Ingrid Minnaar

NANOTECHNOLOGY
for pollination studies

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Dr Corneile Minnaar won the British Ecological Society's Robert May Prize for his paper describing the pollen-tracking technique.

Applying what you've learnt about nanotechnology in cancer research to your own pollination studies... training bees to take part in your lab experiments by rewarding them with a sustained supply of artificial nectar (i.e. sugar water)... designing and 3D printing your own microscopy equipment, then making the plans freely available so that others can try it too – this is the kind of innovation, dedication and collaboration that deserves to receive recognition.

Dr Corneile Minnaar, currently a postdoctoral researcher at Stellenbosch University, was recently awarded the Robert May Prize for the best paper by an early career researcher published during 2019 in *Methods in Ecology and Evolution*, one of the British Ecological Society's journals.

Doses of quantum dot-labelled pollen grains are applied to flowers using a micropipette.

The research for his winning paper was carried out for his PhD, supervised by Prof. Bruce Anderson, who heads the Biological Interactions research group in the Department of Botany and Zoology at Stellenbosch University. Corneile had relocated from Pretoria in 2015 to join the group, as he explains in his profile on the group's webpage.

"I was fascinated by the work on co-evolution that Bruce had done, and wanted to do a PhD that strongly addressed the role of pollen vectors in shaping floral evolution. Pollen vectors are clearly vital to the reproductive success of most flowering plants, so understanding how they transfer pollen between flowers is crucial in understanding the co-evolution between plants and their pollinators. I soon realised, however, that up until now pollination biologists have been unable to fully understand the role of pollinators in floral evolution, because they have been unable to track pollen grains as they are transferred from flower to flower."

Corneile decided to try to develop a technique that would allow individual pollen grains to be tracked. He had first read about the use of quantum dots in cancer research, and knew that these offered an alternative to fluorescent dyes for biolabelling. Quantum dots are semiconducting nanocrystals that can be made using a variety of different elements, and range in diameter from 2 to 10 nm (there are a million nanometres in a millimetre). They act as artificial atoms, in that they emit energy in the form of light when excited by light or electricity. The colour of the light produced will depend on the size of the particle – because bigger dots emit longer wavelengths (towards the red end of the spectrum) than smaller dots – as well as its composition and structure. Apart from having applications in biological and medical research, quantum dots are increasingly used in solar panels, television and display screens, and LED lighting.

Corneile purchased quantum dots that consisted of a copper indium selenium sulphide (CuInSeS) core and a zinc sulphide (ZnS) shell, making them more 'environmentally friendly' than the more typical versions containing cadmium or lead, both of which are toxic heavy metals. He made up test solutions of quantum dots suspended in hexane and then applied various experimental doses to the ripe, open anthers of flowers, using a micropipette. The hexane evaporated rapidly, leaving the quantum dots stuck to the pollen on the anthers. He tested the method on four species with different flower structures – the bulb *Wachendorfia paniculata*, the iris *Sparaxis villosa*, the Cape daisy *Arctotheca calendula* and the purple woodsorrel *Oxalis purpurea* – and also conducted the bee experiments to check whether the presence of quantum dots influenced pollen transport. His results indicated that the quantum dots have no effect on pollen attaching to bees that come into contact with the anthers, nor on the subsequent attachment to stigmas as the bees move from flower to flower.

But how could he count the quantum dots, given that they're too small to see with the naked eye? Corneile designed a simple fluorescence box that can be placed under any standard dissection microscope. It uses four LED lights as the UV excitation source, and has a small drawer for holding either microscope slides or insects. Corneile constructed the box using 3D printing, and made the design files as well as guidelines for its assembly and use available as supplementary information to the paper. This means that even people without access to 3D printers could send the files to an online service, such as 3D Hubs, to print the parts. A PhD student in New York tweeted his thanks for the technique a few months later.

YouTube

The fluorescent box designed by Corneile for use with a dissection microscope.

The box would typically cost between R5 000 and R10 000 to make, depending on the exchange rate and printing service, while labelling the pollen with quantum dots costs less than 40 cents per anther. The method will allow Corneile and other researchers who apply it to answer various questions relating to pollination biology, such as the degree of pollen loss during various stages of the pollen movement, and the role of specific pollinators in ensuring the survival of plants of economic and conservation importance.

Apart from the prestige of winning the prize, Corneile was awarded £250 and membership of the British Ecological Society. Robert May, after whom the prize is named, was a former president of the society who passed away at the end of April, at the age of 84. An Australian by birth, he obtained his PhD in theoretical physics from the University of Sydney, where he was subsequently employed as a physics professor for a 10-year period. He then switched disciplines to population biology and moved to Princeton University in the USA, remaining there for 15 years. During this time his classic book S*tability and Complexity in Model Ecosystems* was published, which applied mathematical techniques to population dynamics, as well as his 1976 paper in *Nature* that showed how chaos theory could be applied to biology. He also published widely on topics ranging from fisheries management to infectious diseases. In 1988 he relocated to Oxford University in the United Kingdom to take up a joint professorship with Imperial College London. He served as Chief Scientific Adviser to the UK government from 1995 to 2000, and president of the Royal Society from 2000 to 2005. He was knighted in 1996 and granted a peerage for his contributions to science in 2001, taking the title Lord May of Oxford.

Following his death, Corneile tweeted: "Robert May was a rebel. He jumped fields and challenged long-held paradigms using radical computational approaches. As an ecologist, your work undoubtedly contains ripples emanating from his ideas and methods: Be a rebel!"

- Minnaar, C & Anderson, B 2019. Using quantum dots as pollen labels to track the fates of individual pollen grains. *Methods Ecol Evol.* 10: 604–614. https://doi.org/10.1111/2041-210X.13155
- The paper is freely available in a special virtual issue of the journal dedicated to the Robert May Prize: https://www.britishecologicalsociety.org/publications/best-paper-by-an-early-career-researcher/robert-may-prize/
- To view a two-minute video on Corneile's research, search YouTube for 'Tracking pollen with quantum dots'.

Corneile Minnaar

The fluorescing quantum dots show where pollen has adhered to a bee that visited a labelled flower.

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