Practical Pollination Biology

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3.9.1 Pollen germinating on the stigma

Detection of germinating pollen grains on the stigma and pollination tubes in the style is done for the following studies:

- compatibility
- pollen competition and performance
- pollen selection
- pollen allelopathy
- pollination efficiency (see *Section 6.3*)

Protocol 3.27 Counting germinating pollen grains on the stigma

Materials

- slides
- methylene green-phloxine B stain
- cover slips

Method

1. Put the cut stigma or slice of it on a slide.
2. Add a droplet of methyl green (Sigma M 5015) + Phloxine B (Sigma P 8894).
3. Cover with a cover slip and squash.

Note: If there are germinating pollen grains, empty grains stain green and the germination tube red. Non-germinating grains stain dark brown-red. For the best results different ratios of methyl green should be tried (1% in 50% ethanol) to phloxine (1% in 50% ethanol), ranging from 1:3 to 1:10 by volume.

Protocol 3.28 Detection of pollen tubes in the style (Martin 1959; Gurr 1965:268-269)

Materials

- FPA solution (formalin 40%, concentrated propionic acid, 50% ethanol, 5:5:90 by volume)
- 8 N sodium hydroxide (Sigma S 5881)
- 0.1 N potassium acetate (Sigma P 1147)
- aniline blue (Aldrich Chemical 86, 102-2)

- fluorescence microscope with UV filter

Method

1. Fix the excised stigma and style in FPA for 24 h and then store in ethanol 70%. Wash in running tap water before the next stage.
2. Soften the style for 5 h (depending on the species: the right amount of time needed for softening can vary from 1 - 12 h) in sodium hydroxide. Rinse in tap water for 1- 3 h to remove the sodium hydroxide.
3. Stain with 0.1% aniline blue in potassium acetate for 4 h.
4. Squash the stained style under a cover slip and observe under a fluorescence microscope equipped with a filter set (of maximum transmission 365 nm). Both pollen tube walls and the callose plugs should show a distinct bright yellow to yellow-green flourescence.

Note: For variations of this procedure see Kearns and Inouye (1993:126-129).

Data on the foraging behaviour pattern of the pollinators according to the flower age (*e.g.* frequency of visits, distribution of the visits during the flower lifetime).

It is important to assign flowers of each plant to the different flower sets to be used in order to remove intraplant variability.

5.3.7 Standing crop

Nectar standing crop is the amount of nectar present in a flower exposed to pollinators at a given moment. It represents the product of the nectar production rate of the flower by the time elapsed since the flower was last visited by a pollinator if removal does not alter the nectar secretion rate and if the flower is not under a nectar resorption period. For example, if a species has a continuous nectar secretion rate throughout the flower lifetime but ends secretion after a single visit of a pollinator (*e.g. Nicotiana longiflora*, Galetto and Bernardello 1992b; some orchids, Galetto *et al.* 1997), all the visited flowers will show no nectar independently of the time elapsed since the last visit. In contrast, those species that have nectar resorption show a large variance in nectar content or even flowers with no nectar (*e.g. Mandevilla pentlandiana*; Torres and Galetto 1998). In general and for species that present a complex secretion pattern (*i.e.* resulting from the occurrence of secretion, cessation, resumption, and resorption periods), the larger the flower lifetime, the more the variance of nectar content per flower.

Nectar standing crop may produce a significant effect on the way in which nectar-gathering animals influence plant reproductive output (*cf.,* Zimmerman and Pyke 1988a). The amount of reward encountered by animals may affect pollinator behaviour, which in turn determines pollen transport and deposition within a population and the number of seeds produced. When studying the pollinator's point of view of the interaction, it is important to estimate nectar standing crop considering the milligrams of sugar present in the flowers instead of volume, because pollinator foraging decisions are mainly based on energetic balance (nectar gains *vs.* cost of gathering it), which is primarily determined by nectar sugars (see nectar energetics *Chapter 5.3.8* and *Protocol 5.33*).

Plants and pollinators are under pressure to maximize fitness. Plants tend to produce the largest number of the highest quality seeds possible. Pollinators enhance their fitness by maximizing their net rate of energy intake while foraging (*cf.,* Zimmerman 1988). Thus, plants and pollinators maximize fitness through different mechanisms. Taking this general but major statement into account, knowledge about both the nectar secretion pattern throughout the flower lifetime and the mean frequency of visits to each flower by pollinators is needed in order to accurately interpret nectar standing crop data.

The occurrence of resorption, changes in the secretion rate, periods of the day when pollinators preferentially visit the flowers, among other factors, are events that help explaining standing crop patterns. Once the mechanisms that promote a particular standing crop pattern are understood, it is possible to relate them to other reproductive variables, in order to evaluate the reproductive ecology of a species, to hypothesize about the evolutionary process of the plant-animal interactions, *etc.* For an overview of nectar standing crop and related variables see *Table 5.10*.

Protocol 5.32
Measuring nectar standing crop (Torres and Galetto 1998)

Materials

- microcapillary tubes
- hand-refractometer
- distilled water (to clean the refractometer after each reading)
- hand-thermohygrometer

A. *In the field*

1. Measure nectar concentration and volume in a large sample of flowers exposed to pollinators at different times of the day or of the flowering season, *etc.*, according to the objectives of the study.
2. Obtain concentration and volume data from flowers of different ages, of different stems of the plant, of different locations within the plant (*i.e.* distance from the ground, shade/open areas, *etc.*), and of many plants of the population.
3. Measure the microclimate around the flowers at each sampling time. The temperature or humidity, or both, may change dramatically both during the day and along the flowering season.

B. *In the laboratory*

1. Calculate the mg of sugars per flower from volume and concentration data (use *Table 5.6*).

Notes:
1. To interpret nectar standing crop data accurately the following information is necessary:

- the foraging behaviour pattern of pollinators (*e.g.* frequency of visits, distribution of the visits during both the day and the flowering season, *etc.*).

- the entire period of pollinators' activity in order to design the sampling schedule (*i.e.* the total daylight hours if they are diurnal or data from different moments of the flowering season, according to the objectives of the project). For instance, to characterize a species with short-lived flowers we need morning, midday, and afternoon samples registering volume, concentration, and mg of sugars per flower several times throughout the flowering season.
- the nectar secretion pattern during the flower lifetime, with data on variations of volume, concentration, and amount of sugars (see nectar secretion *Chapter 5.25* and *Protocol 5.30*).
- the effects of removals on total nectar production (see nectar removal *Chapter 5.3.6* and *Protocol 5.31*).

2. Usual problems with data analysis and comparisons

- Many flowers depleted of nectar are usually sampled. The high proportions of zeros may prevent data analysis through parametric methods. Alternative non-parametric methods should be considered.
- Generally, the comparison between the amount of nectar present in flowers under nets or bags and in freely visited flowers may be used to indicate actual nectar exploitation in relation to animal activity. This comparison is correct only if the species does not present resorption and if removal shows no effect on production rates. If a species presents resorption or an inhibition of the nectar production rate after removals, the difference between bagged and exposed flowers may be underestimated. Conversely, if the species presents a stimulation of the nectar production rate after removals, the difference between bagged and exposed flowers may be overestimated.

5.3.8 Nectar energetics

The readings in most refractometers are expressed in sucrose equivalents, as milligrams of sugar per 100 mg of solution (*i.e.* mass/mass). They can be expressed in milligrams of sugar per volume (*i.e.* mass/volume) by converting the measured sucrose equivalent to mg/µL using *Table 5.6*. This value must be multiplied by the nectar volume measured in the flower to finally obtain the total mg of sugar present in the flower. Some amino acids (*e.g.* proline) and lipids may be present in very small amounts in some nectars. As they also have caloric values, they may be used directly as a source of energy by pollinators. For calculations of energetics usually only sugars are considered through the following equivalence: 1 mg sugar (sucrose) = 4 cal/mg = 16.8 joule. For an overview of nectar energetics and related variables see *Table 5.10*.

Protocol 5.33 Measuring nectar energetics

Materials

- microcapillary tubes
- hand-held refractometer
- distilled water (to clean the refractometer after each reading)

A. *In the field*

1. Obtain the concentration and volume data per flower (*cf., Protocol 5.32* for standing crop).

B. *In the laboratory*

1. Obtain the mg of sugar per flower by calculating the mg of sugar present per µL of nectar corresponding to a particular concentration using *Table 5.6* and multiplying this value by the volume obtained. For example, for a particular flower 12 µL of nectar are sampled with 32% concentration (mass/mass on the refractometer reading). According to *Table 6*, a value of 0.3637 mg of sugar per 1 µL of nectar (mass/volume; mg/µL) corresponds to this concentration value, which must be multiplied by 12 (µL) to finally obtain the mg of sugar present in that flower (= 4.364 mg).
2. For the energetic calculations this amount of sugar represents 17.46 cal = 73.3 joule (*i.e.* 4.364 mg x 4 cal/mg = 17.46 cal).

Note: For the calculation of the sugar amount per µL of nectar at the different concentrations presented in *Table 5-6*, the Equation A (exponential regression) can be used:

$$y = 0.00226 + (0.00937\ x) + (0.0000585\ x^2)$$

where the value of x is the concentration (*i.e.* the refractometer reading). If a particular value does not appear (*i.e.* 24.3%), it can be directly obtained by using the Equation A.
