Microcolony Landscape Resource Simulation Experiments

Methods and Feeding Protocol

Microcolonies in this experiment are being subjected to a feeding treatment regime meant to simulate different landscapes in terms of resource abundance, and access over time (Figure 1). Rations of pollen and nectar are based of of a 5 worker maximum consumption level (ad-libitum feeding) from Rotheray et al. (2017). There are four treatments, or zones to which microcolonies are assigned, shown below. Feeding occurs every three days (two skip days in between).

Treatment	Description
Zone 1	60% of ad-lib ration for both pollen and nectar. All rations are equally sized and spaced over the duration of the experiment. Simulates a landscape with low levels of food that are constantly available.
Zone 2	100% of ad-lib ration for both pollen and nectar. Simulates a landscape with abundant floral resources that are constantly available over time.
Zone 3	100% of ad-lib ration for both pollen and nectar. However, rations are reduced for the bulk of the feeding dates, and the difference is made up during two "pulses" of pollen and nectar during weeks 2 and 6. Simulates a landscape with low levels of food availability, and then large mass-flowering events.
Zone 4	60% of ad-lib ration for both pollen and nectar. Like `Zone 3`, execpt with reduced food availability.

Figure 1 Concept diagram showing food amount as measured by total food abundance over a given time (e.g., growing season - x-axis) and the variability of food availability over time (y-axis). Actual landscapes are shown in the graph on the right

Total experiment food availability: `Zone 1` == `Zone 4` | `Zone 2` == `Zone 3`

Task Lists

Feed Day Tasks

- [ ] Prepare pollen and nectar rations
- [ ] Feed, mass, assess, and photograph microcolonies
- [ ] Fill humidifer reserviors
- [ ] Wash and sterilize nectar cups and all materials
- [ ] Wipe down all surfaces with bleach solution

Every Third (Weekly) Feed Tasks

- [ ] Remove all pollen previously provided and mass remaining pollen. Record on MC data sheet.

Other Tasks

- [ ] Ensure nectar wicks are prepped
- [ ] Fill huimidifer reserviors

Ration Preparation

Rations should be prepared the day of feeding, or the day before at most. Make enough pollen dough for the feeding needed. If existing pollen dough is available, use that first. Pollen dough should only be kept refrigerated for 1 week at most. Ration amounts to be prepared are laid out on the ration data sheet for each week.

1. Mass pollen dough for each microcolony to the nearest .05 grams. Record the mass of each pollen ration for the colony on the ration data sheet (see below).
2. Massed dough should then be rolled into a ball, and sealed using honey bee wax. Keep pollen balls in order in a weigh boat (1 boat for each zone).
3. Prepare nectar reserviors on a lunch tray. Use a catheter syringe to alloquot the amount given by the ration data sheet.
4. Place lids on reservoirs and place fully soaked cotton nectar wick in the feeder tube.

Microcolony Feeding and Data Collection

Prepare the rearing room bench by turning scale on, getting all necessary tools, and placing lunch tray on scale and taring the scale. The steps of feeding and recording data on the

microcolony are important. Follow them exactly! Each microcolony has its own data sheet.

1. Record the date, time, initials, and room conditions.
2. Mass new nectar reservoir that will be provided to the MC (full with nectar wick included).
3. Mass MC and record mass on MC data sheet.
4. Record the number of worker deaths, number of new males, number of males total.
5. Remove males and place into labeled vial. Record the number that were removed of total (e.g. 3/5)
6. Replace workers as needed. Record the number that were replaced of total worker deaths (e.g. 1/1)
7. If needed (every third feed), remove old pollen balls and place into labeled 2oz plastic cup. Mass each (after taring scale for 1 empty 2 oz cup) and record on each microcolony data sheet
8. Provide new pollen ball to colony.
9. Replace MC on shelf with new nectar reservoir.
10. Mass remaining nectar reservoir and record.
11. Repeat 1-10 with all remaining MC's.
12. Transcode mass of pollen rations from diet prep data sheet to each microcolony data sheet under pollen provided column.

Extra material location

Nectar: Located in fridge in gallon jugs
Pollen: Located in freezer door - reserve supply in styrofoam cooler on bottom shelf of freezer.
Nectar wicks: in cabinet above diet prep area. Remember that wicks need to be pre-saturated with nectar before they are added to nectar cups.
2 oz plastic cups: in cabinet above diet prep area, and in box atop cabinet in diet prep area - labeled: "2oz soufflet cups"