**Measuring bee life-history and health**

Lab safety

*Required PPE*

* Gloves
* Goggles
* Lab coat

*Materials of concern*

* Methanol - flammable; toxic if swallowed, inhaled, or if in contact with skin; causes damage to organs
* Chloroform - acutely toxic if swallowed or inhaled; skin and eye irritant; carcinogenic; reproductive toxicity

Tracking larval development and survivorship

*Materials*

* Bees
* Dissecting microscope
* Scale (measures to milligrams)
* Weigh boat
* Microcentrifuge tubes
* Datasheets

*Methods*

1. Perform this protocol daily starting at 14:00.
2. One at a time, take well-plates out of the incubator to view underneath the dissecting microscope.
3. Document the date at which the bees reach following stages:  
   *NOTE:* See ‘Osmia\_lignaria\_identification’ doc for pictures of these life stages.

* **Egg:** white, shiny, and sausage-shaped
* **1st instar:** encased in the chorion of the egg, recognized by clear poles at the apical and posterior end of the sausage shape
* **2nd instar:** larva starts eating with a pronounced head capsule
* **5th instar:** indicated by the first sign of frass, usually brown to black, rod-shaped, and slightly flat; development of setae (but this is often hard to see with the way larvae are oriented inside the wells)

1. Additionally, track mortality. When a bee dies, it will look deflated.
2. As bees reach the 5th instar stage, measure the wet weight of the larvae.

Determining larval wet mass & fat content

*Materials*

* Larvae
* Chloroform
* Methanol
* Microcentrifuge tubes
* Scale (must be able to measure to milligrams)
* Freeze drier
* Chemical fume hood
* P200 micropipette
* P200 micropipette tips

*Methods*

1. Tare labeled microcentrifuge tubes
2. As larvae reach the fifth instar, add bees to a labeled microcentrifuge tube
3. Weigh the wet mass of the larvae
4. Freeze larvae at -20°C until all bees reach the fifth instar
5. With the caps of the microcentrifuge tube open, freeze dry for 24 h at -105°C and 0.027 mbar larvae to remove the water content of the bee
6. Weigh larvae
7. In a chemical fume hood, add 20-fold the volume of 2:1 chloroform-methanol solution (e.g., 1 g larvae → 20 mL chloroform methanol solution)  
   NOTE: Assumes tissues have the same specific gravity of water
8. Let the samples sit in the 2:1 chloroform-methanol solution for 24 h
9. After 24 h, use a micropipette remove the used chloroform-methanol solution
10. Repeat steps 7-9 two more times
11. With the caps of the microcentrifuge tube open, once again, freeze dry for 24 h at -105°C and 0.027 mbar larvae to remove the water content of the bee
12. Re-weigh larvae
13. Calculate the proportion of larval fat content:

dry mass prior to lipid extraction - dry mass after lipid extraction

dry mass prior to lipid extraction

*Sources*

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