Planning Pollination Experiments

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In the last few years, the first author has been contacted several times by aroiders, colleagues or students interested in studying the pollination ecology of an Aroid species, in the field or in garden conditions. According to their request, the best experimental design adapted to answer their question was proposed. Of course, many experimental designs associated with pollination exist. In the following article, we present different simple and useful experiments classified according to their time investment

Three levels of observations and experiments on pollinators/pollination biology, which are more or less time-consuming, can be performed according to time available. Examples of various experiments are given below but this list is not exhaustive.

1. Opportunist collections

   - Bag some young (non-open) inflorescences and see later if inflorescences abort or mature (count seed production if possible). In general you bag an inflorescence to prevent insect visits in order to see if apomixis (e.g. production of seeds without pollen) and/or self-pollination occurs, or to do hand-pollination experiments.
   - Look at all open inflorescences and collect the insects that are inside or on them. For "derived" aroids with a floral chamber and often a 24h flowering cycle such as *Philodendron*, the best collection period is the second day of anthesis (before pollinator release). For "basal" aroids with exposed inflorescences which attract the pollinators every day of anthesis, pollinators must be checked at different periods of the day.
   - Collect inflorescences in order to measure the size and the zone lengths and count the number of flowers, to look at resource allocation according to the vigour of the plant (reproductive effort).
   - Collected inflorescences and insects can be preserved in 50–70% alcohol, but don't forget to label the vial(s): precise location, date, "supposed" aroid species (in case of insects, it's the host name), "supposed" insect name, collector name.

2. For a longer stay in the field (Natural History).

   - Mark inflorescences and follow their flowering phenology each day (or two days) in order to establish the length of anthesis, duration of male and female phases, stigma receptivity (stigma drop formation, changes in stigma color), pollen release with or without resin production.
   - Odor and heat production, arrival and departure time of insects. You can then distinguish between simple visitors (as trigone bees in *Philodendron*) and real pollinators (*Cyclocephala* beetles), as you'll know which insects just come to the inflorescence

at one stage (pollen collectors) and which ones move from male to female phase inflorescences and may achieve pollination. You can also look at pollen loads under a stereomicroscope.
- Collect or count the pollinators, relationship between size/number of the inflorescences and number of captured pollinators or number of visits in "derived" aroids with floral chamber. For "basal" aroids with exposed inflorescences which attract the pollinators every day of anthesis, pollinator visits (frequencies, numbers) are more appropriate. Inflorescences may only be attractive during one period of the day.

3. Floral biology and pollination experiments

(You need to mark and follow the phenology of the inflorescences in order to know their exact stage as in 2 - You also need "correct" sample sizes: a minimum of 20–30 individuals).

- Bag an inflorescence prior to its opening to manipulate pollination: emasculate or tape the stamens and bag the inflorescence again to assess if apomixis is possible.
- Hand-pollinate bagged inflorescences with the pollen of another inflorescence of the same individual to test for geitonogamous pollination.
- Hand-pollinate bagged inflorescences with the pollen of another inflorescence of another individual (manual cross-pollination) to test for pollination limitation in comparison with open-pollinated inflorescences (natural pollination).
- Introduce one or more emerging pollinator(s) (from male-phase inflorescences) into a bagged female-phase inflorescence to test the number of pollinator(s) necessary to pollinate all the flowers.
- Mark insects (dyes, physical marks,..) in order to estimate pollinator movement and pollen flux.

For many of us who are not familiar with insect taxonomy, establishing even the Order to which the insect may belong will be difficult at first. The general appearance of each will become more familiar, but you may need to embark on a self-taught voyage to speed this up. There are many keys to the level of order available on line, some easy to use and some more difficult. The following are all worth checking to see if the approach used is understandable.

- http://insects.about.com/gi/o.htm?zi=1/XJ&zTi=1&sdn=insects&cdn=education&tm=147&gps=522_252_1676_810&f=10&tt=14&bt=0&bts=0&zu=http%3A//pick4.pick.uga.edu/20/q%3Fguide%3DInsect_orders
- http://insects.about.com/gi/o.htm?zi=1/XJ&zTi=1&sdn=insects&cdn=education&tm=20&gps=506_338_1676_810&f=10&tt=14&bt=0&bts=0&zu=http%3A//www.sci.sdsu.edu/classes/bio462/easykey.html%23onepair
- http://www.earthlife.net/insects/orders-key.html
- http://www.backyardnature.net/in_order.htm
- http://www.amentsoc.org/insects/what-bug-is-this/adult-key.html
- http://ipm.ncsu.edu/AG136/key.html

If you are lucky enough to have an acquaintance at a university who is experienced in insect identification, you can get pointers there, not only on the naming, but also on capture and preservation of the visitors.

So much work on this area of aroid biology is still to be done, so every contribution will be of value.