SDB Bootcamp 2012 - Drosophila unit

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The goal of this set of activities is to introduce you to the fly as a model system. We will cover some basic genetic techniques and will also do some dissections. In addition to providing technical experience, these manipulations will also illustrate how epithelial patterning during oogenesis determines the morphology of the fly eggshell and how various mutations can disrupt this process. An overview of these techniques is provided below; detailed protocols are available upon request from Laura Nilson (laura.nilson@mcgill.ca).

Background: We will be working with mutant alleles of three genes, *gurken, capicua* and *squid*, which are known to be required for the production of a normally patterned eggshell. These genes function in the ovary, and work by determining cell fates in a tissue called the follicular epithelium, which surrounds the developing oocyte and ultimately produces the eggshell. Since the follicular epithelium generates the eggshell, failure to induce a normal pattern of cell fates in this tissue results in the production of an abnormally patterned eggshell. We will be looking at two ways in which this patterning process can be perturbed, and seeing how these changes can be visualized either by looking at the resulting eggshells or at the epithelium itself.

A. Setting up a genetic cross

The purpose of this section is to teach you to distinguish males from females, and to recognize virgin females. We will be setting up crosses to generate flies that are heteroallelic for our mutations of interest.

1. Using CO2 to anesthetize the flies, inspect flies using a stereomicroscope and collect males and females of the appropriate genotypes.

2. Place the flies together in a vial containing cornmeal/agar fly food.

B. Collecting progeny for phenotypic analysis

The purpose of this section is to introduce you to additional phenotypic markers and to demonstrate how we collect fly eggs.

1. Using the chart in Appendix 1 as a guide, collect homozygous mutant females and set aside. As a control, collect heterozygous sibling females and set aside.

2. Transfer each of these genotypes, together with 5 males of any genotype, to an "egg collection cage", which consists of perforated plastic beaker covered with a petri dish containing an apple juice/agar mixture topped with a bit of dry yeast.

C. Analysis of eggshell production by wild type and mutant females

The purpose of this section is to show you what wild type and mutant eggshells look like.

1. Obtain the agar plates from previously prepared egg collection cages and observe under the stereomicroscope.

2. Note any differences in eggshell appearance between control and mutant collections.

D. Ovary dissection and mounting

The purpose of this section is to introduce you to fly dissection and to allow you to observe the large scale structure of the ovary. You will see that the ovary is made up bundles of repeating units called "ovarioles", and that each ovariole consists of a series of individual units called "egg chambers", each of which will produce a single mature egg.

1. Anaesthetize female flies with CO2, then transfer a single anaesthetized fly to a depression-containing glass slide. Place a drop of PBS (phosphate-buffered saline) in the depression and place the fly, wings-down, adjacent to the depression. Place the slide on the stage of a stereomicroscope.

2. While looking through the microscope, grasp the thorax of the fly gently with a fine-tipped forceps. Grasp the posterior tip of the abdomen with another fine-tipped forceps and pull gently to remove this tissue. Often this manipulation will pull the ovaries out of the abdomen; if not, use the same forceps to gently tear through the abdomen to expose the ovaries. After removing the cuticle and other debris, place the ovaries in the drop of PBS.

3. After discarding the carcass of the fly, gently tease apart the ovarioles using a fine tungsten needle. This step allows the tissue fixative to access the tissue more uniformly during the subsequent fixation step (which we will not be doing today).

4. Note the structure of the ovary and ovarioles, and their constituent egg chambers. See Appendix 2 for a general illustration.

E. Visualization of ovarian gene expression patterns

The purpose of this section is to allow you to observe the structure of the ovary at higher magnification, and to see the pattern of expression of a cell fate marker related to eggshell production.

1. Obtain previously prepared slides containing dissected ovaries that have been fixed using paraformaldehyde and then stained with an antibody that recognizes a protein encoded by the *Broad* gene. Ovaries will have also been stained with fluorescently-labelled phalloidin, which binds to filamentous actin and allows visualization of egg chamber structure.

2. View slides using a wild field fluorescence microscope equipped with a 40X objective.

3. Note the wild type Broad expression pattern (see also Appendix 3). How is this pattern different in ovaries from mutant females? How does this pattern compare to the eggshell phenotypes you observed in part C?

Appendix 1: Genetic Crosses

Cross #1

parents	grk^{HK}, cn bw/CyO x grk^{2B6}, cn bw/CyO	
progeny	grk^{HK}, cn bw/CyO	*red eyes, curly wings*
	grk^{2B6}, cn bw/CyO	*red eyes, curly wings*
	grk^{2B6}, cn bw/ grk^{HK}, cn bw	*white eyes, straight wings**

Cross #2

parents	yw; FRT82Bsqd^{j4B4}/TM3, Sb x +; FRT82Bsqd^{ix50}/TM3, Sb	
progeny	yw/+; FRT82Bsqd^{j4B4}/TM3, Sb	*red eyes, short bristles*
	yw/+; FRT82Bsqd^{ix50}/TM3, Sb	*red eyes, short bristles*
	yw/+; FRT82Bsqd^{j4B4}/ FRT82Bsqd^{ix50}	*red eyes, normal bristles**

Cross #3

parents	cic^{T6}/TM3, Sb x cic^{U6}/TM3, Sb	
progeny	cic^{T6}/TM3, Sb	*red eyes, short bristles*
	cic^{U6}/TM3, Sb	*red eyes, short bristles*
	cic^{T6}/cic^{U6}	*red eyes, normal bristles**

*homozygous mutant genotype of interest

Appendix 2: Structure and organization of the ovary

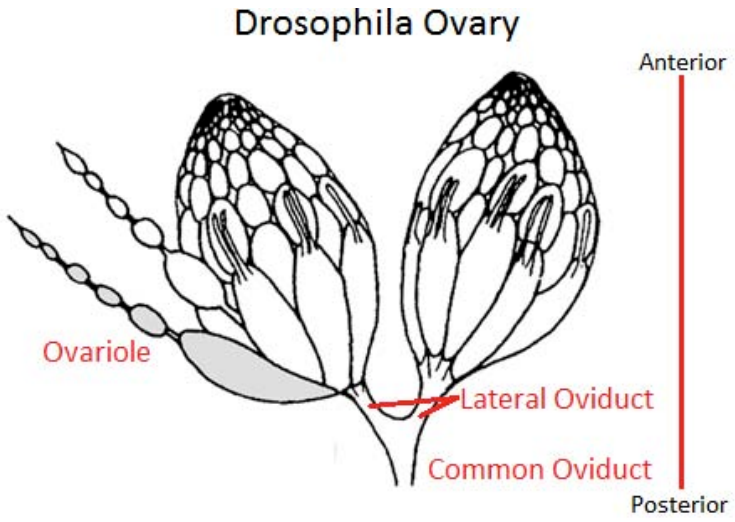
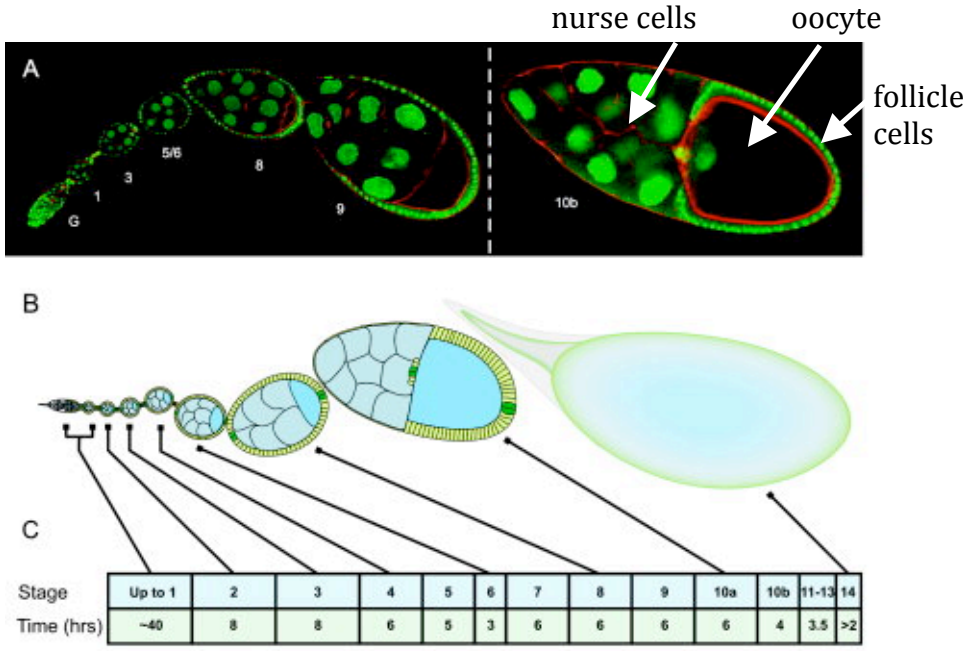
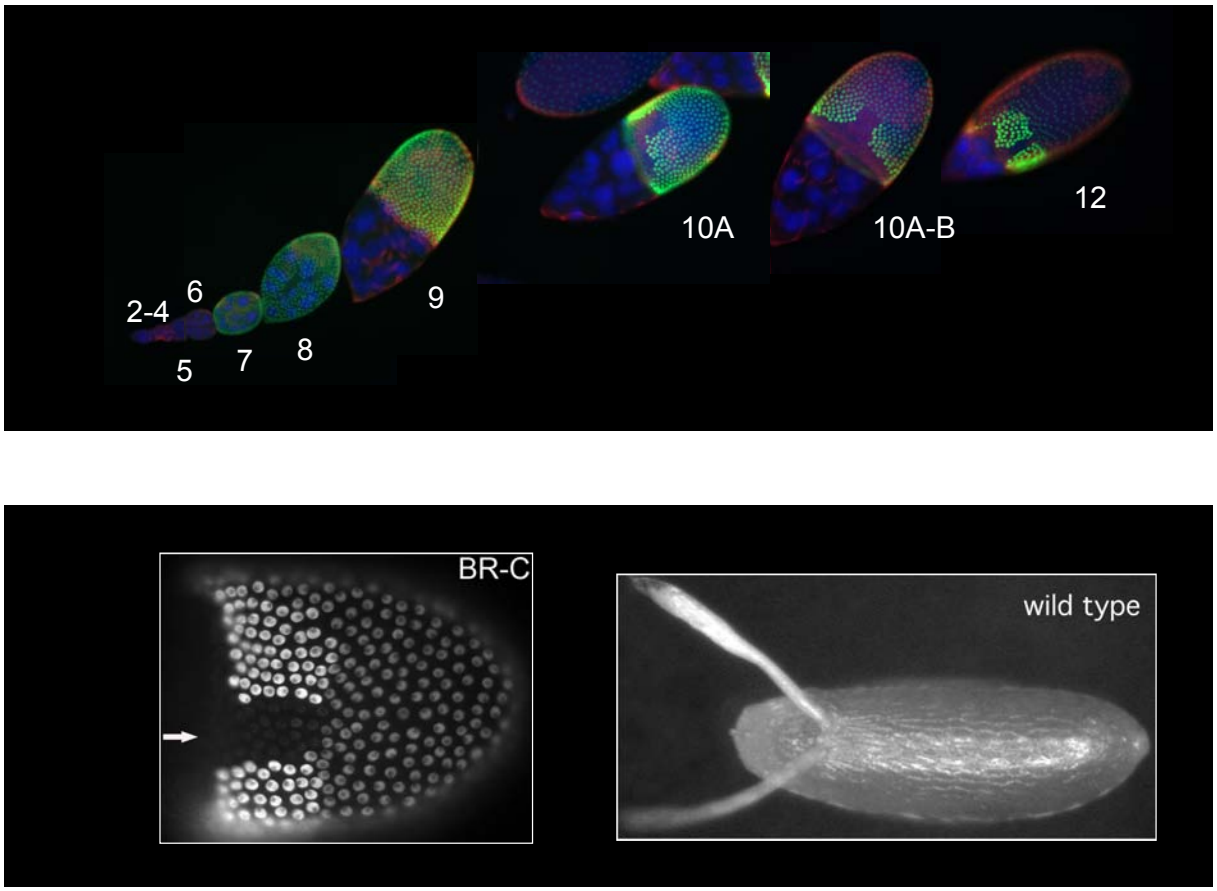


Diagram of a pair of ovaries, illustrating their overall structure and highlighting their organization into individual ovarioles. (http://en.wikipedia.org/wiki/File:DrosophilaOvary.png)



Micrograph (top) and drawing (bottom) of ovariole structure, indicating numbered stages of egg chamber development; anterior is to the left. Each constituent egg chamber consists of cluster of germline cells (the posteriorly localized oocyte and its sister nurse cells) surrounded by an epithelium of somatic follicle cells. By stage 14, the follicle cells have secreted the eggshell and have disappeared. (Diagram from Horne-Badovinac and Bilder, Dev. Dyn. 232, 559, 2005.)

Appendix 3: Expression of the cell fate marker Broad and its relationship to eggshell structure.



Top: Expression pattern of the Broad protein (green) in the follicular epithelium surrounding the developing oocyte. Bottom: The domains of high Broad expression mark the cells that will later secrete the eggshell appendages. (Images from Nilson lab).