Manual

Working with Nasonia

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Nasonia vitripennis is a small parasitic wasp that is involved in a symbiotic relationship with a host organism known as *Sarcophaga bullata,* or a flesh fly. More specifically, Nasonia is a "parasitoid", which is a parasite that completely destroys its host. By interrupting the life cycle of Sarcophaga, Nasonia is able to use its host for nutrition and shelter.

I. Sarcophaga Life Cycle

The Sarcophaga host, which is harmless to humans, is associated with animal carcasses. A non-virgin female Sarcophaga will lay a large number of eggs on a carcass. The eggs rapidly develop into larvae (maggots), which will feed on the dead animal tissue. The larvae infest the carcass for nine to ten days before they wander into the soil surrounding the carcass in search of a dry place to pupate. Pupation involves the development of a protective "shell" or casing around the body of the Sarcophaga. Within the casing, a pupa develops into an adult fly in another nine to ten days (figure 1).

As stated earlier, the parasitic Nasonia is capable of interrupting the Sarcophaga life cycle. An adult female Nasonia will lay her eggs within the Sarcophaga pupal casing. This provides a well-protected, nutrient-rich environment for developing Nasonia as they feed on the tender Sarcophaga pupa. The environment within the Sarcophaga pupal casing creates ideal conditions for the completion of the Nasonia life cycle.

II. Nasonia Life Cycle

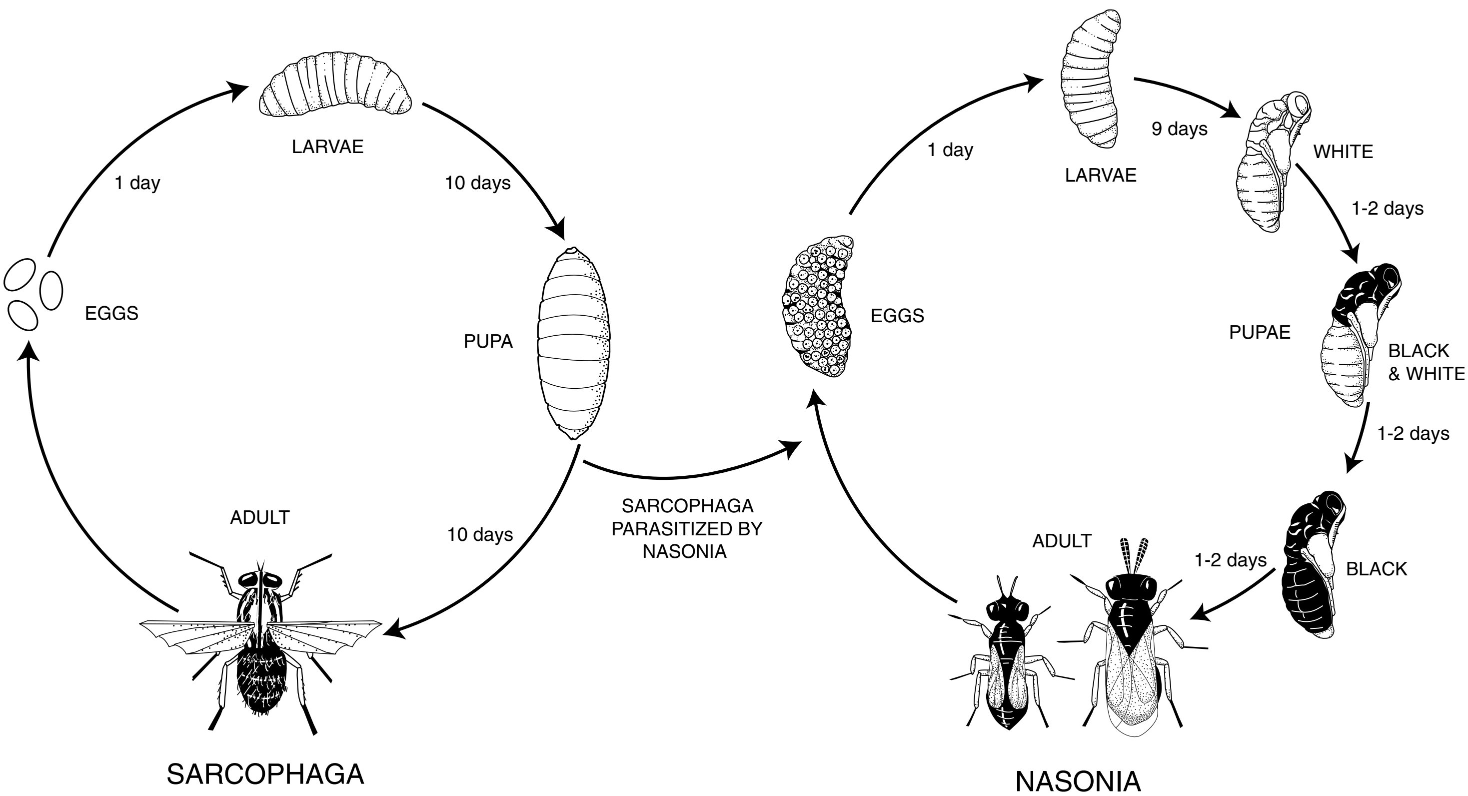
Nasonia have a relatively short life cycle depending on environmental temperatures and exposure to a light source (figure 2). A female Nasonia lays her eggs in a Sarcophaga pupa by distending a long, thin structure, known as an ovipositor, from her abdomen. The female then deposits thirty to fifty eggs through the ovipositor into the pupal casing. The eggs develop into larvae within one or two days. The larvae then feed on the Sarcophaga pupa as a nutritional source. The larvae will continue to develop over the next eight to nine days, and then pupate, forming a protective casing around their bodies. There are three developmental stages of Nasonia pupae: "white", "black and white", and "black" stages, and each stage is more developed than the previous. Nasonia remain in the pupal stage for three to four days. In a process known as eclosion, "black" pupae will break free from their pupal cases as adults. These adults eventually emerge by chewing a hole through the host casing.

Figure 1

Sarcophaga Life Cycle

Figure 2

Nasonia Life Cycle



III. Benefits of Using Nasonia for Scientific Research

There are many reasons why *Nasonia vitripennis* is an excellent research organism. For example, its small size, short life cycle, and ability to produce many offspring are advantageous in the study of animal behavior and genetics. Many scientists prefer to work with Nasonia over other research organisms because they are very simple to culture, handle, and maintain.

A. Arresting Development of Nasonia and Sarcophaga

The developmental rates of Nasonia and Sarcophaga are dependent upon temperature and exposure to light. Standard culturing conditions are 25°C and 24 hours of light per day. Cooler temperatures and less exposure to light will slow down the development of both organisms. A temperature of 4°C is ideal for arresting development. By controlling temperature and exposure to light, scientists are able to carefully plan their experiments for maximum efficiency.

B. Observation of Developmental Stages and Timing

The various stages of Nasonia development can be observed at anytime by cracking open a parasitized Sarcophaga pupa. Each stage is an indicator of the amount of time before the Nasonia reach adulthood. This ease of observation makes it convenient for scientists to schedule their laboratories around weekends and holidays, etc.

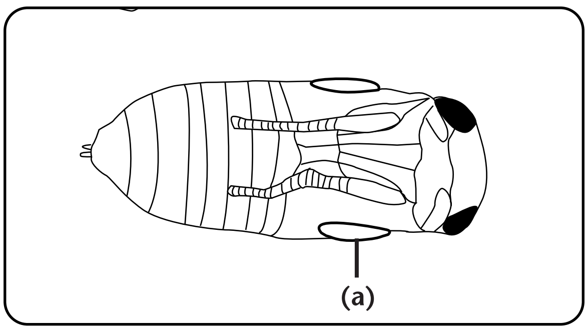
C. Virgin Isolation and Sex Determination

Virgin Nasonia can be identified while they are still pupae. This allows scientists to easily collect, isolate, and store virgins until needed. Collection and isolation are simple in any of the three pupal stages because the Nasonia are immobile. There are often several distinct differences between male and female Nasonia in the pupal stage (figures 3 and 4). Male pupae are smaller in body size and have short wings (a). Female pupae have a larger body size, long wings that wrap around the abdomen (b), and a visible ovipositor (c).

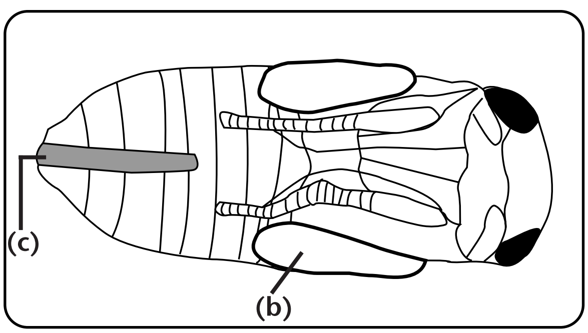
Figure 3

Abdominal View

Male and Female Pupae



Male

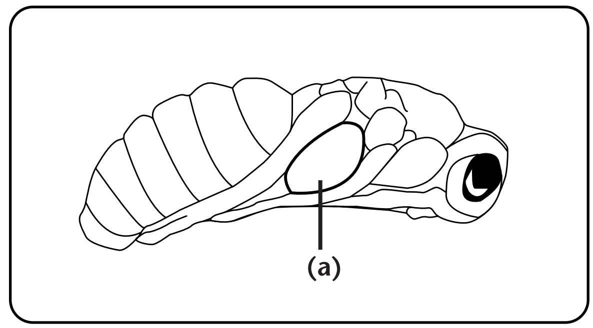


Female

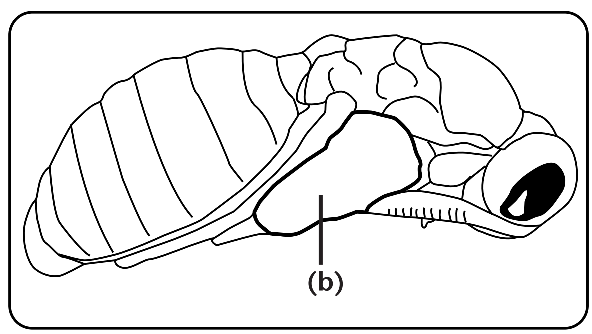
Figure 4

Lateral View

Male and Female Pupae



Male

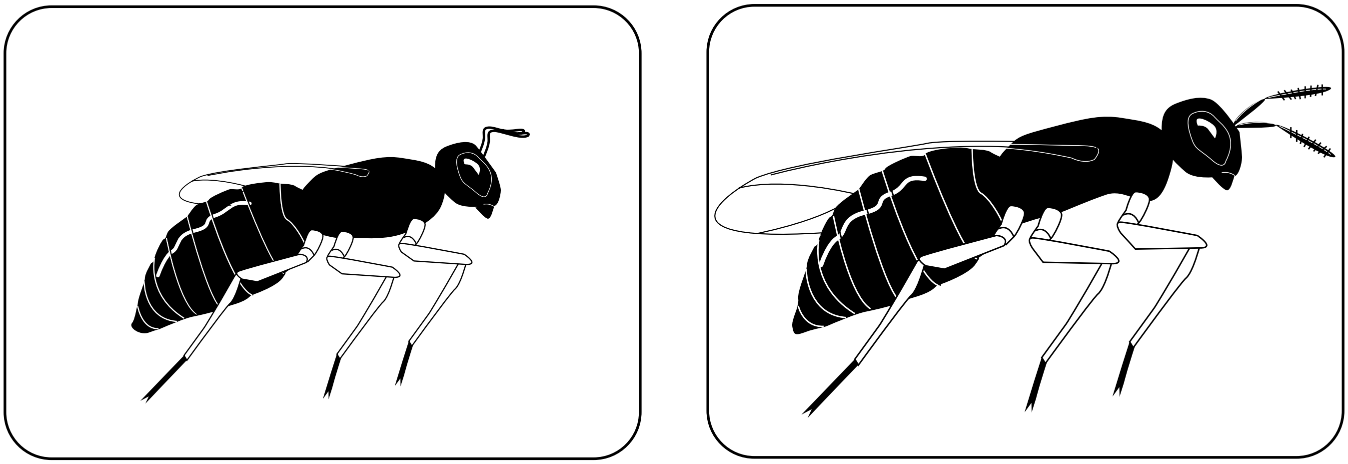


Female

Once Nasonia reach adulthood these differences remain except that female wings now extend beyond their abdomen (figure 5). There are also other differences between males and females that become apparent, such as body color, leg color, and type of antennae. Females have a dark-colored body, legs, and antennae. Males have a body with a green sheen, yellowish legs, and light-colored antennae.

Figure 5

Lateral View
Male and Female Adult



Male Female

IV. Culturing and Handling of Sarcophaga and Nasonia

The basic requirements for culturing and handling the host organism, Sarcophaga, and Nasonia are as follows:

> ***Important!*** *The Nasonia and Sarcophaga are both harmless to humans, although there have been some reports of allergic reactions to the hemolymph in the flesh fly pupae. Teachers and students are encouraged to wash their hands before and after handling Nasonia and Sarcophaga.*

A. Sarcophaga Hosts

1. The non-parasitized Sarcophaga hosts are sent in the early pupal stage. Refrigerate the hosts immediately upon arrival. Refrigeration at 4°C will keep the hosts viable for three to four weeks.

   Important! *If unrefrigerated, adult Sarcophaga will emerge in approximately ten days.*

   Keep the hosts refrigerated at all times. This will ensure that they remain as young and tender as possible for parasitization.

2. At the time of culturing, remove only the number of hosts needed.

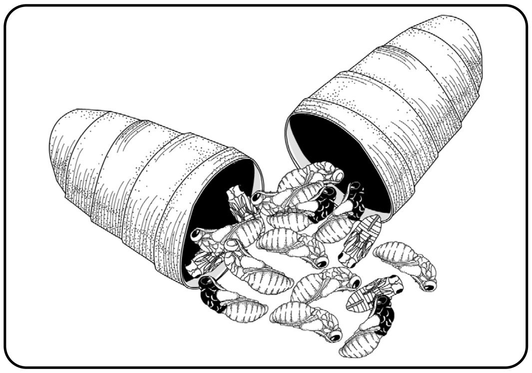
B. Nasonia

> ***Note:*** *Nasonia are shipped in the pupal stage while still in the host.*

1. Upon receipt, you may want to check the stage of the Nasonia. To do this, remove a parasitized host from the culture tube. Gently crack open the thin pupal casing of the host which contains the Nasonia pupae (figure 6).

Figure 6

Cracking Open Host



2. Once the Nasonia pupae are exposed, note the stage that they are in. This should be the stage that the rest of the culture is in. Place the cracked open host containing the Nasonia pupae back into the culture tube.

   Note: *The pupae may be at varying stages, so it is important to note the stage that the majority of the pupae are in for planning purposes. If larvae are present, they will not develop after the host has been cracked open. If adults are present they can be used immediately for sub-culturing. Assume that these adults are non-virgins, so do not use them for experiments needing virgins.*

3. If you need virgin Nasonia for your experiments, they should be sexed in the pupal stage (see Section III, Part C: Virgin Isolation and Sex Determination).

4. Adult Nasonia will be needed for culturing, so you will need to incubate the pupae at room temperature until they become adults. Use the guidelines in table 1 to plan accordingly.

   Note: *Ideally, while incubating, Nasonia should be exposed to cool, indirect light for 24 hours a day. Fluorescent light works well. Light that emits too much heat will harm the Nasonia.*

Table 1

Pupal Stage Upon Arrival	Days until Adulthood (if incubated at room temperature)
Whites	3 – 4
Black and Whites	2 – 3
Blacks	1 – 2

Time Consideration: *If, at any time, you want to slow down the developmental rate of the pupae to fit into your schedule, you can place the culture tubes in the refrigerator. A refrigeration temperature of 4°C is ideal.*

5. Once they emerge as adults, allow the males and females at least 24 – 48 hours together before sub-culturing. This will ensure that the males and females have had a chance to copulate.

6. In order to subculture, obtain a clean, empty culture tube.

7. Obtain the stock culture of Nasonia and tap the tube on the table so the Nasonia fall to the bottom. Remove the cotton plug from the stock and invert the empty culture tube over it. Nasonia will naturally crawl up from the stock culture tube into the empty culture tube. Make sure that the lips of the tubes are lined up so that the Nasonia do not escape.

8. Allow some Nasonia to enter the empty culture tube. Make sure that females are present because they will be the Nasonia that parasitize the hosts (see Section III, Part C: Virgin Isolation and Sex Determination).

   Note: *Alternatively, you can temporarily immobilize them by placing the culture tube in the refrigerator for fifteen to twenty minutes. This will slow the Nasonia down enough so that you can transfer them by hand to new culture tubes, etc.*

9. Quickly replace the cotton plugs in both tubes.

10. Tap the new culture tube on the table so the Nasonia fall to the bottom. Remove the cotton plug and add several hosts. Replace the cotton plug.

11. Label the new culture tube appropriately with the Nasonia strain and the date.

12. Allow two or three days for the females to lay their eggs. Depending on conditions, the larvae will pupate after 9–10 days. After this time, a parasitized host can be cracked open, and the Nasonia offspring can be isolated before reaching adulthood (see Section III, Part C: Virgin Isolation and Sex Determination).

13. Within 14–15 days after the pupa is parasitized (if incubated at room temperature with 24 hours of light), adult offspring will emerge.

    Important! *If you are working with various strains of Nasonia, make sure that you only work with one strain at a time. Thoroughly clean your work station of one strain before working with other strains.*

14. Dispose of Nasonia as needed (see Section IV, Part D: Disposal).

C. Nutrition

1. Adult Nasonia can live without food for three or four days, so plan accordingly. If you need to feed the emerged Nasonia, you can add a drop of 4% sugar solution (4 g sucrose/100 ml of water) or honey to the culture tube. For best results, use the Nasonia as soon as they reach adulthood so feeding is not an issue.

D. Disposal of Nasonia and Sarcophaga

Nasonia and Sarcophaga can be disposed of using two methods:

1. Create a "morgue" by partially filling a container with 10% isopropyl alcohol. Drop the Nasonia and Sarcophaga to be euthanized into the alcohol and cover the container with a lid.

OR

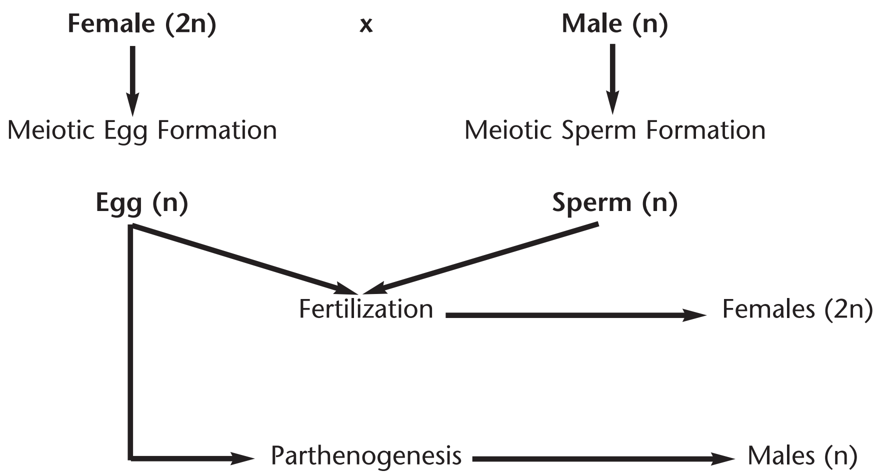
2. Place Nasonia and Sarcophaga to be euthanized into a container and cover it with a lid. Place this container into a freezer and let it sit for at least two hours.

3. After euthanization, the container used for either method can be disposed of in a wastebasket.

V. Haplodiploid Inheritance in Nasonia

The unique reproductive strategy of Nasonia is known as haplodiploid inheritance and is also common to many other insects, such as bees. Nasonia exhibit a form of asexual reproduction, known as parthenogenesis, in which an organism is able to develop from an unfertilized egg. As a result, when the eggs are not fertilized, offspring will be haploid (n) and male. In turn, when the eggs are fertilized by a male, the chromosome number is restored to the diploid state (2n) and the offspring will be female (figure 7).

Figure 7



Once a female has copulated, she will store sperm until needed. She is able to “choose” when to fertilize her eggs. For example, the offspring from a non-virgin female are usually 95% female and 5% male. This occurs because the female chooses to fertilize 95% of her eggs with the sperm she has stored, resulting in diploid female offspring. The other 5% of her eggs remain unfertilized and therefore develop as haploid males.

VI. Genetics of Nasonia

When performing experimental genetic crosses, be sure to keep careful records of all experiments, giving dates of hosting, results of crosses, and comments about technique.

As an example, a virgin female wild type Nasonia is crossed with a male scarlet eye mutant Nasonia. The dark eye of the wild type is a dominant trait and is represented by “D”. The scarlet eye is recessive and is represented by “d”.

Since the F_1 generation is the result of two homozygous strains of Nasonia, the wild type parent has only one possible characteristic in the gametes, wild (D). The scarlet parent also has only one possible type of gamete (d). The F_1 generation can therefore be diagrammed as follows:

Note: *The male Nasonia is haploid, so the chromosome carries only one allele for the scarlet-eye trait, indicated by the "d" in the diagram. The "–" represents the missing chromosome in the haploid male. The female Nasonia carries two alleles for the dark-eye trait, indicated by "D". When the male and female alleles combine, they produce dark-eyed females ("Dd"). The female Nasonia is also able to produce dark-eyed male offspring through parthenogenesis, represented by "D–".*

Scarlet-Eyed Male (P)

Wild-Type Female (P)

	d	**–**
D	***Dd***	***D–***
D	***Dd***	***D–***

Since the dark-eyed trait is dominant, all of the F_1 Nasonia will appear to be of the wild type, even though some ar not homozygous strains.

A brief survey of methods for working with Nasonia is all that has been possible within the scope of this manual. W carry a full line of activities using Nasonia, and we urge you to take advantage of these activities, which will provid greater detail and a more in-depth look at the versatile Nasonia.