Nasonia vitripennis: *A* Drosophila *Alternative*

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Abstract: Our introductory biology students use the parasitoid wasp, *Nasonia vitripennis,* to investigate independent assortment, epistasis, linkage and crossing over in eye color of F1 and F2 offspring. The advantages of *Nasonia vitripennis* over other commercially available model organisms are: 1) these insects can be sexed in the pupal stage; 2) development can be arrested by refrigeration (induced diapause); 3) induced diapause can also be useful if you wish to maintain your own stock; 4) recessive alleles are easily "visible" in these haplodiploid insects through the haploid male and 5) the life cycle is approximately 14 days.

Introduction

In the span of approximately one month, our introductory biology students use the parasitoid Jewel Wasp, *Nasonia vitripennis*, to investigate independent assortment, epistasis, linkage and crossing over in eye color of F_1 and F_2 offspring from gray and scarlet-eyed parents. The advantages of *Nasonia vitripennis* over other commercially available model organisms are: 1) these insects can be sexed in the pupal stage (no more 2 a.m. harvesting of virgin females); 2) development can be arrested by refrigeration (induced diapause); 3) induced diapause can also be useful if you wish to maintain your own stock; 4) recessive alleles are easily "visible" in these haplodiploid insects through the haploid male, and 5) the life cycle is approximately 14 days.

Nasonia vitripennis, parasitize the pupa of the flesh fly, *Sarcophaga bullata.* The female *Nasonia* kills the *Sarcophaga* pupa by injecting venom, and then she deposits 20-50 eggs in the *Sarcophaga* pupa through her ovipositor. Her developing offspring will use the fly pupa as a food source. She also feeds on the fly pupa. Using her ovipositor, she constructs a feeding tube that allows her access to the fly pupa's hemolymph. After one day the eggs hatch and the larvae eat and develop for the next nine days, after which they begin to pupate. Adult *Nasonia* eventually break free of their own pupal casings and chew a hole through the *Sarcophaga* puparium wall to emerge.

A variety of eye color mutations are commercially available. We use the mutations scarlet-eye and oyster-eye (which we call gray-eyes) in our experiments. Wild-type *Nasonia* have dark purple eyes. After becoming acquainted with the life cycle and learning how to distinguish male from female pupae, our students cross scarlet-eye virgin female *Nasonia* with gray-eye (oyster) males. *We guide students through three alternative hypotheses:*

1) Eye color is determined by one gene with multiple alleles and a dominance hierarchy.
2) Eye color is determined by one gene with multiple alleles and incomplete dominance.
3) Eye color is determined by two genes with epistatic interactions.

While the F_1 generations are still pupae, our students isolate virgin females. When the F_1 generations eclose, the students add *Sarcophaga* hosts to the virgin females to generate the F_2 generation of all-male (haploid) *Nasonia*. At that same time, the students discover that the diploid females of the F_1 generation have dark purple eyes, supporting the epistasis hypothesis. New hypotheses and predictions concerning the F_2 generation are then formed:

1) The two genes assort independently.
2) The two genes are linked.

When the F_2 generation emerges two weeks later, the students sort the F_2 males and discover that the linkage hypothesis is supported.

Instructor notes

Accurate identification of male and female pupae and adults is essential for student success. In the pupal stage, female wasps can be distinguished from male wasps using the following characteristics:

1) females are usually larger than male wasps,
2) females have longer wings that on lateral view wrap around the abdomen and are slightly separated from the body, and
3) females have an ovipositor. (The ovipositor is not easily identified in early pupal development --white to black-and-white stage.)

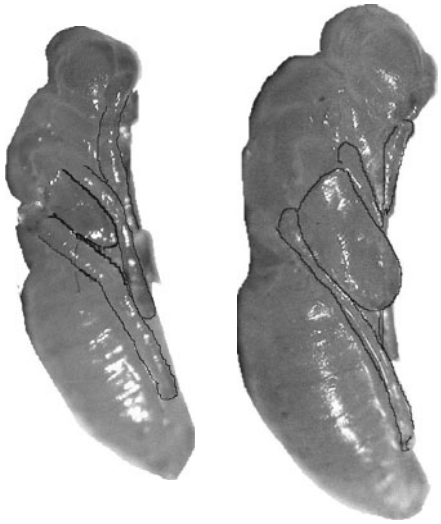

Figure 1. Lateral view of male (left) and female (right) *Nasonia vitripennis* pupae. Wing, antennae and legs have outlined. Note that the difference in body and wing size. Plate magnification 25X.

When students select F_1 female pupae that are used to generate the F_2 haploid male generation, it is important that they select females of approximately the same stage of maturation. (See Figure 2.) We encourage students to select pupae late in the pupal development process (black pupae) since these pupae will eclose in approximately one to two days at which time the students add the host, *Sarcophaga bullata* pupae. The time to adulthood from the white stage is four to six days and from black-and-white, two to four days.

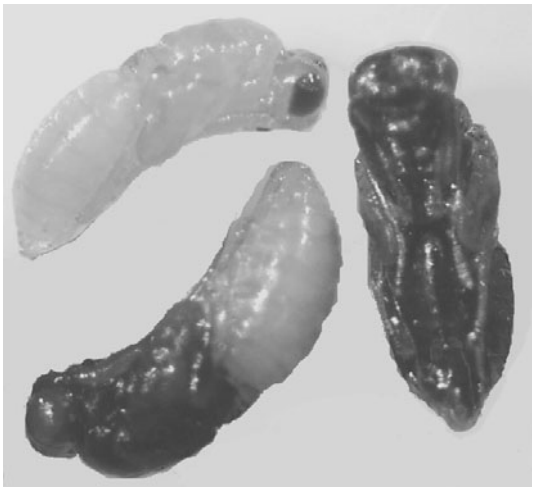

Figure 2. Female *Nasonia vitripennis* pupae in various stages of development. From youngest to oldest counter-clockwise from top left: white (4-6 days until adulthood), black-and-white (2-4 days until adulthood) and black (1-2 days until adulthood).

Before students make predictions, you must decide what nomenclature you will use. This can be difficult because a scheme that works for the hypothesis of a monohybrid cross cannot be used to describe a dihybrid cross. In addition, the monohybrid dominance hierarchy and the dihybrid epistasis hypotheses can have multiple predictions. For our introductory students, we initially limit the predictions to one for each hypothesis. For the monohybrid dominance hierarchy, the prediction made by the student assumes that purple is dominant over scarlet and scarlet is dominant over gray. For the dihybrid epistasis hypothesis, we assume that the gray pigment is formed first in a pigment synthesis pathway. Based on these assumptions, we use the nomenclature found in Tables 1 and 2.

Table 1. Eye color phenotypes and genotypes of parental *Nasonia* wasps we use for monohybrid cross predictions. Note that females are diploid and males are haploid.

	Genotypes for Monohybrid Crosses Assuming $G>g^s>g$	
Phenotypes	Female wasps	Male wasps
Purple	G_	G
Scarlet	g^sg^s, g^sg	g^s
Gray	gg	g

Table 2. Eye color phenotypes and genotypes of parental *Nasonia* wasps we use for dihybrid cross predictions assuming that the gray pigment is formed before the scarlet pigment. Note that females are diploid and males are haploid.

	Genotypes for Dihybrid Crosses Assuming Biosynthesis Pathway: Gray→Scarlet→Purple	
Phenotypes	Female wasps	Male wasps
Purple	G_S_	GS
Scarlet	G_ss	Gs
Gray	ggS_, ggss	gS, gs

Introductory students can find making F_2 generation predictions for the dihybrid epistasis hypothesis difficult because there are four possible genotypes (GS, Gs gS and gs) but only three eye colors (purple, scarlet and gray). We include a schematic (Figure 3) to help them. Usually with careful examination of this figure, students easily can see that purple-eye males must have the genotype GS; scarlet-eye males have the genotype Gs; and gray-eye males have the genotype gS. However, sometimes they need coaching to understand that wasps that inherit both mutations (gs) will have the eye color of the first pigment formed in the eye-color synthesis pathway. If the gray pigment is formed first, then these double-mutants will have gray eyes.

The scarlet-eye and gray-eye (oyster) mutations we use show very tight linkage, (~0.3 cM apart). Purple eye males in the F_2 generation are rare, and introductory students are often biased toward an independent assortment hypothesis. It is helpful to have euthanized adult *Nasonia* with the various eye colors available so that students can compare the eye colors. Gray (oyster) eyes can be differentiated from the dark purple of wild type eyes by placing the wasp on its dorsal or ventral

surface and looking for transparency in the facets of the eye. Wild type eyes are an opaque dark purple. Other eye color mutations are commercially available including black eyes and orange eyes. High light intensity is needed to differentiate black eyes from purple and our students found it very difficult. However, orange eyes are easily differentiated from scarlet, gray and purple eyes. When crossed, the commercially available scarlet and orange strains show independent assortment in the F2 generation (χ^2= 1.07, p = 0.587, N=522). With this cross, students can determine the mode of inheritance and also deduce which pigment is formed first, i.e., orange.

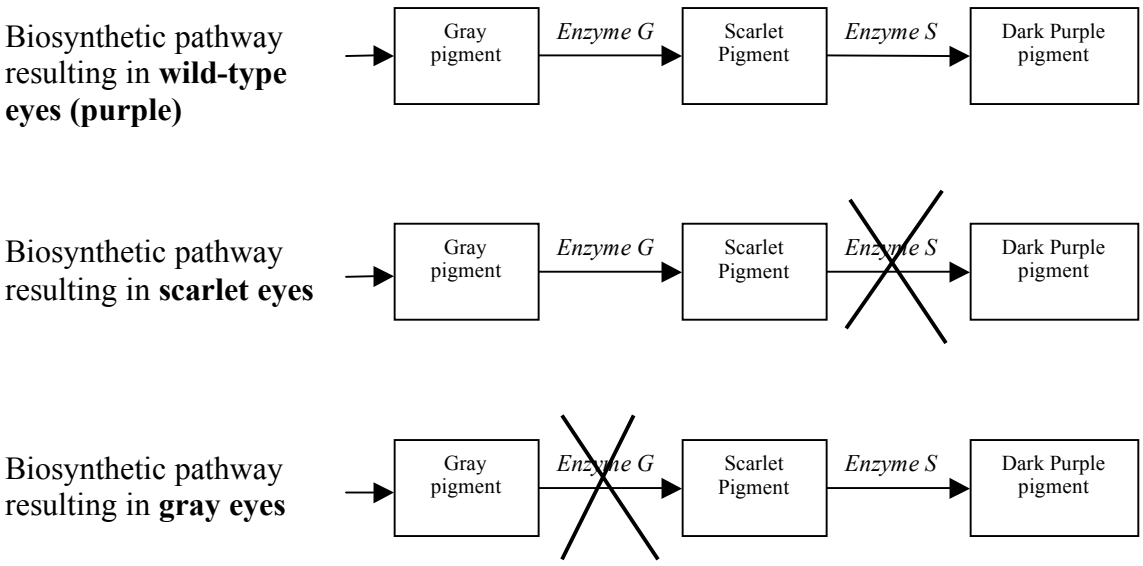

Figure 3. An example of a possible *Nasonia vitripennis* eye color synthetic pathway in which two genes, one producing Enzyme S and the other producing Enzyme G, have epistatic interactions. Enzymes sequentially transform intermediate products until the wild-type pigment (purple) is formed. A loss-of-function mutation in a gene coding for one of the assembly line enzymes results in an assembly line that is blocked at that point. The last successfully produced eye-color pigment accumulates and becomes the eye color phenotype. A non-functional enzyme and a stopped pathway is indicated by an **X**. Illustration adapted from Ward's Natural Science *Nasonia* project kit and used by permission.

Nasonia Handling and Care

Individual living adults and pupae are best handled with a paintbrush. Adults usually crawl, seldom flying. Adult escapees can be captured quickly by placing an inverted tube over them and allowing them to crawl up the tube. A needle probe can be used to move euthanized pupae and adults but can damage living *Nasonia*. Sorting can be done in a Petri dish or on paper. By creasing the paper in half, you can funnel living pupae or euthanized adults easily into a secondary container.

To euthanize pupae or adults for sorting, place the culture in a -20^{0}C freezer for at least two hours or 20 minutes in -80^0 C freezer. You can kill living pupae (both *Nasonia* and *Sarcophaga)* you no longer need by freezing them or disposing in a 10% isopropyl alcohol morgue.

To maintain cultures, transfer male and female adults into new vials or test tubes and add fresh *Sarcophaga* hosts after allowing 18-48 hours for mating. Incubate at 25^0 C under continuous

fluorescent lights. We use 12x100 mm test tubes for low-density (6-8 wasps) cultures or *Drosophila* vials for larger populations. Low-density cultures result in predominantly female populations. Higher-density cultures result in more equal numbers of males and females. Male-only populations can be generated by sexing and isolating virgin female pupae, and adding hosts after the pupae eclose.

To transfer adults, invert one test tube or vial over the other and shake the adults down into the new tube or vial. When using a *Drosophila* vial, we use coarse netting inserted between the new and old vials to catch and remove the used *Sarcophaga* puparia. Test tubes are capped with absorbent non-sterile cotton balls. Foam stoppers are used with *Drosophila* vials.

Adult *Nasonia* can live up to three to four days without food. Fresh *Sarcophaga* hosts can be refrigerated up to several months at 4^0 C before use. Hosts only will be parasitized if in certain developmental stages (from the white to yellow to brown eye stage). If the body has begun to darken or bristles developed, the host is too old. Hosts that you open to assess must be discarded. (Werren, 2000) If you do not intend to add hosts immediately to adult *Nasonia,* feed the adult wasps 4% sucrose. We place a drop or two on the absorbent cotton balls that plug test tubes.

The rate of development is influenced by temperature. To speed up development, increase the temperature up to 28^0C. Development can be slowed with cooler temperatures; the development of pupae and adults can be successfully arrested by refrigeration at 4^0C. We place the vials in airtight containers in the refrigerator to reduce desiccation and have successfully used pupae that have been refrigerated 6 weeks and adults refrigerated 2 weeks. Longer periods of time of refrigerated storage have been reported for both developmental stages. Werren (2000) describes how to induce diapause and has successfully refrigerated cultures for up to 18 months.

Materials

- Dissection microscope
- Box magnifier or Petri dish containing euthanized male and female pupae, adult gray-eye male and scarlet-eye female wasps and adult purple-eye wasps.
- 1 12x100-mm test tube of 3 living adult gray eye males for mating
- 1 12x100-mm test tube of 3 living adult scarlet eye virgin females for mating
- Refrigerated fresh *Sarcophaga* pupae
- 1-2 Petri dishes
- Quartered paper
- Paintbrushes
- Needle probe
- Forceps
- Empty 12x100-mm polystyrene test tubes
- Cotton balls
- 10% Isopropyl alcohol morgue
- Fluorescent light banks

Suppliers:

Carolina Biological Supply Company
(http://www. carolina.com)
phone: 1-800-334-5551
• *Nasonia vitripennis* cultures: shipped as white wasp pupae inside host puparia. Cultures contain 3-5 puparia.
  ○ FR-17-3425 Oyster eye: We call these gray eyes because it is more descriptive to our land-locked students.
  ○ FR-17-3428 Scarlet eye: The results are the same as Ward's scarlet eye: epistasis, linkage with crossing over.
• S*arcophaga* pupae: FR-17-3480: 100-150 pupae
	Wards Natural Science
	(http://www.wardsci.com)
	phone: 1-800-962-2660
	• Box magnifier (4X clear plastic box) 25 W 1480
	• *Nasonia vitripennis* cultures: shipped as white wasp pupae inside host puparia. Cultures contain 3-5 puparia.
	  ○ 87 W 6752 Scarlet eye: The results are the same as Carolina's scarlet eye when crossed with Carolina's oyster eye (gray eye): epistasis, linkage with crossing-over.
	  ○ 87 W 6753: Orange eye: when crossed with scarlet eye shows epistasis, and independent assortment with the orange pigment formed before scarlet.
	• S*arcophaga* pupae: 87 W 6701: 50 pupae

References

Werren, John. 2000. "*Nasonia*." University of Rochester, New York. http://www.rochester.edu/College/BIO/labs/WerrenLab/nasonia/ accessed 5/06.

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About the Authors

Cheryl Knox received her Ph.D. in Plant Physiology from Texas A&M University. Her career at the College of St. Benedict/St. John's University has taken her from faculty member of the biology department to Academic Dean. She returned to her first love, teaching, in 2004 as an Associate Professor and currently teaches introductory biology and non-major's biology courses.

Carol Jansky received a B.S. in biology and medical technology from the College of St. Benedict. After a career in medical technology she returned to her alma mater to coordinate introductory biology laboratories in the joint biology department of the College of St. Benedict and St. John's University.