

PhD/ MSc: Investigating handedness in flowers

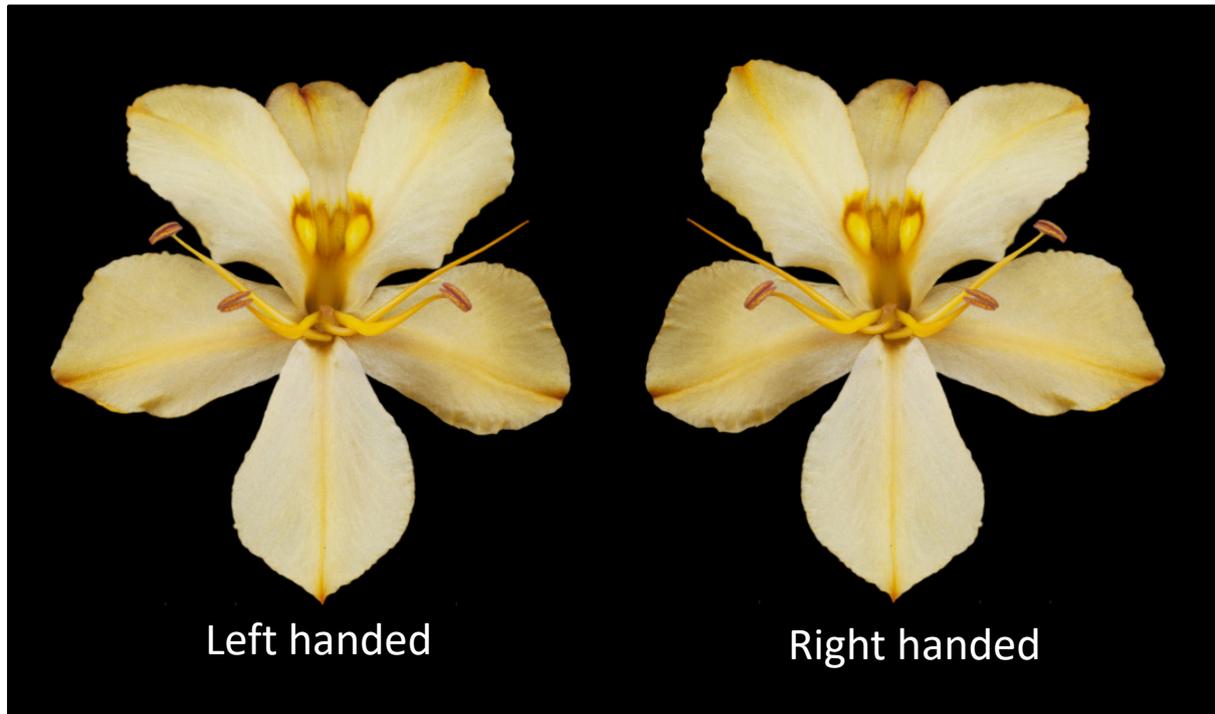


Figure 1. Left and right handed morphs of *Wachendorfia paniculata*

The primary function of flowers is to mediate the movement of pollen. Despite this, very few studies have managed to directly assess pollen movement in plants because there has been no easy way to mark and track pollen. Using cutting edge nanotechnology, our lab recently established a method which, for the first time, allows biologists to track pollen grain movement. To track pollen, we first mark grains on intact flowers using nanocrystals called quantum dots which fluoresce in different colours. We are then able to determine the origins of any pollen grains which have been marked with the quantum dots, by looking at flower stigmas or pollinators (see Figure 2) under a modified dissecting microscope. The method was first applied to a handed or enantiostylous plant, *Wachendorfia paniculata* to study the movement of pollen. Enantiostylous flowers can be either left or right handed depending on the direction that their stigma faces (see Figure 1). Darwin was clearly beguiled by floral polymorphisms—“*I do not think anything in my scientific life has given me so much satisfaction as making out the meaning of the structure of heterostylous flowers*”—devoting an entire book to the subject. Sadly, this book was published before Darwin became aware of handedness in plants. When he first read about left- and right-handed flowers, he was so intrigued that in his very last correspondence (just 8 days before his death), he wrote to a fellow researcher asking for seed from an enantiostylous species so that he could study it. Minnaar & Anderson (unpublished) demonstrated that Darwin's hypotheses about pollen movement in stilar polymorphisms was correct: pollen movement mostly occurs between morphs, thus reducing the probability of self-pollination. However, *W. paniculata* has three anthers all in slightly different

positions and Minnaar and Anderson found that in one population, most of the pollen transfer between morphs occurs via the top anther. Furthermore, the lower stigma-side anther contributes half of its pollen to flowers of the same plant (selfing), apparently contradicting the very reasons for the evolution of enantiostyly.

The application of quantum dots to pollination biology promises to finally open the long-closed doors for research on pollen movement and has raised many new questions about the functions of different anthers in handed plants. Here I appeal to either MSc or PhD students to contact me if they are interested in using quantum dots to help answer some of the many fascinating questions which have arisen as a result of our first preliminary study. Below I outline some of the key questions which we hope to answer and I give some brief descriptions of how a student may begin to answer them. In reality, there are more potential questions than could ever be addressed in a single MSc or PhD and giving prospective students the opportunity to tailor the project to their interests. You may also contact me if you have your own ideas for using quantum dots.

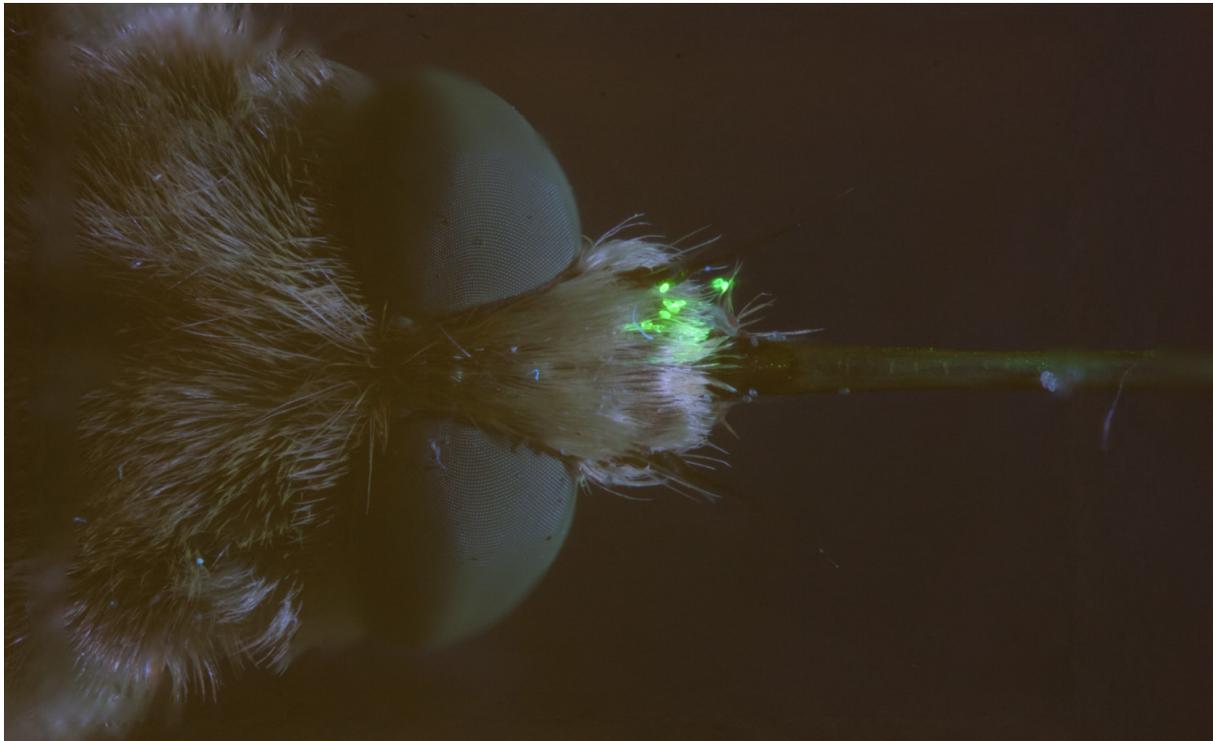


Figure 2. Pollen grains from *Lapeirousia anceps* fluoresce green on the head of a long proboscis fly after it has visited a labelled flower.

Potential project questions

1. Are the positions of upper and lower anthers adaptations to different pollinators?

Upper anthers are placed wider than lower anthers and they appear to place pollen on the wings of large carpenter bees but not on honeybees which are much smaller. In contrast, lower anthers can easily place pollen on both honeybees and carpenter bees. However, the position of this pollen is poorly matched to the position of the stigmas (see figure 1) and so when carpenter bees are present, lower anther pollen does not frequently contribute much to pollen received by the stigmas. We would like to investigate the roles of upper versus lower anthers with respect to the different functional pollinators and we propose that the upper anthers may be specifically adapted to carpenter bee pollination and the lower anthers specifically adapted to honeybee pollination as carpenter bees often do not visit *W. paniculata* populations.

- A) If anther positions are adaptations to different pollinators, we may expect that their relative contributions to stigmas may change under different pollinator environments. We suggest an experiment to test this, working in a population that has both carpenter bee and honeybee pollinators. The anthers of focal plants will be marked with q-dots so that each anther has a unique colour. We will work within small plots where we will be able to manipulate pollinator visitation by capturing arriving pollinators before they visit plants within the experimental plots. Plants within the plots will be exposed to three different pollinator environments: visitation by carpenter bees and honeybees, visitation by carpenter bees only and visitation by honeybees only. At the end of a day of pollinator foraging under these three environments, we will harvest stigmas of plants within the plot and determine whether contributions by the different anthers differ under each pollinator environment. We expect that upper anthers will export most pollen when carpenter bees are present but that lower anthers will export most pollen when carpenter bees are absent.
- B) Limited data suggests that populations with carpenter bee pollinators have larger flowers and very strong stigma-anther separation (so much so that neither the stigma nor the outermost anther contacts honeybee wings). However, we have also noticed that populations without carpenter bees have smaller flowers with small anther-stigma separation distances so that the stigmas and anthers make contact with visiting honey bees. We would like to see whether these preliminary observations hold true. We hypothesise that geographic variation in stigma-anther separation is associated with the type of pollinator visitors. Here, we hope to visit multiple different *W. paniculata* populations, measure stigma-anther separation and then record the pollinator fauna visiting *Wachendorfia* plants in each population.

2. Does the stigma-side anther function as reproductive assurance?

One possible function of the stigma-side anther is that it allows plants to self-pollinate and set seed when compatible mates are uncommon. This is likely when plants colonize new areas and set up founder populations. One line of reasoning suggests that this is unlikely because a history of outcrossing is likely to result in selfed progeny having exceptionally high

inbreeding depression. On the other hand, it could be argued that a history of mild selfing (we have calculated selfing rates of 8%) has allowed deleterious recessives to be purged. In support of a selfing role, the stigma of *W. paniculata* appears to move slowly towards the anther during the course of the day. While the two never touch, it suggests that later in the day, plants may compromise the quality of seeds in order to ensure seed production.

- A) We plan to set up an experiment to determine the levels of inbreeding depression in *W. paniculata* by comparing seed production, seedling growth and mortality between progeny produced via self-pollination versus outcrossing. Inbreeding depression in *W. paniculata* could be contrasted with inbreeding depression of its putative sister species *Wachendorfia brachyandra*, which has stigmas and anthers that touch, suggesting a long history of inbreeding.

W. brachyandra looks very similar to *W. paniculata*, except that it appears to be a habitual selfer and its anthers make contact with its stigma. It is possible that this species is simply an ecotype of *W. paniculata* which is adapted to a selfing life history. If so, we would expect that a phylogeny of *Wachendorfia* will demonstrate that *W. brachyandra* is embedded in the *W. paniculata* clade and that selfing may have evolved numerous times from *W. paniculata*. If this is the case, we expect *W. brachyandra* populations will not form a distinct cluster and will be paraphyletic.

- B) To experimentally test whether the stigma-side anther has a reproductive assurance role, we would like to set up isolated *W. paniculata* populations with different density and morph ratio characteristics using potted plants.

a) low density populations with plants separated by large distances, both morphs present

b) high density population with both morphs present

c) high density population with only one morph present

d) The three populations above with the lower anther removed

Using quantum dots, we will mark the stigma-side anthers of left and right flowers with different colours and at the end of each day harvest all stigmas in the experimental populations. The pollen contribution of stigma-side anthers vs other anthers will be compared between treatments. We expect that stigma-side anthers may contribute more to pollen movement when populations are monomorphic or sparse. We will also collect seeds and compare fitness of seedlings between treatments. Treatment **d** will be carried out in all three of the above treatments (**a**, **b** and **c**). Because flowers only last one day, experiment **d** can be alternated with the other experiments on alternating days. Seed set differences can then be quantified between treatments with the expectation that seed set will decline the most in small or sparse populations which have had their stigma-side anthers cut off. However, seed set will not decline when the anthers have been cut from dense, dimorphic populations.

3. Does frequency dependent selection maintain left and right-handed morphs?

Stylar polymorphisms like enantiostyly are theoretically underpinned by frequency dependent selection: the rarer a morph, the fitter it is. This is because, as a morph becomes relatively less common, it has relatively more reciprocal mates and vice versa. Consequently, morph ratios tend to be maintained close to 50:50. While frequency dependence is constantly predicted for plants with stylar polymorphisms that promote outcrossing, it has seldom, if ever been demonstrated. Here we hope to examine frequency dependence through the lens of both male and female fitness. Because each flower only produces a maximum of 3 seeds, we expect that frequency dependence may not act easily through female fitness (it does not take much pollen to fertilize all the seeds in a capsule). However, the amount of pollen exported (an important component of male fitness) may be more likely to be influenced by relative frequency. We plan to work in a small population and each day manipulate the ratio of left to right handed flowers by harvesting either left or right-handed flowers so that the ratio varies between days. By marking anthers using quantum dots, we will be able to determine pollen export by different morphs by counting the marked pollen on stigmas at the end of the day (male fitness). Similarly, we will be able to calculate seed set of different morphs by marking flowers and counting their seeds once they are developed (female fitness). We expect to find that male fitness may increase sharply with morph rarity. However, we expect that correlations between female fitness and rarity may be less pronounced.

4. Wing placement of pollen

Wachendorfia is also interesting in that it is one of the only recorded examples of a flower that places pollen on the beating wings of its pollinators (See Figure 3). Pollen is placed on the undersides of the wings as they beat against the anthers of the flowers. We suspect that this placement may be particularly efficient for bee pollinated flowers because bees usually harvest pollen from their bodies and the undersides of their wings appear hard to groom. We think that the underside of wings may be a "pollen safe site" a place where bees are unable to groom. We would like to test this by collecting *Wachendorfia* pollen and placing it in a small container. Then we would put a bee in the container, allowing it to become covered with pollen. Then we would allow bees time to groom themselves. Pollen attachment could be compared between bees that have had time to groom and bees that have not had time to groom. If bee wings are pollen safe sites, then groomed bees are expected have pollen on their wings but little pollen on the rest of their bodies while ungroomed bees would have pollen all over their bodies.

We expect that pollen which attaches itself to the wings of bees may need to be especially sticky. It could be interesting to compare the stickiness of *Wachendorfia* pollen with the stickiness of pollen from relatives which do not place pollen on bee wings. If pollen is particularly sticky, stigmas may also need to be unusually sticky to remove that pollen. We would also like to determine whether *Wachendorfia* stigmas are stickier than stigmas of other flowers that do not attach pollen to bee wings.



Figure 3. Pollen grains from the *Wachendorfia paniculata* adhering to the wing of a honey bee

5. How Does *Dilatris* Work?

Dilatris is in the same family as *Wachendorfia* (Haemodoraceae). Like *Wachendorfia*, it also has three anthers. One anther is deflected in the same direction as the stigma and the other is deflected in the opposite direction. Both of these anthers are orange. However, the third anther is not deflected left or right and it lies in the centre of the flower—it is yellow. Furthermore, the stigma of *Dilatris* alternates between left- and right-facing within an inflorescence. By marking anthers with different colour quantum dots, we will track their pollen and determine whether the fates of pollen from the three different anthers is dependent on their relative positions. We expect that anther position will have a profound effect on how pollen is moved in *Dilatris*. We hope that this study will shed light on how stelar polymorphisms in *Dilatris* function and whether they also play a role in promoting outcrossing.

Renumeration: I have an MSC and a PhD bursary available (R50000 and R70000 p.a. respectively), but students are encouraged to come with their own funding. Project expenses will be covered by the supervisor.

Deadlines: If you are interested in the project, please send me (Bruce Anderson) a CV and a cover letter motivating why you would like to do the project. These should be sent to banderso.bruce@gmail.com. If you would like to find out more information, please contact me on the same email address. CVs should be submitted by 15 Dec 2017. I will let you know if your application was successful by 1 Jan 2018.

General information: Most *Wachendorfia* populations flower between spring and early summer (September–November) and this will be the primary field season for the project. Most species are widespread in the Western Cape and are common in recently burned areas. Students would register with Stellenbosch University. Please visit my website for more information about me and our lab: <http://www.biointeractionslab.com/prof.-bruce-anderson.html>

A full list of my publications can be found at <https://scholar.google.com/citations?user=TQUTOpAAAAJ&hl=en>