

Biology 322 Fall 2012

Wasp Genetics: Genetic Heterogeneity and Complementation Revisted

Required Reading: *Deaf by Design* Nature 431: 894-896 October 21, 2004
<http://fire.biol.wvu.edu/trent/trent/Naturedeafdesign.pdf>

Introduction: Wild-type eye color in *Nasonia vitripennis* is a deep reddish-brown. You have received a shipment of a scarlet-eyed mutant, called *stC*, from Carolina Biological Supply. No one at Carolina seems to know much about this mutant so **you need to determine yourself if this mutant strain represents a newly discovered gene or if it carries is an allele of a previously identified scarlet gene** -- either the *st318* gene, which maps to chromosome V or the *stDR* gene located on chromosome I.

→ → We will set up crosses on Tuesday Nov. 13th. Your google.doc for this lab period should address these questions:

1. What is the simplest way to address the question underlined above? For now, assume that all mutant alleles are recessive to wildtype.
 2. Generally outline the crosses that you would do. What are the predicted results if *stC* is an allele of the *stDR* gene or the *st318* gene? If *stC* is an allele of neither gene? BE sure to review the info about haplodiploidy and indicate whether you will score male only, female only or both sexes.
 3. What is the relevance of the *Deaf by Design* article to this laboratory exercise?
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Planning your Experiment

→ → See last page of handout for important instructions on handling these wasps and tracking your experiment

Phenotypes of mutant strains

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

Strains that we will be working with:

- Wildtype
- scarlet mutant: *st318* (gene located on chromosome V)
- scarlet mutant: *stDR* (gene located on chromosome I)
- scarlet mutant: *stC*

1. Does the test for allelism depend on whether the mutant scarlet allele is recessive or dominant to the wild-type allele? How would you test for dominance?
2. What crosses are necessary to address the question at hand?
3. Does the sex of the parent carrying the mutation make a difference? Does the sex of the progeny make a difference? See info on haplodiploid reproduction below.

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 15°C to 30°C. The generation time is similar to that of *Drosophila melanogaster*: ~ 14 days at 25°C and one month at 18°C. 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.

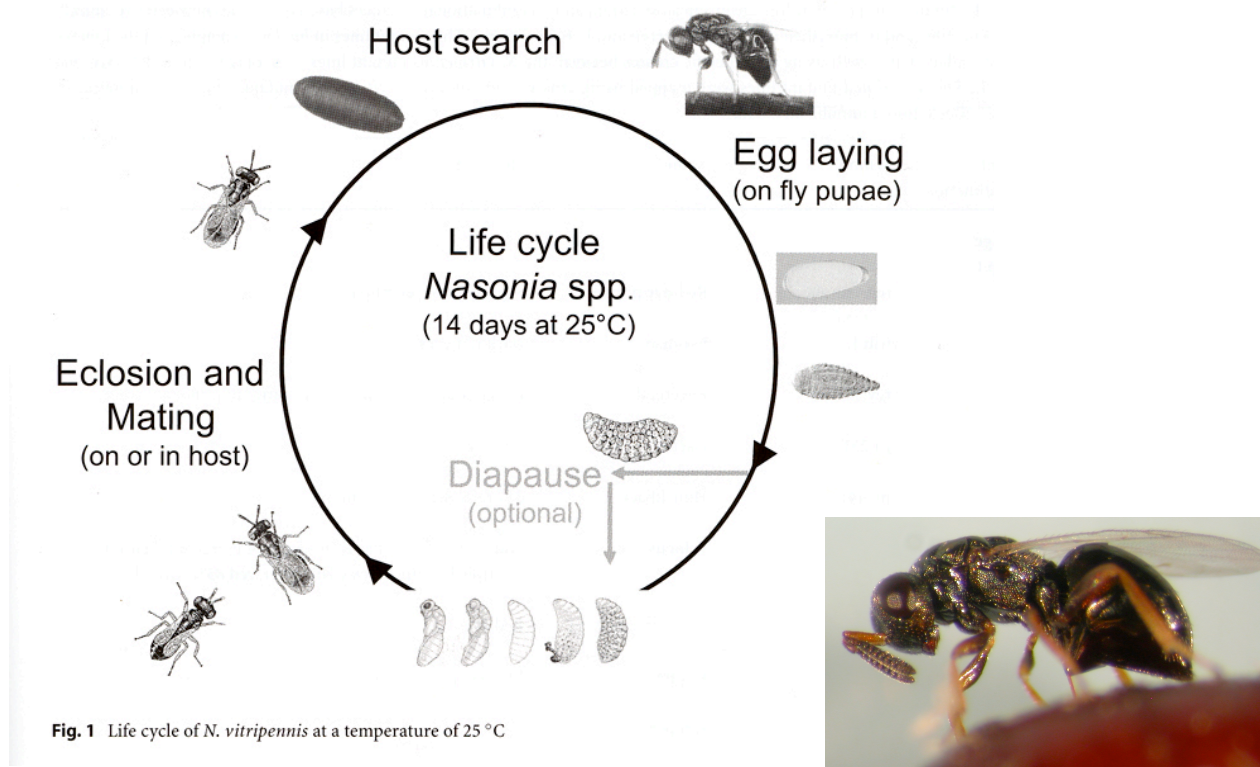
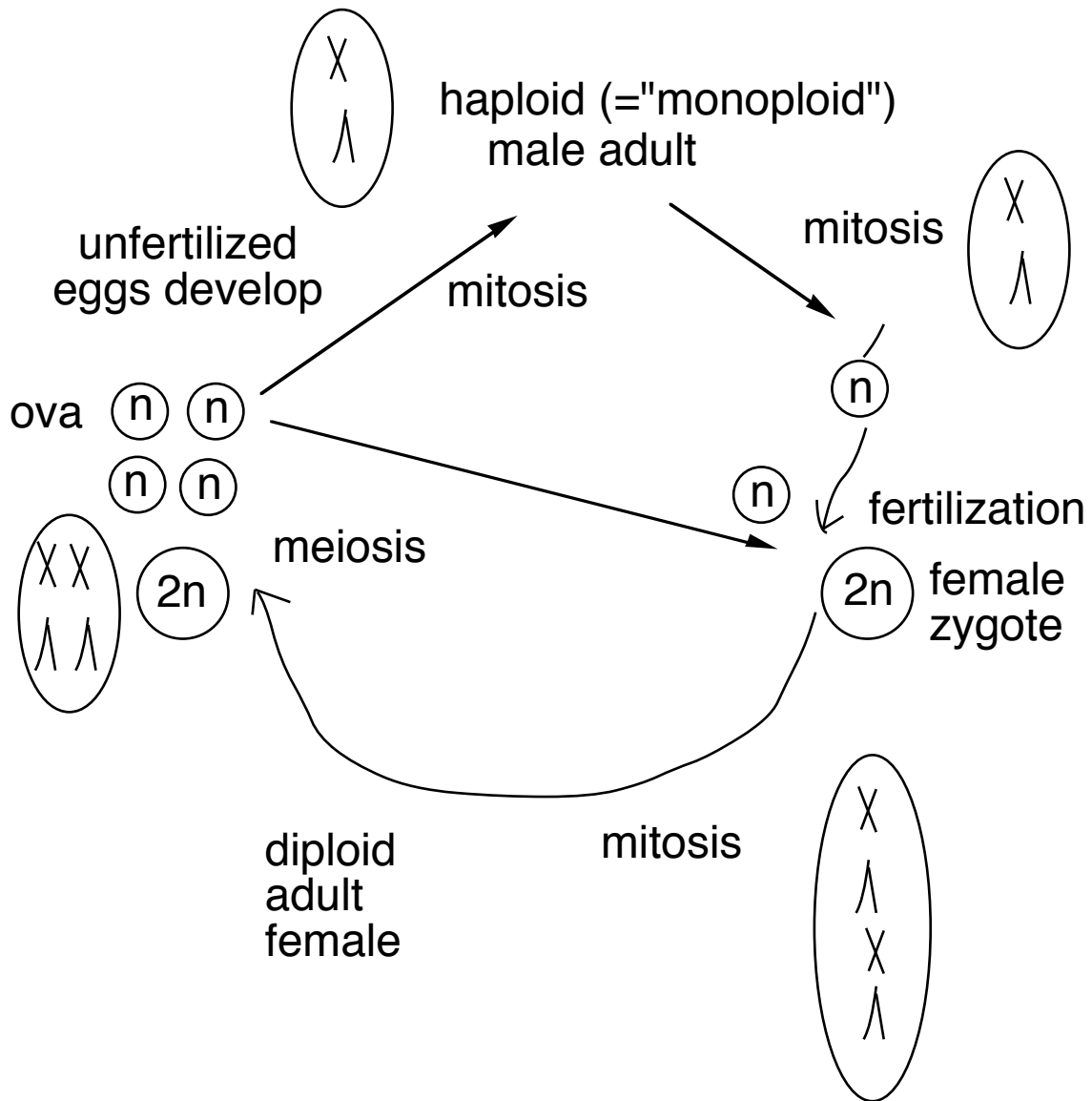


Fig. 1 Life cycle of *N. vitripennis* at a temperature of 25 °C

The predominant mode of sex determination in the order Hymenoptera (bees, ants, wasps and sawflies) is haplo-diploidy. In the simplest version of haplo-diploidy, fertilized eggs produce females and unfertilized eggs produce males (see figure below). As a consequence, females are diploid and males are usually haploid; no sex chromosomes are present the genome of this order.

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

Life cycle of bees and wasps



Handling Wasps

Viewing wasps with the stereomicroscope:

- Set up for stereomicroscope will be different
- Replace glass stage with opaque stage
- Since wasps are not transparent like worms, light should be incident not transmitted

Handling wasps and setting up mating

- Each student set up crosses (see board)
- Tap females to bottom of vial (on pad of paper) before introducing males
- With brush, transfer a single male from the male vial and place in the vial of females. [Males have been refrigerated so they won't move too fast]
- Watch male to ensure that he mates
- If he doesn't mate within 5-10 minutes, introduce a second male

After mating

- Tap wasps to bottom of vial and using blunt forceps place 1 fly pupae per female in the vial (check board to see if this instruction has changed)
- Replace cotton plug and check to make sure there are no tunnels in the cotton -- wasps will find their way out
- Store vials in plastic basket encased in a knee-high hose
- Males will die off in a couple of days and females will lay eggs in the pupae and will also suck pupal juices

Rate of Wasp Development

- We have a 28° and 18° incubators – see below for info on rate of wasp development at these two temperatures
- Pupae can be "held" at 4° effectively stopping development

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

Table 1 | Pupal stages of *M. vitripennis*.

Pupal stage	Yellow stage	Red-eyed stage	Half-pigmented stage	Fully pigmented stage	Eclosed adult stage
Time after egg lay at 18 °C (d)	18-22	23-26	26-28	28-30	30
Time after egg lay at 25 °C (d)	7-9	9-11	11-12	12-14	14
Time after egg lay at 28 °C (d)	5.5-7	7-8	8-9	9-10	10
Maximum time can be stored at 4 °C	2 months	2 months	0	2 weeks	Several days
Appropriate for RNAi against embryonic patterning genes?	Yes, ideal	Yes, latest recommended	No	No	Not tested

You are responsible for independently tracking the development of the offspring of your cross and sexing/scoring/storing them at the correct stage.

Tracking progress of wasp experiment:

Thursday Nov 15

- check fly pupae to see if females have laid eggs and drooled all over them
- If yes, remove females and discard in morgue
- If no or not sure, leave females in vial until next Tuesday

GOAL: Progeny ready to score no later than Thursday 11/29 (when we will score/collect class data and figure out genotypes)

- ***MONITOR the development of the wasp progeny with this goal in mind***
- Consult developmental timetable in handout and note effect of temperature on larval and pupal development --we will look at photos of pupae in lecture
- track development of progeny and peek inside fly pupal case when you first think that you might have yellow pupae
- track crosses until you see females at the vested or black pupal stage* (why females?) → transfer to 4°C (put in bin marked 322 wasps in frig with clear glass doors)
*the yellow pupal stage is too young because wild-type eyes are red at that stage – follow animals until they are vested and/or black but don't let them eclose (crawl out of their pupal case) as we are not set up to knock them out for scoring
- if necessary shift between 28°C and 18°C so you catch the animals at the correct stage.