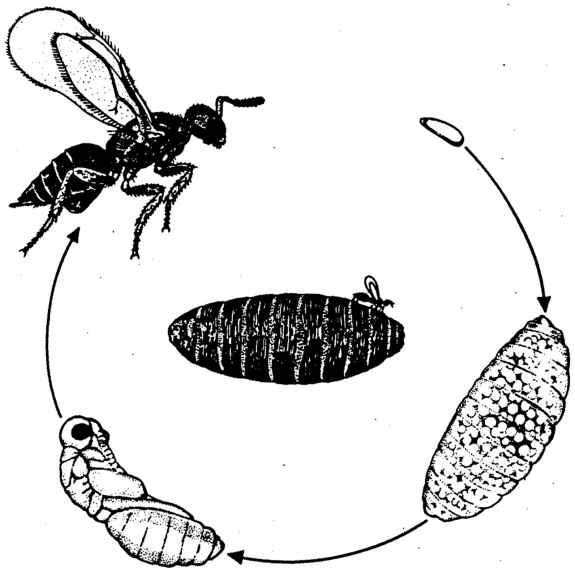


Figure 2. The life cycle of Mormoniella vitripennis. The Sarcophage bullata shown in the center of the drawing is 12 mm long.

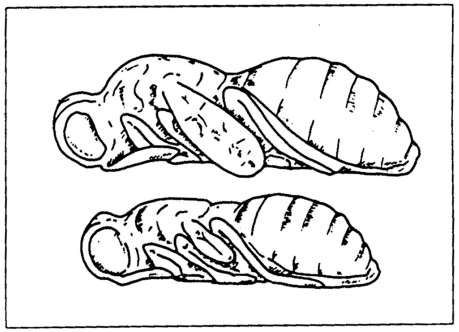
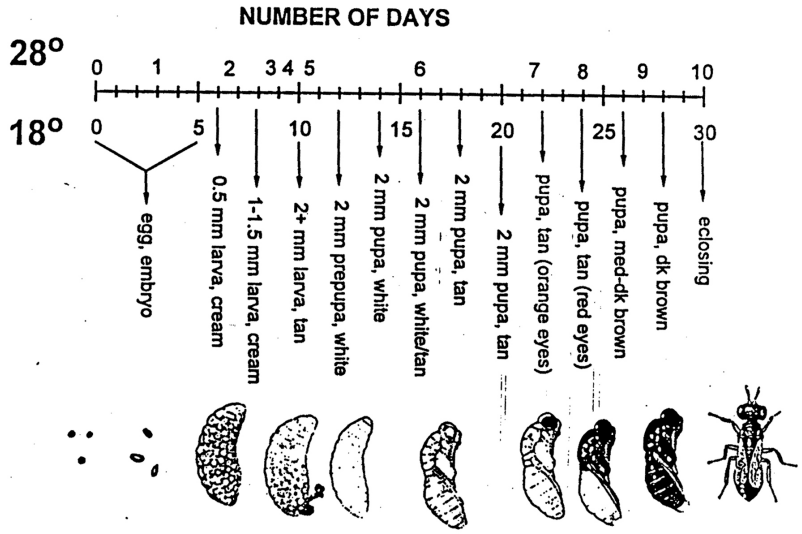
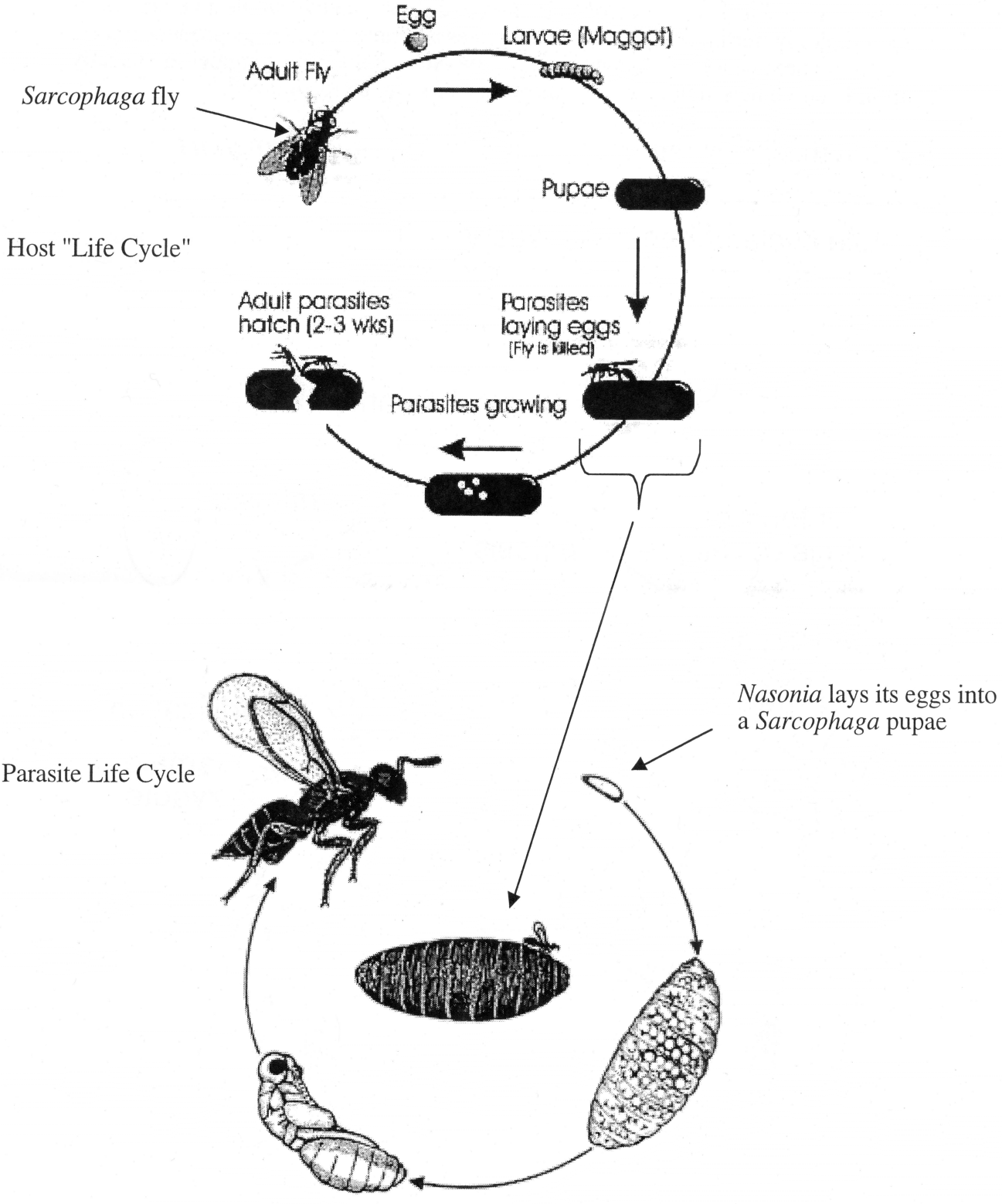


Figure 10 *Female* (top) *and male jewel wasp pupae.*



RATE OF WASP DEVELOPMENT AT 18° & 28°

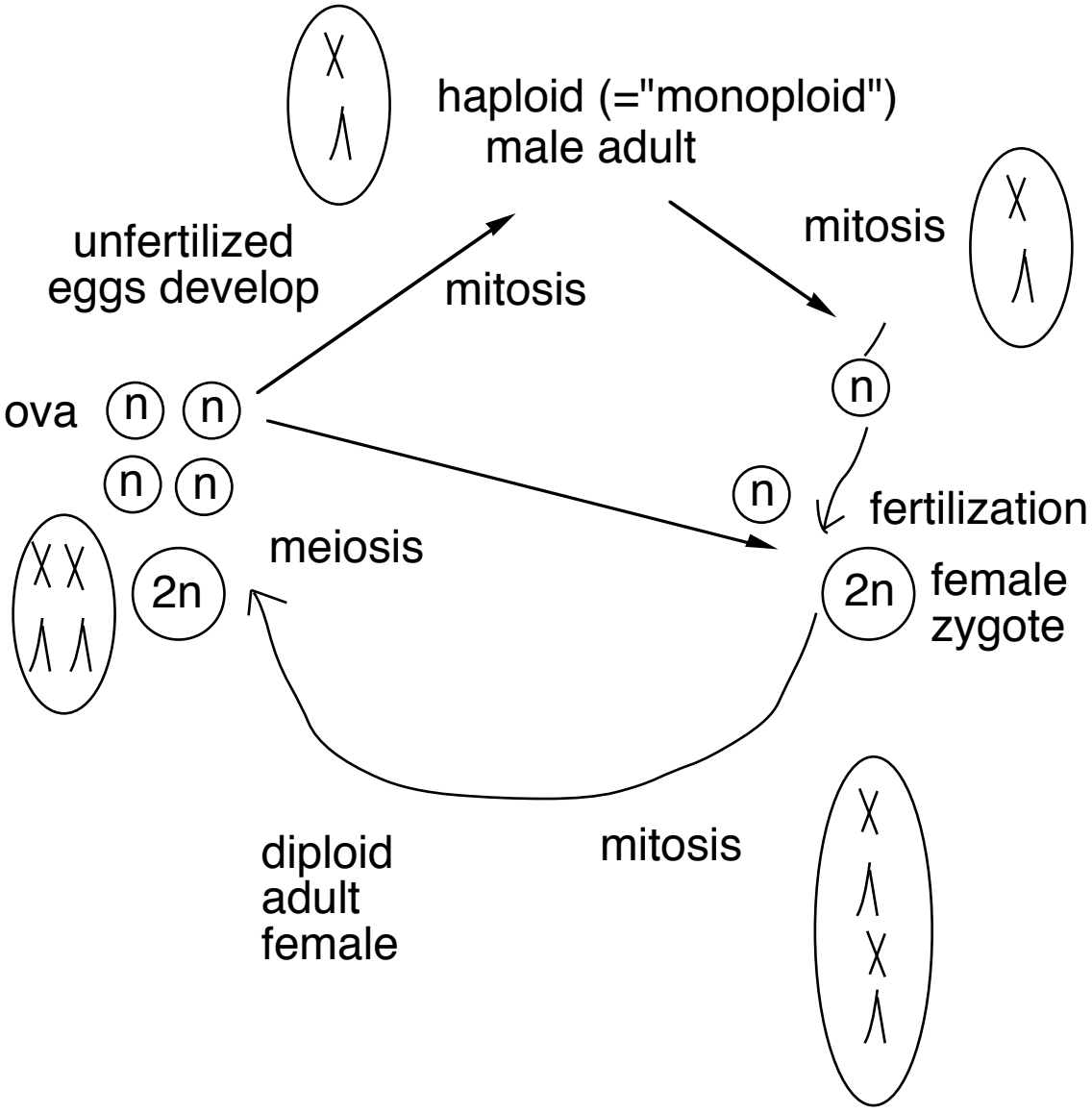
Figure 4: The rate of wasp development was correlated for 18° and 28°. At 18°, wasps develop from egg to adult in approximately 30 days, whereas adulthood is reached in only 10 days when raised at 28°. Rate of development was determined by periodic examination of growth; wasps were examined every two days at 18° and every day at 28°. This scale was utilized in analyzing the temperature shift data seen in Figure 5.


Egg
Larvae (Maggot)
Adult Fly
Sarcophaga fly
Pupae
Host "Life Cycle"
Adult parasites hatch (2-3 wks)
Parasites laying eggs
(Fly is killed)
Parasites growing
Nasonia lays its eggs into a *Sarcophaga* pupae
Parasite Life Cycle

The predominant mode of sex determination in the order Hymenoptera (bees, ants, wasps and sawflies) is characterized by haplo-diploidy. In the simplest version of haplo-diploidy, fertilized eggs produce females and unfertilized eggs produce males (see figure next page). As a consequence, females are diploid and males are usually haploid and no heteromorphic chromosome pairs can be recognized.

n=2 in the following figure. n=5 in *Nasonia vitripennis*.

Life cycle of bees and wasps



Experimental Cross Planning

Strains that we will be working with:

- st^{DR} st^{DR} is located on chromosome I
- st^{M} scarlet eyes
- st^{N} scarlet eyes
- st^{+} wildtype eyes

Questions to ask your self:

1. Is the mutation recessive or dominant? How do you test for dominant/recessive genes?

2. How does complementation analysis depend on whether the gene is recessive or dominant?

3. For two genes, diagram the crosses necessary to determine if the complement or not, given they are both recessive.

4. For two genes, diagram the crosses necessary to determine if the complement or not, given they are both dominant.

5. For two genes, diagram the crosses necessary to determine if the complement or not, given one is recessive and one is dominant.

6. Does the sex of the parent carrying the mutation make a difference? Does the sex of the progeny make a difference? Can the haplo/diplo system help you make any shortcut? If so (and hint), it is so, how?