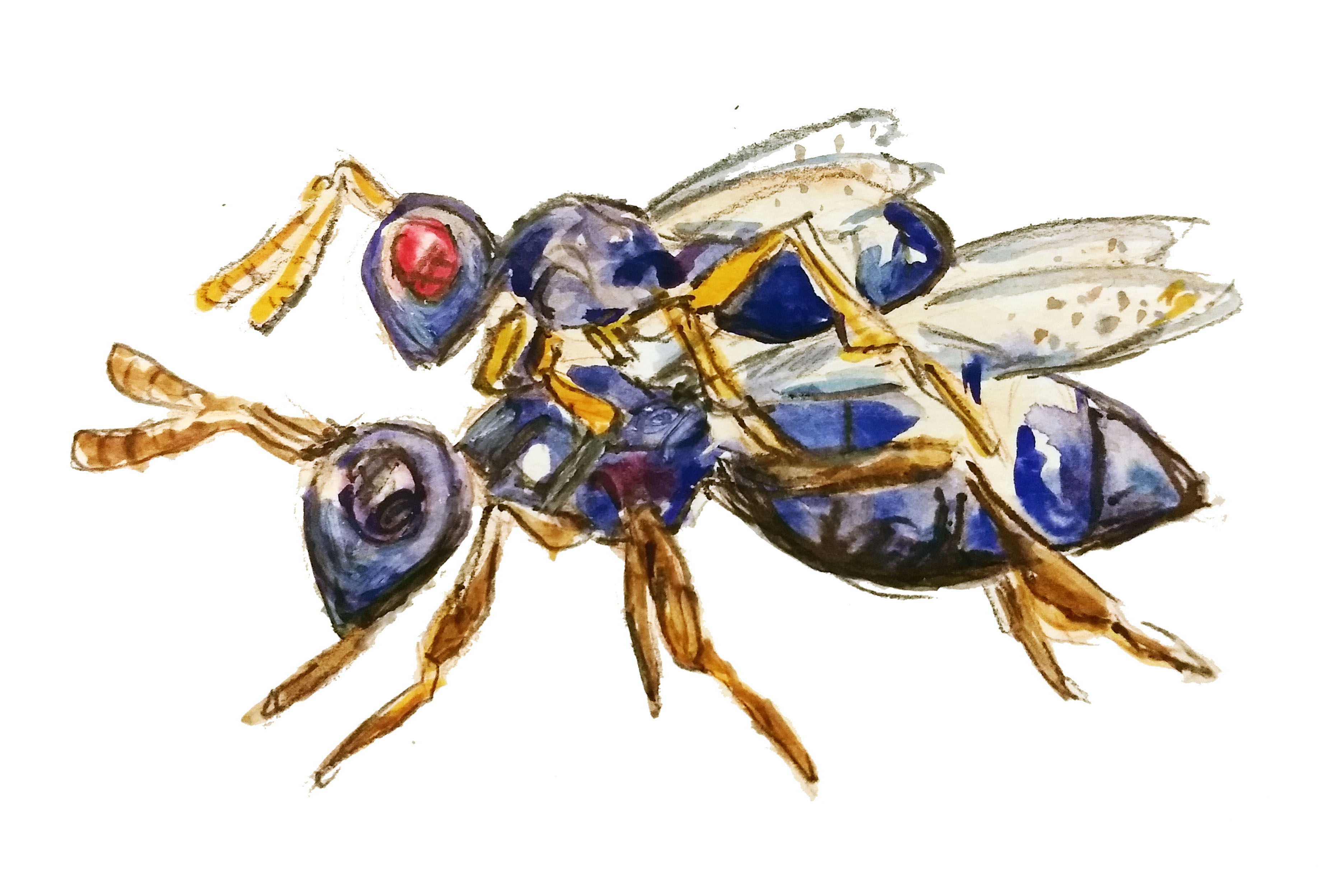
**Haplodiploid Inheritance**

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**Outline Note to instructor:** If the practical is being conducted with pre-existing data only and a physical experiment will not take place, in the student version delete sections that are in red and add the sections that are in blue.

**Activities**

Part 1: Read background on sex determination and inheritance. Answer questions about background. Follow instructions to conduct experiments to learn about haplodiploid sex determination and inheritance using pre-existing datasets.

Part 2: Collect experimental data. Analyze data. Answer questions about haplodiploidy using inferences from data and primary literature.

**Materials**

For each team of 3-4 students:

1 work laptop

1 dissection microscope

3-4 small paintbrushes (for transferring wasps)

40 small vials

Fresh cotton (for plugging small vials)

1 small vial of 50 virgin female *Nasonia vitripennis* of the AsymCx strain (has purple eyes)

1 small vial of 50 virgin male *Nasonia virtripennis* of the AsymCx strain (has purple eyes)

1 small vial of 50 virgin female *Nasonia vitripennis* of the STDR strain (has red eyes; some sources may list this strain as “*scarlet*”)

1 small vial of 50 virgin female *Nasonia vitripennis* of the STDR strain (has red eyes; some sources may list this strain as “*scarlet*”)

1 large vial of fresh blowfly pupae (~250 hosts). Commercially purchased standard hosts are *Sarcophaga bullata* or *Calliphora* spp.

1 rack for small tubes

3-4 micro picks (for opening hosts to collect offspring)

1 marker for labelling tubes and racks

Rolls of masking tape for labelling racks and euthanizing wasps (as many as needed, to share)

Squirt bottles of 70% ethanol for cleaning and euthanizing wasps (as many as needed, to share)

Paper towels for cleaning

**Objectives**

-Learn the principles of haplodiploid sex determination and inheritance

-Learn background of *Nasonia vitripennis* system

-Learn experimental handling of *Nasonia vitripennis* as a laboratory model

-Learn analyses of experimental data

-Infer aspects of parasitoid and *Nasonia vitripennis* biology from experiments experimental data

-Answer questions on biological knowledge based on literature and experimental data

**Goal**

Enhance knowledge of students on parasitoid biology, experimental design, and data analyses.

**Feedback**

Instructor will provide feedback on: interpreting primary literature of given materials to answer questions, following the scientific method to design and conduct an experiment, and drawing conclusions from experimental data.

**Haplodiploid Inheritance – part I**

Maleness and femaleness in sexual species is conserved across the animal kingdom, but the underlying mechanisms for sex determination are surprisingly diverse. Consider just the example of the Vertebrata (Figure 1 below). Generally, the sex of mammals and birds is determined by an individual being heterogametic or homogametic for sex chromosomes that are strongly distinct in size, shape, and genetic content (mammals XX= female and XY=male; birds ZW=female, ZZ=male). In some species the Y or W chromosome is absent (XO=male,, ZO=female), the “O” indicating “zero”, Reptile, amphibian, and fish species have various additional sex determination mechanisms that can rely on environmental cues, such as temperature, photoperiod, or food availability.

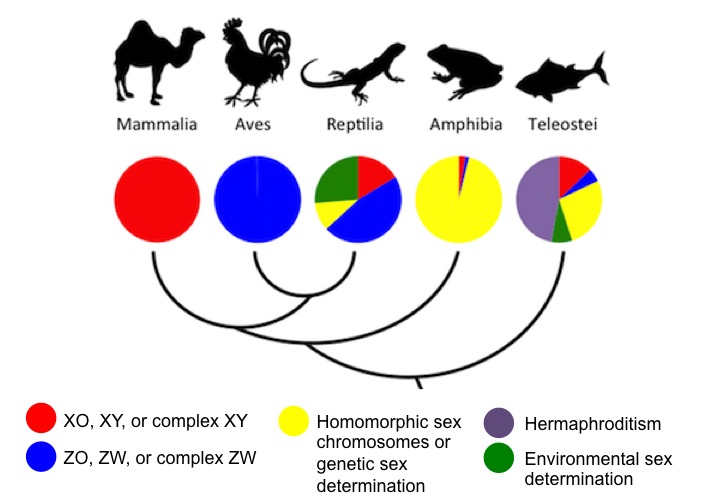
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Figure 1. Diversity of sex determination in the Vertebrata. Mammals and birds have heteromorphic sex chromosomes that are divergent in size and genetic content. Homomorphic sex chromosomes in other groups have fewer differences and are more difficult to distinguish. Hermaphrodites have both male and female reproductive characteristics. Environmental sex determination includes both abiotic factors (temperature, photoperiod, location in an aggregate of individuals) and biotic factors (resource availability, population density). Figure adapted from Bachtrog et al., 2014.

For the Invertebrata, sex determination modes are even more diverse, even among closely related species. The sex determination mechanism determines to a large extent how traits are inherited from males and females to their offspring. For example, genes on the Y chromosome are only inherited from father to sons and genes on a father’s X chromosome can only be passed to daughters (Table 1). The expression of the gene depends on whether it is dominant or recessive. In diploid organisms, dominant traits are visible when an allele is present in a single copy, but recessive traits are only visible when both alleles are present. Both dominant and recessive traits are directly visible in a haploid. In this practical we explore a specific mode of sex determination and inheritance with the parasitoid wasp *Nasonia vitripennis*.

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| **Box 1 - *Nasonia vitripennis* biology**    *Nasonia* are small parasitoid wasps (Hymenoptera: Pteromalidae) that parasitize blowflies and fleshflies (see Figure 2 for the life cycle). The best-studied species is *Nasonia vitripennis.* These wasps have become a popular model organism for evolutionary biologists and geneticists. They are easy and quick to culture (generation time of 14 days at 25ºC), their sex is easy to determine, and they can be handled without anesthesia. Similar to the *Drosophila* fruit fly system, there are also many known genetic mutations with easily observable phenotypes, which is helpful for tracking inheritance patterns. For example, there are mutations that cause different eye colors, wing morphology variations, and body colors (with many that have been mapped to specific chromosomes).  In nature, *Nasonia* uses different fly species as its host (mainly *Protocalliphora* spp*.*). The fly larvae feed on blood of bird chicks and pupate in the bottom of nests. After young birds have fledged, *Nasonia* parasitize the fly pupae. However, in the laboratorium, *Nasonia* can instead be reared on commercially available hosts such as *Calliphora* spp. and *Sarcophaga bullata*.      Figure 2. Life cycle of the parasitoid wasp *Nasonia* *vitripennis* and their *Protocalliphora* fly hosts. The fly larvae live inside bird nests and carcasses. After pupation they are vulnerable to parasitisation by *Nasonia*. A *Nasonia* female lays about 10-40 eggs per fly pupa, the wasp larvae will feed on the fly pupa. The wasps will emerge after approximately two weeks at 25ºC (unless they are in developmental diapause, a winter survival strategy that occurs when mothers are exposed to shorter photoperiod, in which case they can be kept in 5ºC storage for two years), The males emerge first. The males have short wings and cannot fly. They mate with the emerging females, which in nature would disperse and colonize new hosts. Flies are ~5-10 mm. The wasps are 2-3 mm. |
| Box 2 – How to handle Nasonia Even though *Nasonia* are wasps, they cannot sting people. Males and females of *N. vitripennis* are easily distinguished: males have short rudimentary wings, while females have larger wings that extend over the tip of the abdomen (see Figure 3). Males also have yellow antennae and legs females have black antennae and darker legs. Additionally, females have an ovipositor at the ventral side of the abdomen.  Adult wasps can be shaken in small numbers from the main vial onto the working surface. The wasps are more visible on a light-colored background. When the wasps are walking around on the working surface, you can isolate them by placing a small vial over them. Most of the times the wasps will walk up the vial. If necessary, the wasps can be pushed by using a small paintbrush. The vials are closed off with cotton wool and placed in Styrofoam trays. The wasps are cultured in a climate-controlled room at 25ºC (Figure 4). You may euthanize loose wasps with masking tape or spraying and wiping with 70% ethanol, and wasps still in tubes by freezing overnight.  Differences between *Nasonia vitripennis* ♂ and ♀   |  |  | | --- | --- | | ♂ | ♀ | | Short wings, shorter than abdomen | Large wings, longer than abdomen | | Yellow antennae and legs | Black antennae and partially black legs | | Tip of abdomen rounded | Tip of abdomen pointed with ovipositor |   Description: femaleDescription: male  Figure 3. Morphological differences between the *Nasonia vitripennis* sexes. Photo by P. Koomen    Figure 4. *Nasonia* cultures incubated in plastic vials in an incubator |

**Table 1** Mendelian inheritance of mutations in diploid XY sex determination systems (adapted from <https://medlineplus.gov/genetics/understanding/inheritance/inheritancepatterns/>, accessed 29 November 2020)

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| --- | --- | --- |
| **Inheritance Pattern** | **Description** | **Graphic representation** |
| Autosomal recessive\*  A=unaffected allele  a=mutant allele  \*autosome=any non-sex chromomsome | Both copies of the gene in each cell have mutations. Carrier parents of an individual with an autosomal recessive condition each carry one copy of the mutated gene, but they do not show the mutant phenotype. |  |
| X-linked recessive  X=unaffected chromosome  Xa=mutant chromosome | In males (who have only one X chromosome), one altered copy of the gene in each cell is sufficient to cause the condition. In females (who have two X chromosomes), a mutation would have to occur in both copies of the gene to cause the disorder. A characteristic of X-linked inheritance is that fathers cannot pass X-linked traits to sons. |  |
| Y-linked  Y=unaffected chromosome  Ya=mutant chromosome | The mutated gene that carries the disorder is located on the Y chromosome of a male’s cells. Because only males have a Y chromosome, a mutation can only be passed from father to son. |  |

**Question 1** Using Table 1, for a diploid species with XY sex determination, give the percentage of offspring of each phenotype/genotype combination for a mutation that follows A) autosomal recessive inheritance, for two carrier parents B) X-linked inheritance, for a carrier mother and unaffected father C) X-linked inheritance, for an effected mother and an unaffected father and D) Y-linked inheritance, for an effected father and a unaffected mother.

1. *25% AA unaffected, 50% Aa carrier, and 25% aa mutant (offspring will be half male, half female, with autosomal genotype/phenotype percentages being the same for each sex)*
2. *25% XaX carrier female (half of the females), 25% XX unaffected female (half of the females), 25%XaY effected male (half of the males), 25% XY unaffected male (half of the males)*
3. *50% XaY effected male (all males), 50% XaX carrier female (all females)*
4. *50% XX unaffected female (all females), 50% XYa effected male (all males)*

**Exercise: Conduct experiment to investigate sex determination and inheritance modes in *N. vitripennis***

Above you will find two boxes describing the biology of *N. vitripennis* (Box 1)and how to handle the wasps (Box 2). Before you start the experiment, **read these two boxes.** Divide yourself into groups of 3-4 students. Each group will receive vials with virgin males and virgin females of the STDR and AsymCx isolines, and fresh hosts (fly pupae). Read the following note about *Nasonia* isolines, and then conduct the experiment below (label all tubes specifically and carefully).

*Nasonia* isolines

Like most animals, *Nasonia* produce haploid gametes (male sperm and female eggs) through meiosis. Meiotic recombination is normally reflected in new genotype and phenotype combinations appearing in offspring. However, in isolines such as AsymCx and STDR, inbreeding has occurred over so many generations that there is no longer any variation for genetic loci. When breeding within the isoline, offspring all have the same genetic background and so express the same traits even if recombination occurs. This consistency is advantageous in breeding experiments, as phenotypic changes can easily be linked to the introduction of novel genetic material if the isoline is outcrossed to a different background

Use the experimental data provided to answer the questions in Part II.

1. First note the phenotypic difference(s) between STDR and AsymCx. This should be discernible without a microscope but examination at 2.5X with individuals still in the tube may be helpful.
2. Host N=5 to N=10 virgin females of each background separately, on three hosts each (a smaller sample size makes it easier to complete data collection but gives less statistical power).
3. Put a virgin STDR male and a virgin STDR female in a small tube together for up to 10 minutes. The male should climb on top of the female to court her, she accepts him and raises her abdomen, he climbs down to copulate, he climbs up and performs post-copulation head-nods, and he walks off. This will indicate a successful mating, and the female is then hosted on three hosts. If this does not occur within 10 minutes, replace the male or female with a fresh individual. Repeat 10 times. To save time, group members can run matings concurrently.
4. Do step 4 for the AsymCx background.
5. Do step 3 for a virgin STDR male and a virgin AsymCx female each cross.
6. Do step 3 for a virgin AsymCx male and STDR female each cross.
7. Have the instructor place your racks in standard conditions for the next two weeks.

**Haplodiploid Inheritance – part II**

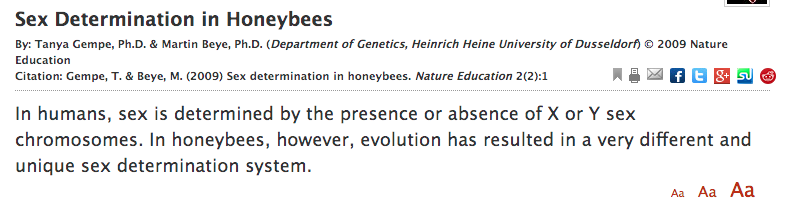
Two weeks later, using the information in Box 2, sort male and female offspring of each female and according to phenotypic difference(s) noted from step 1) of the experiment. Record the data for your individual group, and add to the pooled class datasheet.

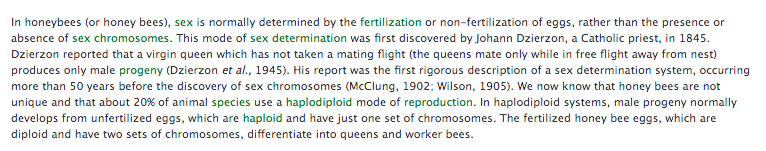
**Question 2:** Based on the data (individual groups and the pooled class),do *Nasonia vitripennis* have diploid chromosomal sex determination (e.g. XY)? Why or why not?

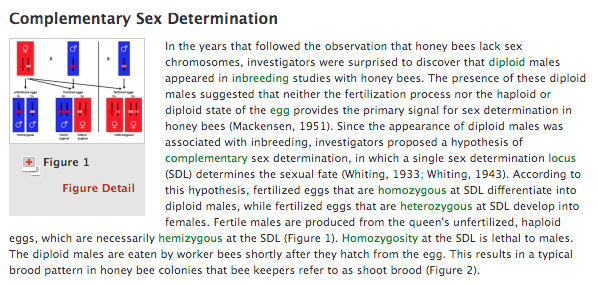
*For both AsymCx and STDR backgrounds:* N. vitripennis *does not have diploid chromosomal sex determination because virgin females produce offspring (all male) by themselves. Otherwise, they would be incapable of reproducing. Since they produce only haploid eggs, males are haploid. The mated females, which fertilized their eggs from sperm provided by a mate, have mostly female offspring that are diploid, but a small proportion of male offspring will still be produced. These are haploid offspring that resulted from some eggs going unfertilized. It is possible that individual group data is skewed towards higher male production due to variation in handling, but the pooled class data should compensate for this. Students may infer at this point that this is the “haplodiploid” mode of reproduction in the title of this practical (but if not, should do so in question 4).*

**Question 3**: Based on the data (and Table 1 and answer for Question 1), what is the inheritance pattern of the phenotypic marker(s) you noticed in part 1) of the experiment? I.e. is it (are they) sex-linked?

*Students should have data indicating 100% red eye offspring for STDR (males only for the virgin females and for both male and female offspring for the mated cross). Similarly they should note 100% purple (or dark-eyed offspring) for AsymCx, again all male for the virgin females and for both sexes for the offspring of the mated females. The STDR male and the AsymCx female cross will have the same type of offspring as the AsymCx mated cross, and the AsymCx male and the STDR female cross will result in a large number of purple (dark-eyed) female offspring, and a small proportion of red-eyed male offspring. Based on their answer for question 2, students should be able to rule out X-linked or Y-linked inheritance, because N. vitripennis does not have sex chromosomes, and because the males and females of the STDR male and AsymCx female cross have the same phenotype. From the AsymCx male and the STDR female cross, the red-eyed male offspring indicate that the red phenotype is only apparent in hemizygotes (haploids) or homozygotes (diploids) (the female offspring of the pure STDR cross) for this marker. Therefore the inheritance pattern is autosomal recessive with red being the recessive phenotype and purple being the dominant (wildtype) phenotype.*







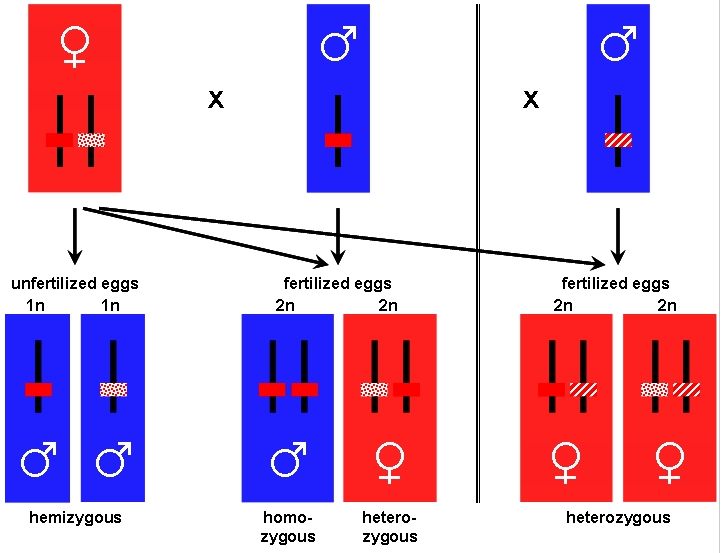


Figure 1 (of Gempe & Beye 2009). Genotypes and sexual fate under the system of complementary sex determination found in many hymenopteran species (ants, bees, wasps, sawflies). Males derive from unfertilized eggs and have only one sex determination allele (marked by different colored bars). Fertilized eggs with two different sex-determining alleles (heterozygous) develop into females. Diploid males arise from fertilized eggs that are homozygous for the same sex-determining allele. These diploid males arise most commonly under inbreeding conditions in which the father has an allele in common with the mother.

**Question 4** *Nasonia vitripennis* belongs to the order Hymenoptera (bees, ants, wasps, sawflies). After reading about the best-studied hymenopteran species, the honeybee, and considering your own experimental results, A) what mode of reproduction do you think *N. vitripennis* has? Why? B) Do you think *N. vitripennis* has complementary sex determination? Why or why not?

*A) Based on the experimental results,* Nasonia vitripennis *has a haplodiploid mode of reproduction like the honeybee. For both the AsymCx and STDR backgrounds, virgin (unmated females) produced all male offspring that can be assumed to be haploid. The mated females are like honeybee queens in that a large number of their eggs were diploid and fertilized, and developed into female offspring.*

*B) Students should conclude that* N. vitripennis *does not have complementary sex determination. The isolines AsymCx and STDR have already been inbred for many generations without producing diploid males. Furthermore, in their own experiments when they crossed isoline females with males of their own background, which have the same genetic background at every locus albeit at a hemizygous haploid state instead of a homozygous diploid state, they also did not produce diploid males. However, students may suggest the possibility of undetected male diploidy e.g. lethality at early developmental stages corresponding to complementary sex determination. While this does not actually occur for* Nasonia*, it would be a valid suggestion, and students can suggest a means of detecting it. Inbred females that are outcrossed to a different background should have more male offspring, for example (recovering the male fraction that would have died from diploidy).*