

CARE AND FEEDING OF THE BUCKEYE LARVAE AND BUTTERFLIES

I. Caterpillars

We generally rear the larvae, eggs and adults at the same temperature and light regime: 25 C day: 20 C night, 15 hours day: 9 hours dark. This seems to work pretty well, though you can vary it depending on how fast you want them to grow.

Eggs hatch in about 5 days, although this varies with the temperature. You can keep a couple hundred caterpillars in a large petri dish for the first few days, just putting in fresh leaves. After 3 days, however, you should start dividing the larvae up into smaller batches.

There are a few different ways you can rear them--it depends what you are using them for. For our regular culture, we keep the larvae in groups and rear them in large petri dishes (14 cm diameter, 2.5 cm high). You can also rear them individually in the small creamer type containers that you can get from Bioserv. Either method works well.

You will need to keep the caterpillars clean, and they will be producing a lot of frass by the time they reach the 4th instar (there are 5 total instars, and they grow A LOT in the last instar). They will pupate just fine on the top of these petri dishes. However, if they are not being fed, the hungry larvae will eat the pupae.

After the pupae have hardened (a day after they pupated), remove them from the dish, carefully pulling off the silken pad they spin on the top of the dish. We put them in rectangular boxes with paper towel on the bottom and 2 of the sides. The newly hatched butterflies need something to climb up on when hatch. If they don't have something like this, they can't expand their wings. You could also use screen cylinders for this purpose.

We collect the hostplant, *Plantago lanceolata* from various places around campus. In the winter, we collect *Plantago* as long as we can, and eventually switch the larvae to artificial diet (see recipe attached). They do fine on this, but we don't like to feed them on artificial diet unless we have to.

II. Butterflies

Butterflies are raised in the growth chambers at a temp. of 25 C day: 20 C night and daylength of 15 hours (6:00 a.m. to 9:00 p.m.). If you need to have some other temperature, that is fine too. We have used various other temperatures, depending on how fast we want the larvae to grow. Another one is 28 C day and night. That is for fast growth. For slowing down growth you can rear them at 15 C day and night and they do pretty well.

As the butterflies emerge from their pupal cases, they are very fragile for several hours and should be undisturbed until their wings have unfolded and had a chance to dry. If you touch them at this time, their wings can be deformed.

On the first day they hatch, they are still full of meconium, the pupal waste products, and probably won't feed. They should be taken from the pupal boxes and put in mating nets.

The females and males should be separated by sex at this time. The females have a brownish colored tuft at the bottom of their last abdominal segment. Also, the front pair of legs on females are longer and have an orangey-brown tip. If you look at the forelegs under a dissecting microscope you will see that they are also very spiny at the tip. The females use their forelegs to taste leaves to decide if they are going to oviposit, so they have spines to scratch the leaf. Males don't have these spines. Females are often fatter than males but this cannot always be used as an indicator. We mark the wings of the females gently with a sharpie felt tip so we can easily distinguish them from the males in the mating bags.

We mark each mating bag with the following information: Sex, number of individuals, and date. We usually put a paper or cardboard tag at the top of the net by poking a hole through it with the wire hook.

On the day after they emerge they are ready to eat. They feed on a mixture of honey and water 1:4, kept in the refrigerator. Be sure it is mixed well. Heat the honeywater on the hot plate up to a setting of 4. The butterflies don't like to feed on the cold nectar. They will feed more easily if the food is warm. Using a disposable pipet, dispense the honey water to several wells on a cell well plate. Extend the proboscis of the butterfly into the honey water to begin their feeding. Six individuals should fit on the cellwell plate.

We have also had some success getting the butterflies to feed themselves. We put 2 small dishes (the small petri dishes will work, or the little creamer containers) with a piece of green pot scrubbing stuff in the dish and fill the dish with the nectar solution. The egg production isn't as high, but they do seem to learn to feed themselves. You will need to replenish the solution every day or 2.

On the second day put males and females in the same nets to mate, 5 females and 5 males. When the butterflies have been mating for 2 or 3 days, put a leaf of *Plantago lanceolata* for the females to lay their eggs on into the cage. It should be inserted into a water pic to keep it fresh. Although we haven't tried it, you could probably also use *P. major*. When there are 20 or more eggs on a leaf, replace the leaf with a fresh one. Even if the butterflies don't start laying eggs right away, they will soon. If the leaf wilts, replace it. You should also add water to the water pic every day to help keep the leaf fresh. When you remove a leaf with eggs, be sure that the water pic is full and store it upright in a petri dish in a growth chamber until it hatches. Label the dish with the

approximate number of eggs, the date and your initials. Collect a total of 600 to 800 eggs for each generation.

To try and maintain some genetic variation in the population, be sure you collect eggs from all the nets each generation. We have had some inbreeding problems at various time, so this helps prevent that.

To kill extra butterflies, larvae and pupae, put them in the freezer. **Don't forget to clean out the dead ones from the freezer, later.**