Instructor's Notes on Introduction to Bean Beetles and Developing Experiments

The following are the lists of equipment, supplies and preparation instructions for introducing your students to bean beetles and developing experiments. The quantities listed are those needed for a class of 24 with students working in 6 groups of 4 students. We are assuming that each group will develop and initiate an experiment with two experimental treatments. The quantities per group would need to be increased if the number of treatments were greater than two.

Equipment

- Dissecting microscopes (at least 1 per group, minimum 6 total)
- Stand magnifiers (optional, but essential if microscopes are unavailable) (at least 1 per group, minimum 6 total)
- Fine tip sharpies to mark dishes (1 per group, 6 total)
- Soft forceps for handling beetles – at least 1 per group, minimum 6 total

Supplies

- 35mm plastic petri dishes for isolating individual beetles
- 60mm plastic petri dishes for creating mini-cultures of bean beetles
- 150mm plastic petri dishes for culturing bean beetles and/or providing cultures for students to examine
- Dried beans for culturing bean beetles (black-eye peas, pigeon peas, mung, aduki, or hyacinth beans will permit *C. maculatus* to complete its life cycle).

Live Bean Beetles

- General use cultures should be staged 4-5 weeks prior to date of use to ensure a good supply of live adults. See A Handbook on Bean Beetles, *Callosobruchus maculatus* (beanbeetles.org) for details on staging beetle cultures.

Comments on Handling Live Beetles

Bean beetles raised at temperatures between 25° - 30°C will be the sedentary phase, the adult morph that does not readily fly. It is best to work with the sedentary morph adults since the sex differences between the adults is very distinct and easily recognized, and adults are easily handled without using anesthesia. However, beetles will rapidly crawl out of culture dishes left uncovered.

- Remind students to vigorously tap down the culture dish they wish to open and return the dish cover immediately after removing the adult they wish to transfer.
- A small paint brush may be used to move beetles from one dish to another when transferring a number of adults from one culture to another.
- Very dense culture are difficult to control, so dump a small portion of a dense culture to a 150mm dish to make it easier to isolate individual adults beetles and to provide each group with beetles and beans with eggs to observe.

Comments on Experimental Design

- Have students read a published paper on bean beetle microbiome research as a way to get them thinking about potential variables that could influence the microbial communities in the gut of adult beetles.

- Use that reading as either 1) a starting point for soliciting potential experimental questions from your students or 2) providing the context for the question you choose for them to investigate in the two different implementations you conduct.
- In one implementation, you choose the experimental question and in the other implementation, the students will choose the question.
- In both implementations, once a single question is set, guide the students to develop a consensus experimental design for the entire class.
- A simple experimental design with two treatment groups will be more meaningful than a complex experiment. In some experiments, there may not be a true control, but rather two groups that have different characteristics to be compared.
- Each group (4 students) should conduct both experimental treatments and each group should later extract the microbiome of one beetle from each treatment (two beetles total per group).
- At the time that each group (4 students) isolates beetles to prepare microbiome extractions, each group should surface sterilize (see Preparation of Microbiome for Plating) one additional beetle from each experimental treatment (two beetles total), freeze each beetle in a separate sterile microfuge tube for DNA extraction and complete microbiome 16S sequencing. Your students should conduct the partial (half-semester) or complete (full-semester) DNA extraction as soon as the beetles are available (before performing the microbiome extraction and plating). Send the individual DNA samples to the Bean Beetle Microbiome Project for processing.

Important reminders for students

- Please remind students that bean beetles are agricultural pest insects and must be contained in the laboratory environment.
- Beetles and beetle cultures may not be removed from the laboratory
- Culture dishes and other culture containers must remain closed except when adding or removing beetles or other material from the dish or container.
- Adult beetles have the habit of "playing dead" so use care before assuming that a beetle is dead.
- If you are not sure of the sex of an adult beetle, place it in a small petri dish and ask your instructor.
- Disposal of beetles and all materials (disposable dishes and beans) that have been exposed to live beetles must be frozen for 72 hours (3 days) at -20°C prior to disposal. If a culture spills, sweep the contents into a plastic bag, seal the bag and freeze it for 3 days. Everything must be frozen first, then disposed.

Disposal of Cultures and Experiments

Disposal of beetles (live and dead) and all materials (disposable dishes and beans) that have been exposed to live beetles must be frozen for 72 hours (3 days) at -20°C prior to double bagging and disposal as food waste. Protect the environment by preventing accidental releases of this tropical and subtropical agricultural pest species.