# Vacation Laboratory Studentship: Homology of the Daffodil Corona

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Last summer I was fortunate enough to be supported by St Anne's under the 'Vacation Laboratory Studentship' scheme to undertake five weeks working in a lab at the Department of Plant Sciences, here in Oxford. The project I worked on forms part of Dr Robert Scotland's research investigating the homology of the daffodil trumpet (corona). Homology concerns the similarities between characters (physical traits) or DNA sequences due to their shared (common) ancestry.

*Figure 1:* Narcissus bulbocodium
*Photo: www.pacificbulbsociety.org*

In particular, one of the aims of the study is to determine the developmental origin of the corona, since it is uncertain whether it is derived from the stamens or the petals (known in this group of plants as 'tepals'). Dr Scotland had been growing 1,000 bulbs of the hoop petticoat daffodil, *Narcissus bulbocodium* (*Figure 1*), on the roof area of the department since autumn 2007. These plants flowered in spring 2008, and shortly after those flowers died, new ones were beginning to develop deep inside the bulbs, ready to flower in spring 2009. The project involved dissecting these tiny flowers at varying developmental stages from the centre of each bulb, and then viewing and recording images of the specimens using a scanning electron microscope (SEM).

Initially it proved very difficult to find and identify the earliest floral primordia due to their small size of c. 200 microns – a fifth of a millimetre. Given that we were doing this under a normal dissecting microscope, using only razor blades, tweezers and fine mounted needles, it was pretty tricky, to say the least! After many hours of peeling and cutting up the bulbs, however, I gradually developed a technique that resulted in more successful dissections and 'cleaner' specimens. The task demanded a steady hand, a good eye and plenty of patience to succeed – skills which I improved over the course of the project. Confidence was also required to dissect quickly and decisively, before the tissue dried out.

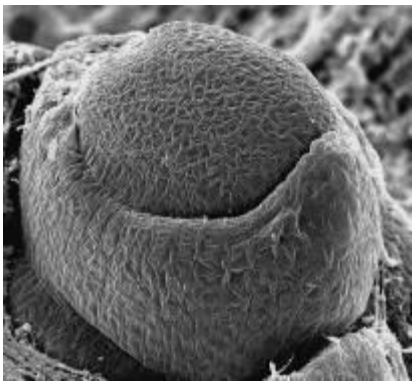

*Figure 2: The bract grows over the meristem.*

As each specimen was dissected, it was put straight into a fixative solution made up earlier in the day, and left in it overnight in a walk-in fridge. The next day these specimens were put through an alcohol series from 40% to 100% ethanol, changing the concentration every hour. The day after that, they could be critically point dried (a process that removes the water from specimens) then carefully mounted onto 'stubs'. After being coated with silver in the 'sputter coater' they were finally ready for viewing in the SEM.

It was possible to identify a number of clear developmental stages in the transition from meristem to mature flower. The first of these is simply the meristem, surrounded by two leaves – one large and one small. Next, a 'bract' is seen starting to grow over the surface of the meristem, initially with a distinctive beak shape that made this stage easy to identify (*Figure 2*). Incidentally, in a mature daffodil the bract can be seen as a brown papery structure at the base of the flower (*Figure 3*).

*Figure 3: The bract in a mature flower. Photo: www.microscopy-uk.org.uk*

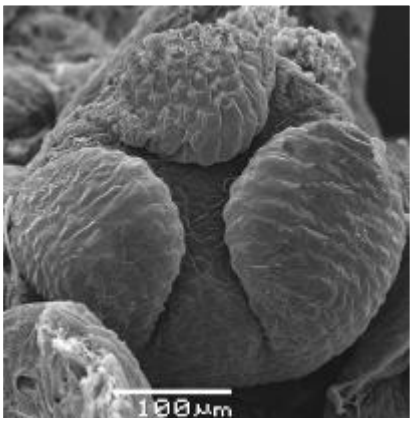


*Figure 4: Bumps on the meristem which will develop into three of the tepals.*

Once the bract had grown completely over the meristem, it had to be removed to observe the subsequent stages, and this was particularly fiddly. Beneath the bract, a number of bumps then develop on the meristematic surface (*Figure 4*); this is the start of the differentiation of the floral parts, with these bumps becoming three of the six tepals.

The next structures seen to develop are the six stamens (*Figure 5*), followed by the three carpels; it is not until the standard floral parts are really quite mature that the corona begins to develop (*Figure 6*).

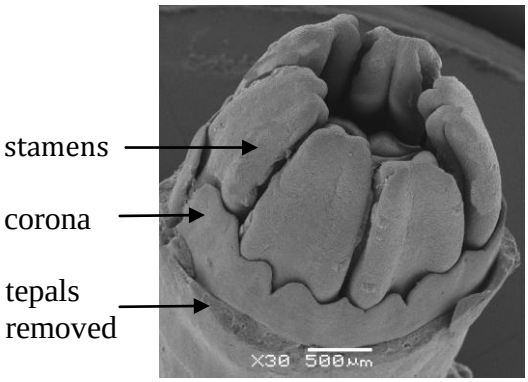


*Figure 5: The tepals have been removed to show the developing corona*


*Figure 6: The six tepals, six stamens and three carpels are visible. The corona is still quite small – it is just visible in the red circle.*

It was interesting to work with *N. bulbocodium* in the lab, having encountered the species in its natural setting during the 3rd year 'Plants' field trip to Portugal that Easter. During the studentship I also did some work using two other species of daffodil, which made for an interesting comparison with *N. bulbocodium*.

Another component of Dr Scotland's research into the daffodil corona involves determining the pattern of evolution of the corona over the whole of the daffodil family, the *Amaryllidaceae*. This confirmed that the corona has evolved independently a number of times within the family. A 'tree-building' program was used to construct the phylogenetic tree of the *Amaryllidaceae*, on which species with different types of corona were marked: staminal (derived from the stamens) and perianthal (derived from the perianth, i.e. sepals and petals). Such tree-building processes are often described and discussed in lectures, but it was a lot

easier to understand it in relation to a concrete example, seeing it through from the raw data to the finished tree.

Other aspects of the project include using '*in situ* hybridisation' to find out whether the genes which are 'switched on' in the corona are those usually expressed in the stamens, or those usually expressed in the petals. It is hoped this will provide further evidence to support Dr Scotland's initial investigations using a Northern blot, which indicated that the *Narcissus* corona is staminal.

During the time I spent in the department I also attended weekly lab meetings, at which any matters relevant to the lab members were discussed. Sometimes, one or two people would make an informal presentation of some progress they had made on their work, report back on a conference, or lead a discussion on a journal article of interest. It was interesting to hear about the kind of projects the PhD and postdoctorate students were working on, even if the details were somewhat beyond me! It also helped me to get a better idea of how it might be to work in a lab atmosphere – getting to know people a little and feeling included as part of the lab team.

The early development of floral organs is something which I encountered briefly in my 2nd year course prior to doing the studentship, but which featured strongly in the 3rd year 'Plant and Microbial Biology' course that I chose as one of my two 'major' options. The 'ABC model of floral development', which is integral to the genetics component of the daffodil project, has been highly influential in the study of development in angiosperms (flowering plants). I found it interesting to be able to tie that theoretical aspect of the work into a study involving microscopy, morphology and homology too.

Despite periods of frustration with the low success rate of peeling the bulbs to the necessary standard, there was a real satisfaction to be gained from seeing the gradual improvement in my ability to get it right. I found the work could be very absorbing, and the moments when I got a further glimpse into part of the daffodil trumpet story gave me the drive to keep going, and the desire to find out more.

Unfortunately, I did not get to see the tail end of the process during the course of my five weeks because the development of the flowers turned out to take place later in the year and more slowly than anticipated. Nevertheless, I gained a huge amount from the experience and really enjoyed contributing to some 'real research'. Working on this aspect of morphology in the daffodil family was a really exciting opportunity to apply modern microscopy techniques to a significant question, which it has only been feasible to tackle relatively recently, because of the practical methods required. It enabled me both to consolidate practical skills I've encountered previously – such as dissection and the use of a dissecting microscope – and to learn new ones, such as using the SEM, something I would certainly not otherwise have got the chance to use in my undergraduate course.

I'm very grateful to St Anne's for supporting me with this studentship. I would also like to thank Dr Scotland for kindly allowing me to be involved in this key stage of his project, and for his time and encouragement over the course of the studentship.

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