

Chapter 10

Investigative laboratories in cell biology using a host-parasitoid model: the tobacco hornworm, *Manduca sexta*, and the braconid wasp, *Cotesia congregata* – Introduction to the system

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Contents

Introduction	181
Materials	182
Standard material needed	182
Material needed for initiation to the model	182
Notes for the Instructor	182
Background information	182
Life cycle of <i>Manduca sexta</i>	182
Life cycle of <i>Cotesia congregata</i>	183
Raising parasitoid wasps	184
Infection of hornworms	185
Initiation to the model of study	
Student Outline	192
Acknowledgements	193
Literature Cited	194
Appendix A: Saline Recipe	195
Appendix B: List of Suppliers and WEB references	196
Appendix C: Ordering/Infection Calendar	197

Introduction

This exercise is totally inquiry-based. No initial schedule is given to the students and at the outset no information is given other than the scientific names of the organisms. The rule is that students first have to make observations and then ask questions on their own that they must try to answer. The life cycle of the parasitoid wasp is briefly discussed. Only after students observe and compare their control and parasitized hornworms (about 2 weeks after the first laboratory) is the life cycle of the caterpillar discussed (See background information below). Students are expected to ask questions and decide (as a group) what they are interested in studying next. This pedagogical technique makes the laboratory lively because students are motivated to respond to their own questions. Because there are two weeks between infection and observations at the start of the semester, the second laboratory can be used to present an overview of the techniques used by cell biologists in the research laboratory. Doing this provides background information for students to later design experiments to test their self-generated hypotheses. However, students in introductory classes may still have a difficult time asking questions and may require more guidance, which the instructor can offer by scheduling the exercises in a more regular manner/order. Further, although the original idea was to bring the students from the organismal to the molecular level, the instructor can guide students in a set path as much as he/she desires. For example, if the instructor is more “molecularly” oriented, he/she can easily direct the students towards the study of DNA or proteins; if he/she is more “cell structurally” oriented, students can be guided towards this aspect of cellular biology (i.e., light and electronic microscopy). Students can also learn basic principles such as osmosis or microscope calibration using this system. In short, this is a model system that can be custom-designed to fit the instructors’ interests and the class level. Significantly, the system is a common and important research model and thus there is abundant background literature

Host-parasitoid wasps

available. The number of students in the laboratory should not be too high (10 is ideal, 15 is still all right, and 20 is difficult to handle without assistance).

The caterpillar, *Manduca sexta*, is easy to raise and eggs or larvae can be purchased from Carolina Biological Supply Company; the parasitoid wasp, *Cotesia congregata*, can be collected initially from the wild and then easily maintained and raised in the laboratory (see “raising hornworms” and “hornworm infection” section below).

Material

Standard Material Needed

* Per class:

- CO₂ tank with regulator and attached Buckner funnel with a cover (a Petri dish works well - if no CO₂ is available, access to a freezer is required, but CO₂ is *strongly* advised)

* Per group: (groups of two students work best)

- Scissors (1)
- Forceps (2)
- Petri dish (1)
- Pasteur pipette w/ bulb (1)
- Small dish (watch glass type)
- Saline for dissections (see recipe in Appendix A)
- Dissecting microscope with *good* light and black background

Material needed for introduction to the system:

- All standard materials listed above
- Non-parasitized caterpillars (2nd-3rd instars - 2 per student)
- Adult wasps

Notes for the instructor

Background information

Life cycle of Manduca sexta

After hatching from the eggs, the caterpillars will go through 5 larval instar stages (a 6th instar may occur). About 20 days after hatching the larvae will wander and then pupate to become adults within a few weeks (Binkley et al., 1975; URL 1). Only the larval stages are of interest to this laboratory.

Life cycle of Cotesia congregata

The adult parasitoid wasp, *Cotesia congregata*, oviposits in the hornworm larvae (2nd or

3rd instar) and at the same time injects symbiotic viruses into the hemocoel of the host. The viruses knock down the internal defensive responses of the hornworm (Buron and Beckage, 1992; Beckage, 1997). The eggs hatch in the host hemocoel within two to three days and simultaneously release special cells from the egg chorion (Buron and Beckage, 1997). These special cells, called teratocytes, grow to become giant cells visible by the naked eye and are the focus of most of the laboratories developed using this system. The function of teratocytes is not yet known but several roles have been hypothesized, such as inhibiting pupation or being involved in the depression of the host's defensive system (see in Buron and Beckage, 1997). Following hatching in the caterpillar, the wasp larvae will undergo 2 molts inside the host caterpillar's hemocoel and, after 12 to 16 days post oviposition, the 3rd instar wasp larvae will emerge out of the caterpillar and spin cocoons from which the adult wasps fly about 4 to 8 days later.

Raising the hornworms

- Set environmental chamber on a 17L:7D photoperiod at 27°C. *Note: Caterpillars can also be raised at room temperature.*
- Obtain *Manduca* eggs (Carolina Biological Supplies # BA -14-3880). *Tip: Obtain the eggs 2 weeks before you want to infect the caterpillars (see Appendix C).*
- Eggs will hatch within 2-4 days – Young larvae need food but can't be manipulated easily. *Tip: Set the eggs on a tilted cover in a larger cup with food and cover. This way the young larvae will not stick to the food after hatching, which is often a death sentence for them.*
- About a week after larvae hatch, isolate each larva in individual small cups (Bioserv # 9052 & 9050).
 - Place a small square (about 1 cm²) of food in each cup. (Ingredients to mix as needed: Bioserv # F9783B or pre-made diet: Carolina Biological Supply Company # BA-14-3908). *Tip: I use 1 liter of food per about 40 larvae per week.*
 - Food needs to be replaced about every 2-3 days. (Keep an eye on larvae – do not allow any mold to grow and remove excrement. *Tip: If the larvae are not well fed their growth will be retarded.*
- About 5 days later hornworms will be too big for the small cups and will need to be moved to larger cups (Bioserv # 9076 & 9077).
 - Caterpillars need to be fed about every other day. Parasitized caterpillars will not feed as much. *Tip: Don't be tempted to give too big a piece of food at once...mold is not far off and is just waiting for you to take a vacation!*

Raising parasitoid wasps

- Place cocoons in small cups in a cage at room temperature (See Figure 10.1). *Note: Wasps will emerge out of cocoons within about 4 days and adult wasps will live about 1 week.*
- Dot honey (wasp food) in a Petri dish; set the dish in the cage. Clean and refill the Petri dish every 2-3 days (see Figure 10.2).

Host-parasitoid wasps

- Place in cage a small cup containing gaze saturated with water (= wasp drink). This must be refilled every 2-3 days (see Figure 10.2).

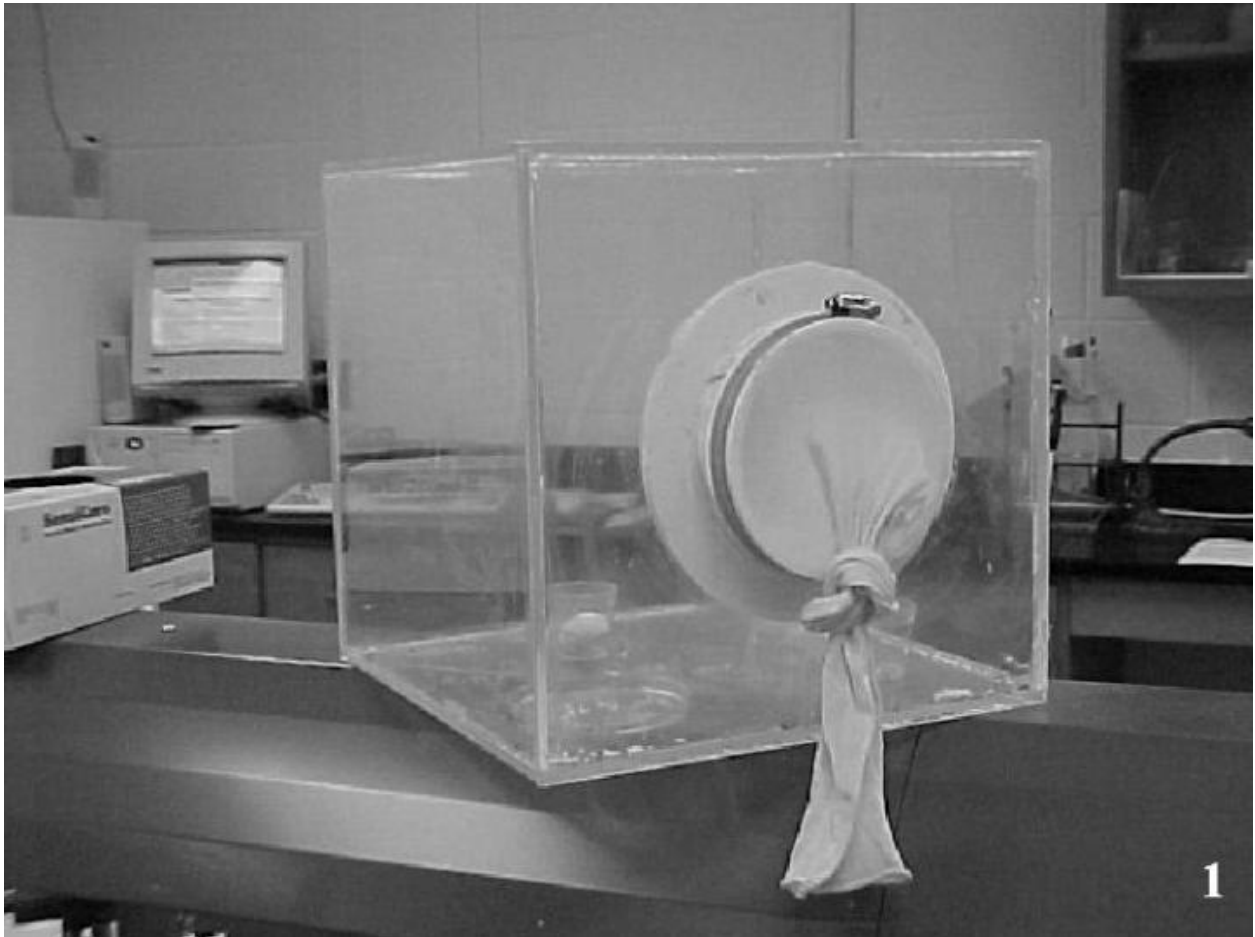


Figure 10.1. Example of homemade Plexiglas cage for raising *Cotesia congregata*.

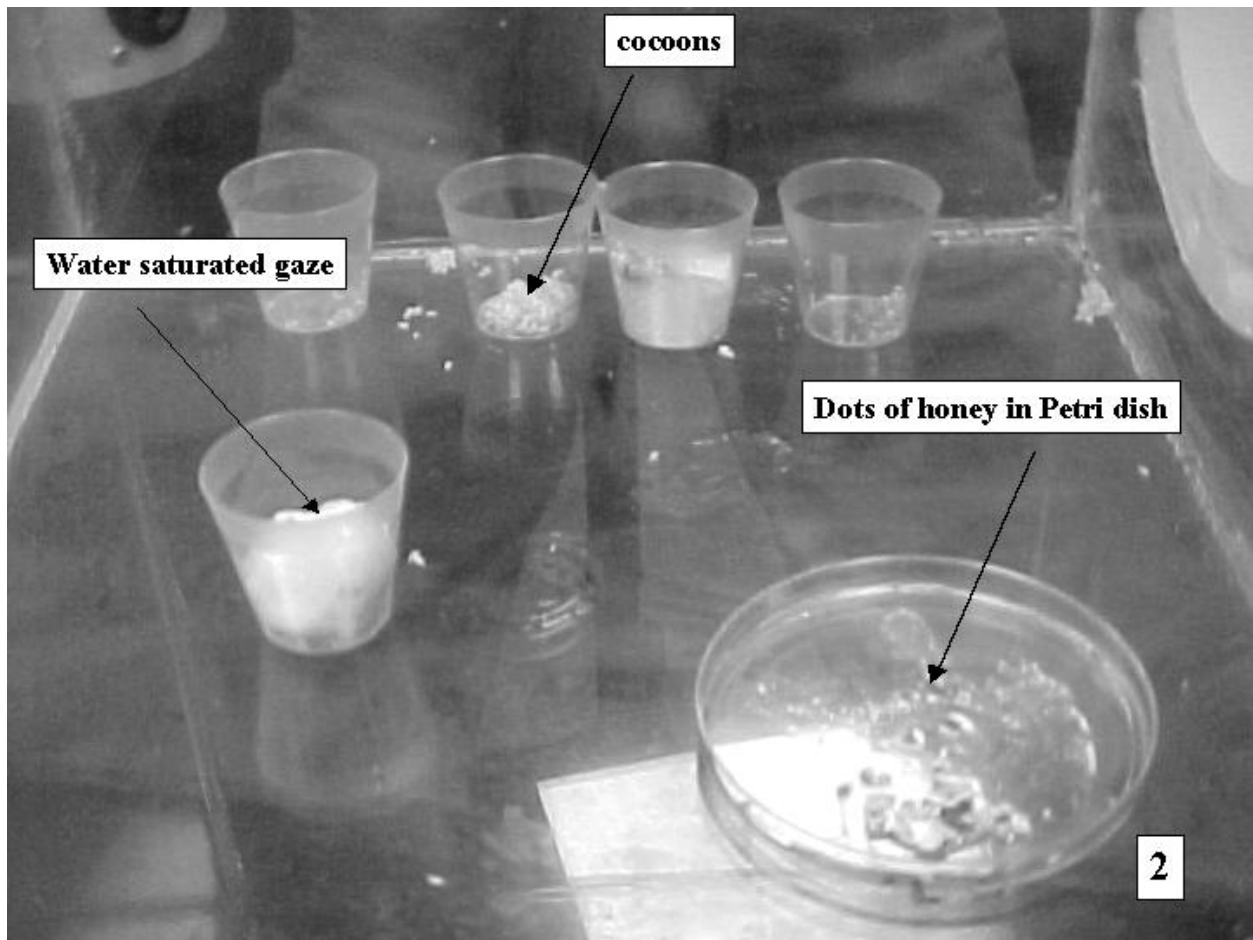


Figure 10.2. Display of cups for water and food inside the *Cotesia congregata* rearing cage.

Infection of hornworms

- Use 2nd to 3rd instar hornworms. (These should be about 2 cm long.) *Tip: Infect 2 week old caterpillars – wasps will emerge two weeks later.*
- Place caterpillars into a cage with adult wasps (several at once is OK).
- Watch wasps oviposit. (It usually takes only about 15 seconds to a few minutes). *Note: If caterpillars are stung too much they will die. Limit oviposition to 1 to 3 wasps per caterpillar if possible by quickly removing the caterpillar.*
- Remove the parasitized caterpillars and replace them in large cups with food.
- Parasitized caterpillars will not eat as much as non-infected ones although they still need food regularly.
- Cocoons will appear about 15 days later. (See Appendix C for ordering/infection calendar).

Introduction to the hornworm-wasp system

Day 1: Infection of caterpillars (See “infection of hornworms” section above.)

Day 15: Observations of effect of parasitism on caterpillars.

Host-parasitoid wasps

Gross observations (color, size, weight of the caterpillars) (Compare Figures 10.3 and 10.4.)



Figure 10.3. 5th instar non-parasitized caterpillar (much larger than parasitized ones).

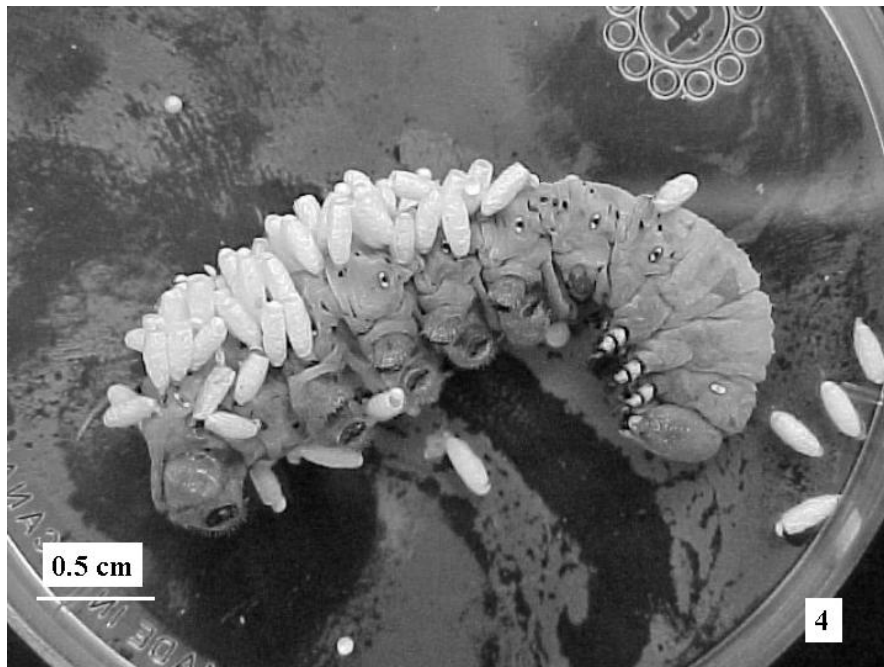


Figure 10.4. Parasitized caterpillar showing wasp cocoons. (The parasitized larvae are smaller and much less active than control caterpillars - not at same scale as Figure 10.3).

Dissections (See Figures 10.5 and 10.6). After anesthetizing caterpillars in CO₂ (See Materials), remove cocoons and dissect caterpillars in Petri dish. Dissections must be done when everything

else is ready because hemolymph will melanize within 10-15 minutes and will then not be usable. Keeping hemolymph on ice will slow down melanization. Begin dissection at the posterior end between the legs. Cut tegument as superficially as possible to avoid touching the intestine and keep the dissection clean. Keep cutting until the head is reached (See arrows). Hemolymph and teratocytes will spill out of the body and can then be recovered in a watch glass using a Pasteur pipette. To isolate lots of teratocytes (See examples of laboratory exercises below), gently flush the cavity of the caterpillar with saline -- more teratocytes will spill out. Teratocytes will settle at the bottom of the watch glass. Gently swirl the watch glass to concentrate teratocytes in the middle and pipette the amount of teratocytes needed for the exercise. *Tip: Don't flush the caterpillar cavity too roughly since pieces of fat body will come loose and teratocytes will be difficult to isolate.*

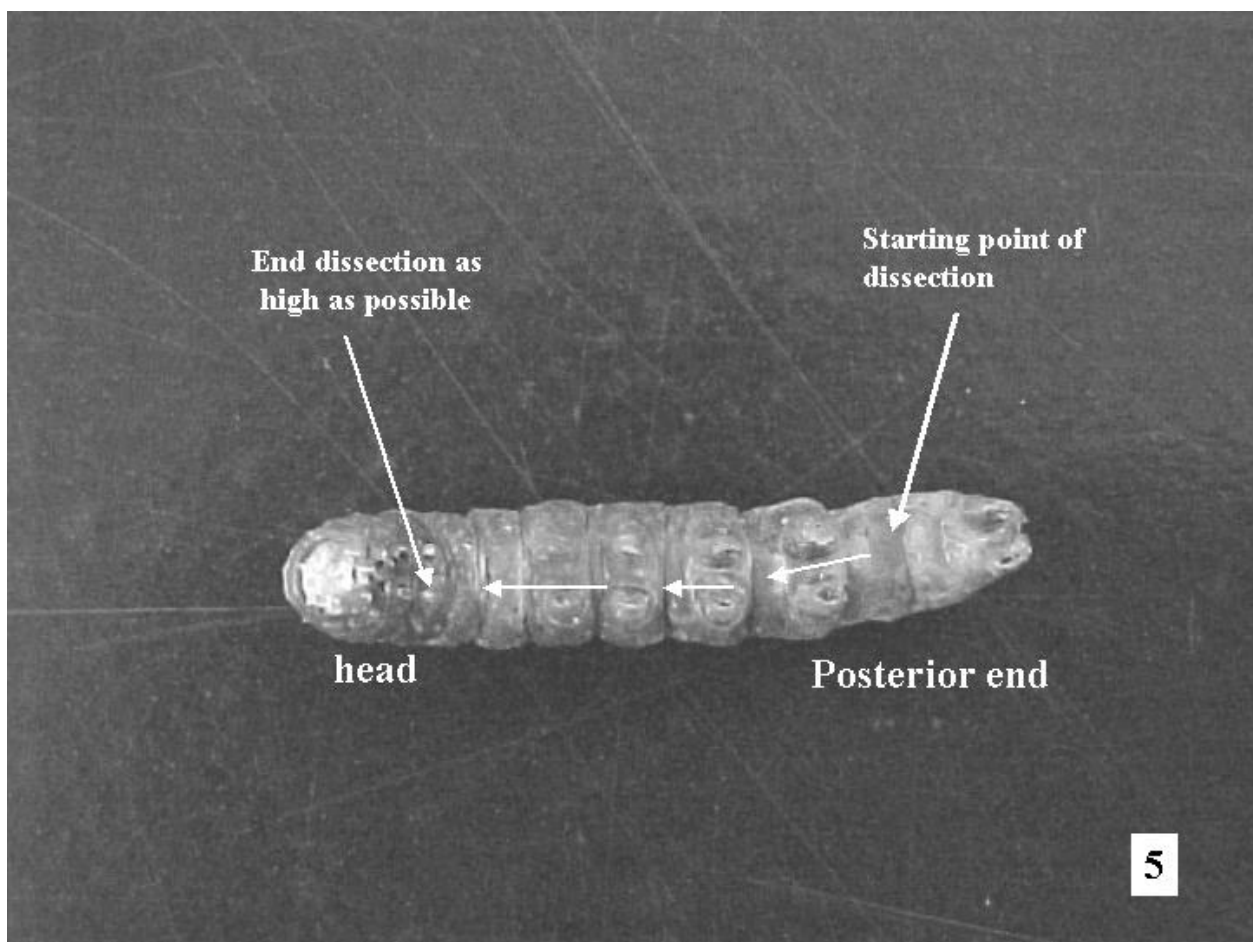


Figure 10.5. How to dissect a caterpillar (ventral view).

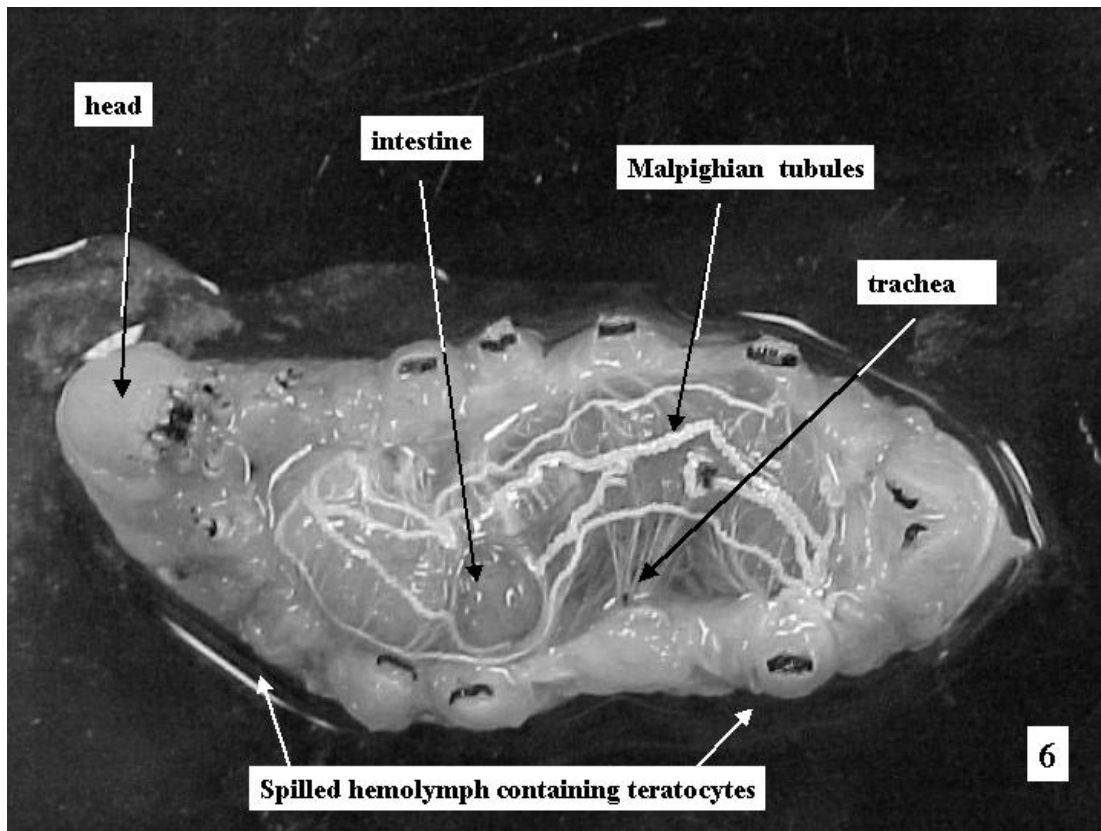


Figure 10.6. Dissected caterpillar (ventral view).

Examples of cell biology exercises possible with this model (Material that works best in parentheses)

- Use of bright field versus phase contrast microscopy / digital image capturing (teratocytes, hemocytes) (Figure 10.7)
- Calibration of light microscope (teratocytes)
- Osmosis (teratocytes) (Figures 10.8a,b)
- Protein electrophoresis using SDS-PAGE (teratocytes, wasp larvae)
- Scanning Electron Microscopy (teratocytes, wasps, caterpillars) (Figures 10.9 a,b)
- In vitro cell culture (teratocytes or wasp larvae in Grace's medium) (Buron and Beckage, 1997). This can be followed by protein electrophoresis of teratocytes and culture media to observe teratocyte products in host hemolymph.

Note: This is a more difficult exercise. Many cells are needed to see teratocyte products and the results may be disappointing to the non-experienced student. Nevertheless this is a good opportunity to talk about the limitations of some techniques (e.g., coomassie blue vs silver staining).

- Histopathology (pieces of infected caterpillars)
- Immunofluorescence (e.g. cytoskeleton of teratocytes or hemocytes)

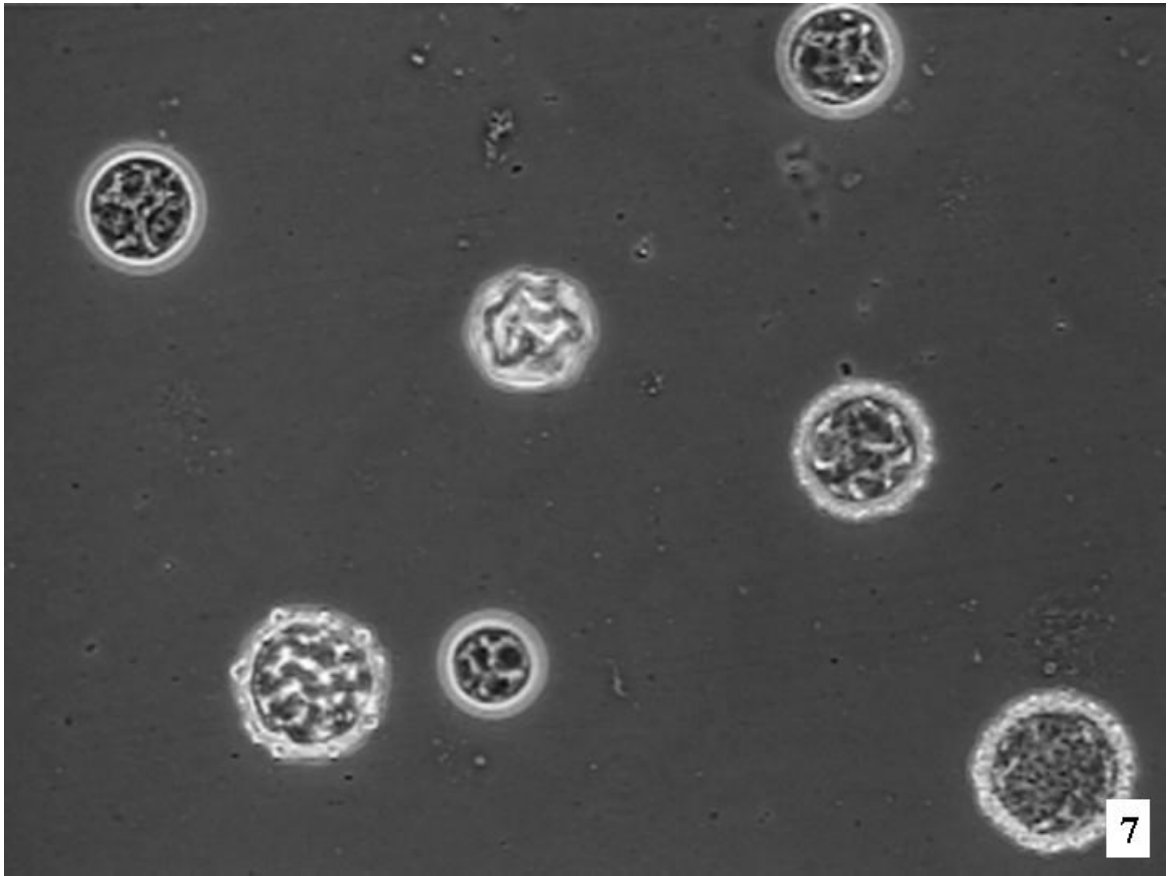


Figure 10.7. Teratocytes in saline observed with phase contrast microscope.

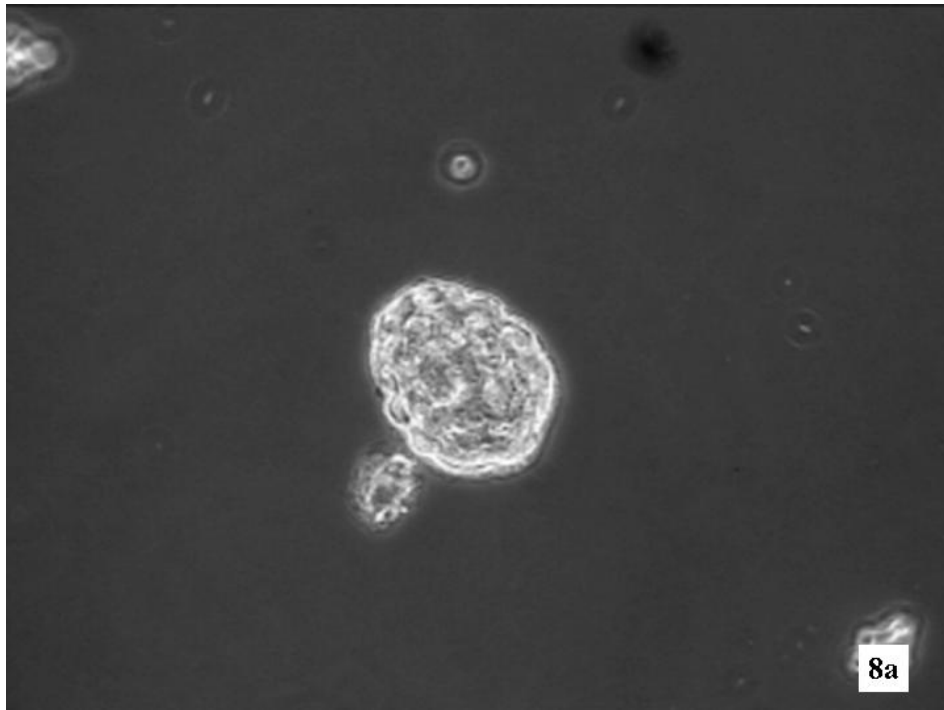


Figure 10.8a. Teratocyte in 20% NaCl solution.



Figure 10.8b. Teratocytes in water showing blebs.

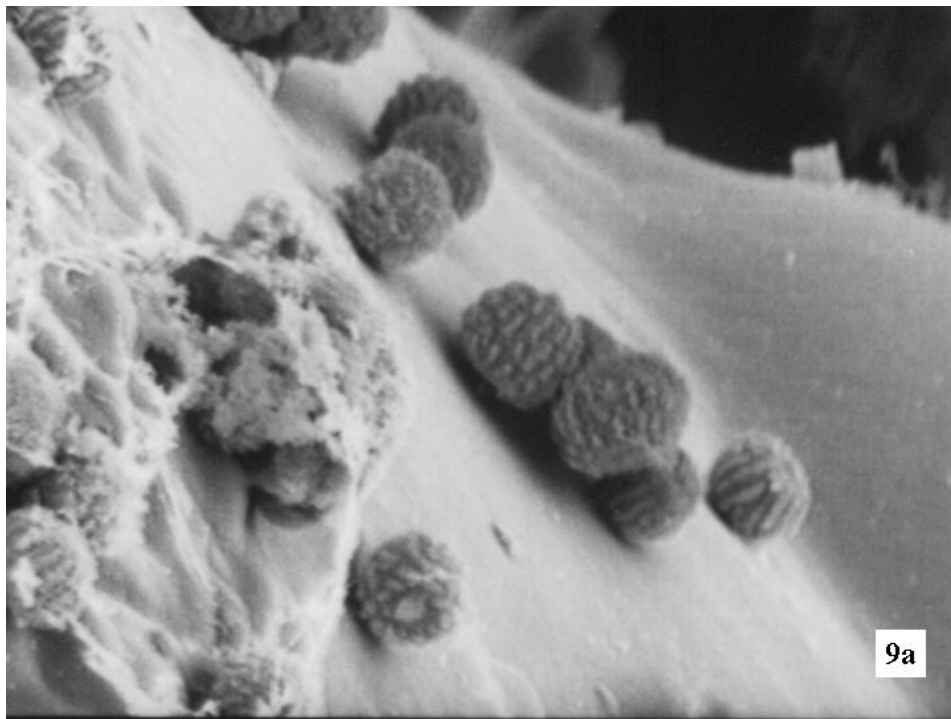


Figure 10.9a. Scanning Electron Micrograph of teratocytes.

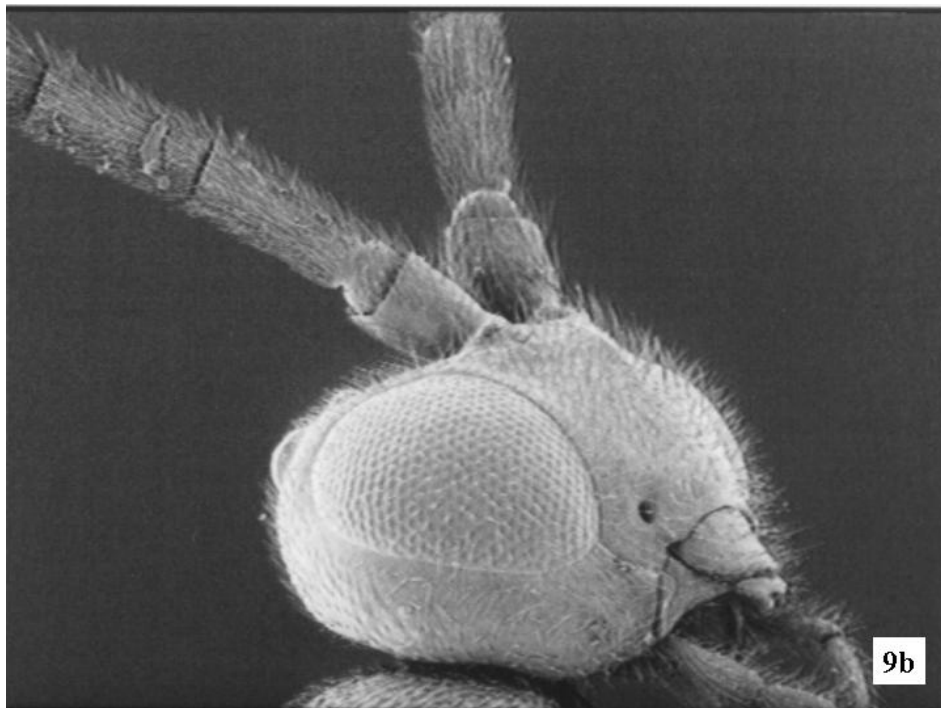


Figure 10.9b. Scanning Electron Micrograph of *Cotesia congregata*.

Student Outline

This laboratory will initiate you to a host-parasite model that you will be using throughout the term. The host is the tobacco hornworm, *Manduca sexta*, and the parasite is a braconid wasp, *Cotesia congregata*. These wasps are totally inoffensive to humans (i.e., they will not sting you). However, they do oviposit into the caterpillars by stinging them. On the first day of this exercise you will have the wasps parasitize the hornworm following the instructions given by your professor. Two weeks later you will observe what happened to the parasitized caterpillars.

Note to instructors: Students will notice a difference in overall sizes. Infected caterpillars are usually lighter in both weight and color and are smaller. However, some students believe that if the caterpillar is full of wasps it should be heavier. It is a good opportunity for the students to manipulate numbers and test their hypotheses. Neither Table 10.1 below nor the following instructions should be given in the handout if instructors want their students to initiate the questions.

Schedule:

Day 1: Parasitization of caterpillars

Day 15: Observations

Gross observations:

Caterpillars	Parasitized (n)	Control (n)
Average weight (+/- Standard Error)		
Average length (+/- Standard Error)		

Table 10.1. Class average size and weight of parasitized caterpillars versus controls (n = sample size).

Build a bar graph showing standard errors. Compare control and parasitized caterpillar weights and lengths using a t-test. Are the differences observed between the two groups statistically significant?

Dissections: Note your observations in the table below. In writing, compare and contrast what you have observed in the infected and uninfected caterpillars.

Parasitized caterpillars	Control caterpillars

Table 10.2. Observations recorded after dissecting the caterpillars.

Based upon your observations, outline one or more questions that you would be interested in pursuing next.

Question A:

Question B:

Question C:

Compare and discuss your questions with the other person(s) in your group. Decide amongst yourselves which question(s) the group would most like to address in future laboratories. Questions should be in the form of a hypothesis. Based upon your understanding of the techniques used by cell biologists (as discussed in lecture and laboratory), do your best to outline an experiment (as a group) to test your selected hypothesis. Be ready to present your hypothesis and an outline of your test to the class.

Your group's selected hypothesis:

Outline of experiment to test your group's chosen hypothesis:

One person in your group will be asked by the instructor to present this information (hypothesis and test outline) to the class. The class will then, as a whole, decide what question will be most interesting to pursue during the next laboratory. The techniques to be used will be discussed.

Next Laboratory Exercise (class decision):

Outline of the experiment to be carried out during the next laboratory:

Acknowledgments

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- Riddiford, L. M., A. T. Curtis, and K. Kiguchi, 1979. Culture of the epidermis of the tobacco hornworm *Manduca sexta*. *Tissue Culture Association Manual*, 5, 975- 985.
- URL 1: <http://www.ifas.ufl.edu/~insect/field/hornworm.htm>

Appendix A *Manduca* Saline (Riddiford et al., 1979)

Stock solution # 1:

100 mL (10 x) concentrated salts – use distilled H₂O

Can be stored for 2 months at 4°C

40mM NaCl

400 mM KCl

180 mM MgCl₂

30 mM CaCl₂

Stock solution # 2:

150 mM PIPES buffer (Sigma P8203) (100mL) pH 6.5

Can be stored for 2 months at 4°C

To make 1 liter of *Manduca* saline:

1 liter volumetric flask

About 500 mL dH₂O

83 g of sucrose (optional)

1 g PVP (Sigma P 6755)

100 mL stock solution #1 (concentrated salts)

10 mL stock solution # 2 (PIPES buffer)

Swirl to mix

Add H₂O to make 1 L.

Autoclave / refrigerate

Host-parasitoid wasps

Appendix B Suppliers and WWW sites of interest

Bio-Serv: www.bio-serv.com
e-mail: rearing@bio-serv.com
tel: 908-996-2155

Carolina Biological Supply Company:
www.carolina.com
tel: 800-334-5551

Fisher: www.fishersci.com
Tel: 800- 926-1166

GIBCO: www.lifetech.com
E-mail: info@lifetech.com
Tel: 800-828-6686

Sigma: www.sigma.sial.com.
e-mail: custserv@sial.com
tel: 800-324-3010

Ted Pella: e-mail: tedpel@aol.com or tedpel@snowcrest.net
1-800-237-3526
tel: 800-324-3010

WEB sites

<http://www.ifas.ufl.edu/~insect/field/hornworm.htm>

<http://ww.acad.carleton.edu/curricular/BIOL/resources/rlink/>

<http://www.researchlink.ferris.edu>

Appendix C Ordering /Infection Calendar

Note: There is variability in emergence time depending on the size of the caterpillars and the number of wasps that have oviposited in each. After a few weeks of this cycle, however, one should obtain a continuous supply of infected caterpillars at different stages of infection. It is advised to start the above schedule at least four weeks prior to the first laboratory meeting with students.

- week 1: Obtain caterpillar eggs (1st batch) / caterpillar eggs hatch (usually within 3 days after you receive them).
- week 2: Set caterpillars in small cups. Place available wasp cocoons in wasp rearing cage (see Figure1).
- week 3: Infect caterpillars and place them in large cups. Order new batch of caterpillar eggs (2nd batch).
- week 4: Caterpillar eggs (2nd batch) hatch.
- week 5: Wasps emerge from 1st batch of caterpillars. Wait two to three days to remove cocoons from infected caterpillars (this minimizes caterpillar “bleeding”). Place cocoons in wasp rearing cage.
- week 6: Adult wasps ready. 2nd batch of caterpillars can be infected. Order 3rd batch of eggs.
- week 7: ...Etc.