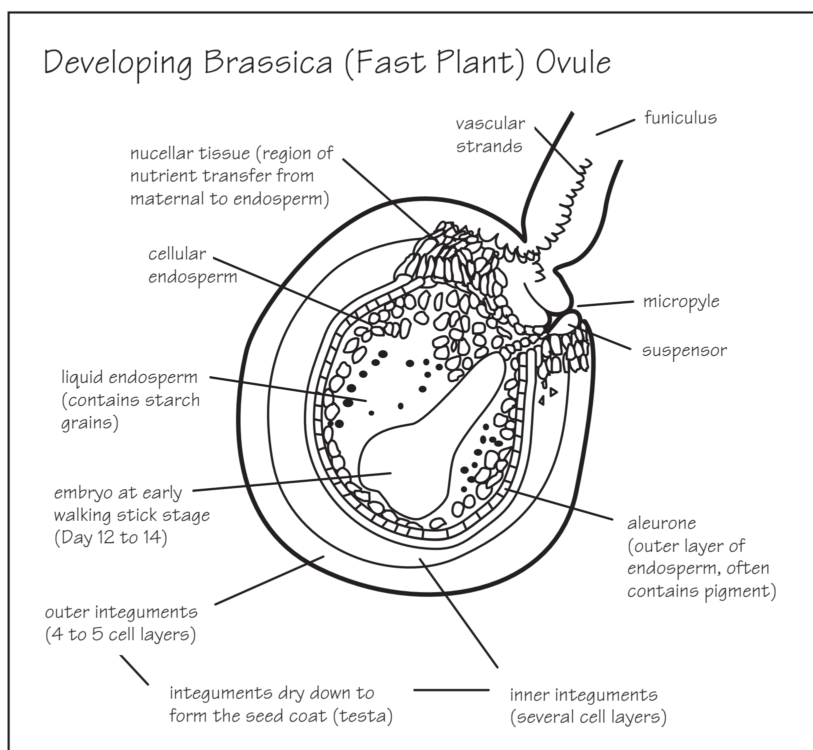
Embryogensis: Going for the Globular!

Introduction

The developmental events following double fertilization is a complex part of the life cycle that culminates in the production of the seed containing a quiescent embryo often accompanied by various parts to aid in its dispersal. The process of seed development involves complex interdependancies amoung the developing embryo, endosperm, and maternal tissues.

Following *double fertilization*, four complex processes are triggered. The ovary wall and related maternal structures rapidly grow to become the fruit tissue (the pod) surrounding the developing seeds. Each ovule within the fruit enlarges to accommodate the developing endosperm and embryo. The various outer cell layers of the ovule (*integuments*) eventually become the seed coat. Within the embryo sac, the triploid endosperm nuclei divides very rapidly to form the nutrient-rich, starchy liquid endosperm. The liquid endosperm provides nutrients to the developing embryo.

Since fertilization, the zygote has undergone several mitotic divisions. The first few divisions produced a strand of eight cells known as the *suspensor*, which is attached to the embryo. The suspensor orients the developing embryo within the ovule and is thought to serve as an "umbilical cord," as it passes nutrients from the endosperm to the embryo cells. The basal cell of the suspensor anchors the developing embryo and orients the embryonic root tip near the *micropyle*, a hole in the integuments where the pollen tube entered. At the tip of the suspensor, repeated cell divisions give rise to the very young *globular embryo*.



Immersed in the nutrient-rich endosperm, the embryo develops rapidly. By Day 7, the embryo becomes flattened and bilaterally symmetric with two lobes which will become the cotyledons. This is the *heart* stage.

As its development continues, the embryo elongates into the *torpedo* stage. At this stage the embryo produces chlorophyll and becomes green. Elongation of the embryonic hypocotyl separates the *root apical meristem* from the *shoot apical meristem*, which is hidden between the embryonic cotyledons. As the embryo enlarges it consumes space formerly occupied by the endosperm. To package the enlarging embryo, the cotyledons fold around the hypocotyl, now curved within the ovule; this is the *walking stick* stage. By Day 20, the walls of the ovule (the *integuments*) harden and become the seed coat (*testa*) and the embryo within desiccates to become a seed (Stages I and J, Life Cycle). As maturation proceeds within the enlarged folded embryo, the starch reserves within the embryonic cotyledons are converted to lipids as the final form of energy storage.

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There are many questions that remain unanswered in developmental embryology – perhaps you and your students can answer some of them.

Questions

- What is the normal sequence of embryo development within the ovules of Fast Plants?
- Can your students identify the various stages in the devxelopmental continuum from embryo to mature seed?
- Can your students record, draw to scale and estimate the relative sizes of the embryo at various stages of development?

Sample Hypothesis: Normal embryogenesis proceeds rapidly within the developing seed from a single-celled zygote through recognizable stages of increasing size and complexity ending in a dried seed.

Design

* At specified intervals following pollination, remove embryos from ovules, draw to scale various stages and measure embryo sizes. Construct a developmental chart of embryogenesis.
- Students will record observations and measurements on the Ovule and Embryo Student Data Sheet.

Time Frame

A period of 36 days from the sowing of seed is required for the growth of the Fast Plants and the completion of these activities. The time required for the embryogenesis activity will vary depending on the amount of class time spent in dissecting embryos. A minimum of two 50 minute class periods is recommended, one for students to practice and develop their dissection and drawing skills and one or more to examine and record the stages of embryogenesis. The number of periods spent on embryo dissection depends on the timing of the dissections students wish to make.

Learning Objectives

In participating in this activity students will achieve a number of learning objectives.

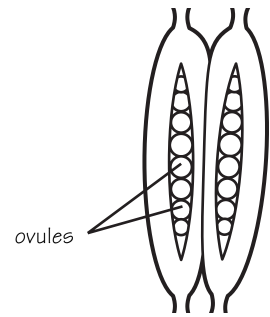
- learn to dissect embryos from ovules in developing pods, improving their hand-eye coordination;
- learn that embryogenesis is a continuum of development from a very small spherical group of cells to a complex multidimensional, multicellular organism;
- learn how an embryo can enlarge within the limited confines of the ovule and become "packaged" in preparation for desiccation and quiescence as a seed (Stage I of the life cycle);
- learn to make accurate descriptive observations of specimens under the microscope, draw carefully "to scale," and record and analyze data obtained from the drawings;
- learn to construct a model embryonic development that can be compared to the development that occurs in plants grown in microgravity.

Materials

- fresh or fixed pods of Fast Plants at various stages of development
  - for a range of embryo stages suitable for initial dissection, ovules sampled between 6 and 12 days after pollination are best, these should provide stages between heart and walking stick
  - after developing dissection skills, sample ovules from plants 3 to 6 days after pollination
  - pods may be fixed in a mixture of .75 ml, 95% ethanol and 25 ml of glacial acetic acid
- dissecting microscope with 20 to 40X magnification
- fine-tipped forceps and fine dissecting needles (e.g., tuberculin syringes with #23 or #25 needles)
- dissection strips
- 2 cm wide clear adhesive tape
- clear double stick tape
- fine scissors or cutting blade
- water and dropper
- IKI solutionfor staining starch, dissolve 2g of KI in 100 ml H_20 then dissolve 0.2 g iodine in the solution. Store in airtight container.

Procedure

1. **At the desired day after pollination (dap)**, students should use fine scissors to remove one pod from one of their two plants. Place the pod on the sticky tape of the dissection strip aligning it longitudinally on the scale.



2. Measure and make a drawing of the pod "to scale" in a lab notebook.

3. Using the dissection needles or a sharp blade, cut along one seam of the pod where the two carpels are fused. Pry open the pod to reveal the ovules aligned within the carpel; each ovule is attached to the vascular strands by its funiculus. You will also see a thin paper-like septum separating the ovules in each carpel.

4. Observe the opened pod with a hand lens or under a dissecting microscope. In a lab notebook, make a drawing "to scale" of what you observe.

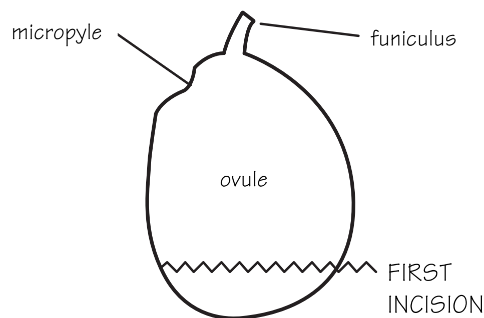
5. Remove an ovule from the opened pod with fine forceps or dissecting needles, keeping a portion of the funiculus attached to the ovule. Then transfer the ovule onto the sticky tape on a dissection strip. Measure and record the length of the ovule next to the first circle on the Ovule and Embryo Student Data Sheet.

6. With a pipette, transfer a small drop of water to cover the ovule on the sticky tape of the dissection strip. Alternatively a drop of iodine potassium iodide (IKI) staining solution can be used in the dissection, in which case any cells or tissues containing starch will turn blue or purple.

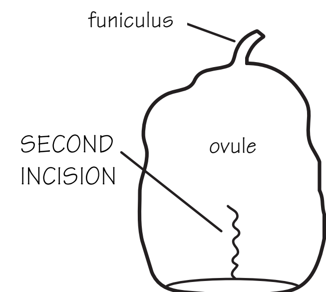
7. Place the opened pod with remaining ovules on moist paper toweling in a covered petri dish. This will keep it fresh for further sampling.

8. Place the dissection strip under a dissecting microscope and observe the ovule, noting the funiculus attachment and the micropyle.

   - If the ovule is illuminated from below, students may be able to see the indistinct embryo within the ovule. This will depend on the stage of embryo development.



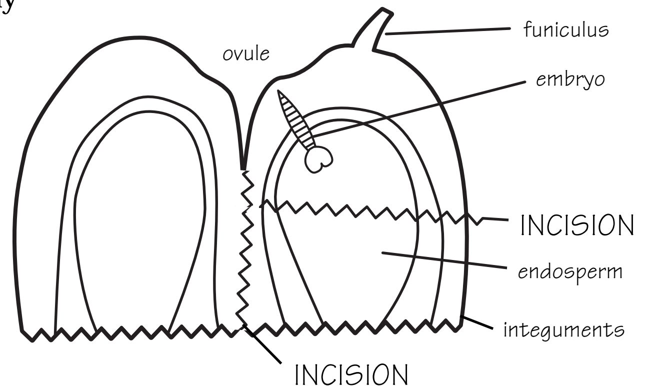
9. With needles make an incision across the ovule at the end opposite the funiculus. As this cut is made, the embryo may float out into the water along with the cloudy starchy liquid endosperm.



   - Make a second incision perpendicular to the first and using dissection needles gently pull open the integuments. Embryos at **9 dap** will generally be visible once the seed coat is open.

- If the embryo is not visible, slowly and carefully remove small pieces of the integuments, working toward the micropylar end.

  Young embryos in the heart and globular stages are found surrounded by a funnel of aleurone and nucellar tissue from the torn embryo sac. The young embryo is immersed in cellular endosperm and is anchored by the suspensor in the integuments at the base of the funnel.



- Continue to carefully tease out the embryo and, if possible, its attached suspensor.

10. Once the embryo has been removed, students may wish to increase the magnification under the microscope for viewing and drawing.

    - Under an appropriate magnification, slide a second dissection strip under the one holding the embryo in the water drop.

    - Align the magnified image of the scale of the second dissection strip across the horizontal diameter of the field of view of the microscope.

11. Draw in the magnified scale on the horizontal line of the first circle on the Ovule and Embryo Student Data Sheet. Be as accurate as possible in the spacing between the scale marks.

    - Draw a scale bar at the top or bottom of the circle representing the distance of 1 mm or some fraction (0.5, 0.25, 0.1) of the magnified millimeter scaling. Indicate the distance represented by the bar on the drawing.

12. Observe the embryo and accurately draw it to scale within the same circle as the scale bar.

13. Identify and record the stage of embryo development (globular, heart, torpedo, etc.).

    - Either from the drawing or directly, measure and record the length of the embryo, excluding the suspensor. Record the magnification of your microscope.

14. Calculate the magnification of the drawing using the following method:

    - Measure and record the actual distance in millimeters between the two ends of the scale bar in the circle of the drawing of the embryo (e.g., 21 mm).

    - Divide this measurement by the distance in millimeters represented by the scale bar in the circle to give the magnification of the scale bar and drawing (e.g., 21 mm/0.5 mm = 42X).

15. Dissect ovules from pods of different dap making drawings and length measurements of the difference stages of development, using the remaining circles on the Ovule and Embryo Student Data Sheet. If students have "spare" ovules at one developmental stage, they can share them with other students or exchange them for ovules at different stages of development.

    Suggested dissection times include 6, 9, 12 and 17 to 20 dap. If dissections are made in a potassium iodide staining solution, note the presence or absence of starch in the ovule at different stages.

Concluding Activities and Questions

In completing the activity , students will have taken their Fast Plants through a complete life cycle, from sowing the seed on Day 0 to harvesting the seed for the next generation. Analyze the data taken on the Ovule and Embryo Student Data Sheets. Have students consider the following:

- Students as a group or as a class may construct a developmental graph or chart depicting the dap on the x-axis and the length of the embryo, size and stages on the y-axis.

- Students may use the embryo lengths of various developmental stages as a quantitative indicator of development.

- How much does the embryo enlarge from the time it is a globular until it is mature?

- What are the relative sizes of the various stages in embryogenesis? With what will you compare those sizes?

- In what stages of embryogenesis is the embryo enlarging most rapidly? What is your evidence?

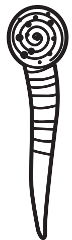
- What becomes of the endosperm? Is there any stage in embryogenesis at which starch is not present in the ovule?

Extension

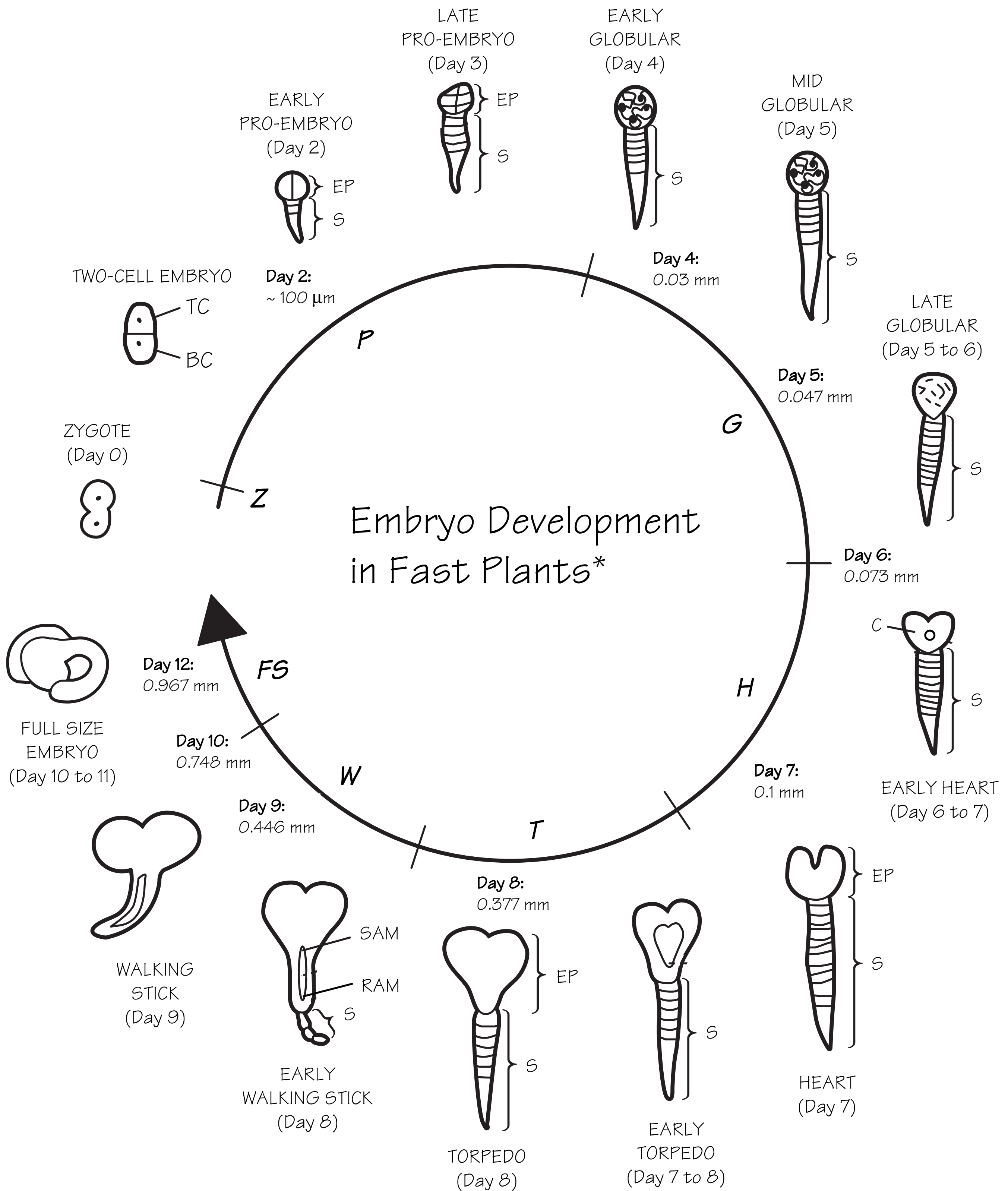
The embryo dissection activity can also be performed using pods that have been harvested and "fixed" in an acetic alcohol fixative on specified days after pollination. Fixed pods can be stored for future use.

Practice makes perfect . . . embryos!

Embryo dissection can be challenging for students, especially when the embryos are at the early stages of development. Prior to beginning the dissection of your experimental embryos, you and your students should practice dissecting embryos at different stages.



Have students plant several film can wick pots of Fast Plants, timed to be different ages the day or two before your class begins its experimental dissections. Pollinate these plants well: the more pods, the more practice!



KEY

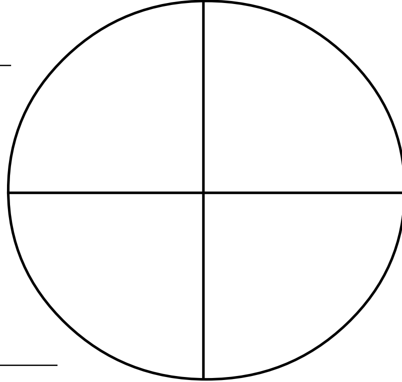
BC = basal cell C = cotyledon EP = embryo proper RAM = root apical meristem
SAM = shoot apical meristem S = suspensor TC = terminal cell
Z= zygote P = pro-embryo G = globular H = heart T = torpedo W= walking stick FS = full size

*embryos not drawn to scale; sizes are samples, measured on embryos from Basic Rapid cycling *Brassica rapa*, Stock: C1-33

Ovule and Embryo Student Data Sheet

Student Name ______________________ Date _______________

dap __________
scale bar length __________
ovule length ___________
stage letter ___________
embryo length ________
magnification
of drawing ____________
magnification
of microscope ______________



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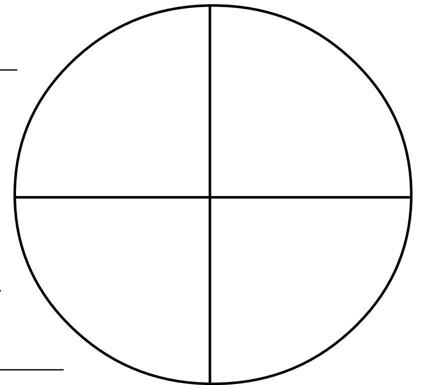

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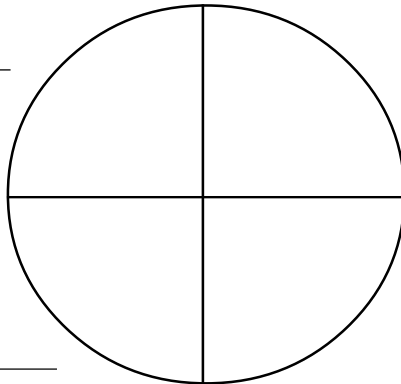

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