Normal Botany

David Baulcombe (2009)

I wrote this short text for the Annual Record of Trinity College, Cambridge.

An introduction to beachcombing

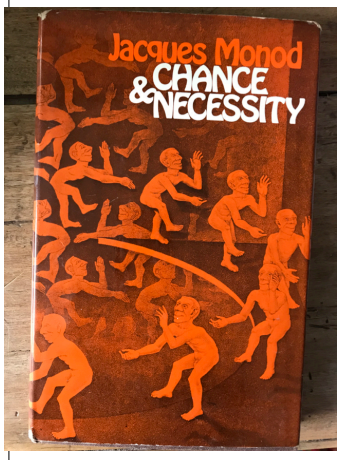
Having reached a certain age I can now look back and reflect on the different approaches to scientific discovery. I see that very few people are visionaries who can both identify the big questions at the frontiers of knowledge and, just as important, see how to find an answer.

The rest of us – practitioners of Thomas Kuhn's "normal science" - are beachcombers. Like Newton we are as "a boy (or girl) playing on the seashore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary". Newton of course was aware of the nearby "great ocean of truth" but the rest of us only see it if we pick up the smoother pebble, if ever.

My life as a beachcomber started when I decided to do a PhD. My tutor at Leeds University advised that I should focus on what I thought is the most important question in biology and that, for me, involved genetic regulation. It would hold the key to many of the mysteries of biology and help meet challenges in healthcare and agriculture.

Genetic regulation is best understood in terms of development: the sequence of events in which a single egg cell differentiates into a multicellular adult. Each of the cells in the adult has the same genes as the egg but different proteins. Crick's central dogma of molecular biology tells us that 'DNA makes RNA makes protein' when a gene is switched on and so there must be an off switch if protein is not being made. Genetic regulation is all about the understanding of these on and off switches.

Figure 1 My undergraduate reading. I never really understood the concept of teleonomy in this book which Monod defines as the characteristic of life associated with being "endowed with a purpose or project".



Jacob and Monod (Figure 1) had started to unravel genetic regulation in bacteria but, when I started my PhD in 1973, we knew virtually nothing about the more complex processes in animals and plants. As a beachcomber I was not quite sure how to go about finding out, but I thought that the best approach would be molecular biology and I chose to go to Edinburgh (Figure 2).

It was a good choice because Edinburgh is a beautiful city and because the University had a lot of people who were interested in the molecular biology of genes. Ken Murray was developing gene cloning and, although I did not use his method, I appreciate its power: it allowed the isolation of DNA from individual genes. Until gene cloning we could study DNA or RNA from a plant or animal but only in its totality

– there was no way of separating the DNA of an individual gene from the other 30000 or so other genes in the genome.

My undergraduate and postgraduate degrees were in botany but, having discovered molecular biology, I almost moved into biomedicine. I was awarded an 1851 Research Fellowship to work in Paris on the regulation of haemoglobin genes (in ducks – for very good reasons) but I bottled out of the transition and went to Montreal to investigate haemoglobin genes in plants instead. From there I moved to Athens (Georgia, USA) and eventually to the Plant Breeding Institute (PBI) in Trumpington.

Figure 2: Edinburgh. I fell in love with Edinburgh and a Southerner. I married the Southerner and now just have occasional visits to Auld Reekie – here in the Botanic Garden.

A few of the various pebbles and shells picked up in these travels were quite smooth or fairly pretty. I was becoming an expert gene cloner and DNA sequencer. I had identified genes that are switched on in the nitrogen-fixing root nodules of soybeans and I found out that plant hormones act by activating and repressing gene expression in shoots and seeds. But none of these smoothish pebbles had yet made me look up to the great ocean of truth. It was all very definitely normal botany.

Avoiding the obvious

Plant breeding is essentially applied genetics but, until I joined the PBI, I was a 'genetics sceptic' because it was not obvious to me how to make a connection between a molecular biologist's DNA and a geneticist's gene. A geneticist does not need anything as crude as a gene in a test tube because information can be inferred by careful deduction. After a year or so at the PBI, however, I was a genetics convert.

I should have picked up sooner on the power of genetics because I had heard Salvador Luria - one of the giants of twentieth century science – give a talk. Luria started by asking the audience whether we wanted a geneticist's talk or a molecular biologist's. A geneticist's talk, he explained, may not have much data but there would be plenty of ideas. A molecular biologists talk, in contrast, would have lots of data…….

Figure 3 *tjuntiwari*. The leaves of this plant are rich in nicotine and are chewed by aboriginals

I also learned from my PBI colleague Enrico Coen who worked on pin and thrum-eyed primroses and bilateral symmetry of snapdragons. He said that it was not a hindrance to work

with difficult species rather than the model organisms used by the mainstream of plant biology. With model species it is easy to do the obvious experiments but, with the other plants, one is more likely to do the experiments that are informative.

In my subsequent attempts to avoid the obvious I have used several species including tobacco, potato, tomato, *Chlamydomonas* and an Australian weed – *Nicotiana benthamiana* (Figure 3). One of my preferred journals likes to use common names rather than Latin binomials and the galley proofs of our papers would always come back with *N. benthamiana* changed to 'tobacco' (*N. tabacum*). We corrected these errors but then, in the final proof, the tobacco references were restored. Eventually the editor conceded defeat when I found out that the indigenous name for *N. benthamiana* is *tjuntiwari*.

Bryan Harrison from Dundee also had a big influence on my research. He is a virologist and I contacted him because I had been reading about viruses. Viruses are packets of highly specialised genes and I thought that they could be useful tools in my quest to understand genetic regulation in plants. There was ample precedent for this approach from animal virology.

Virology is a relatively young branch of the life sciences and Bryan is a bridge with its early pioneers. He had worked at the Rothamsted Research Institute with FC Bawden and NW Pirie who had discovered that tobacco mosaic virus contains RNA. This finding contradicted the erroneous Nobel Prize winning work of Wendell Stanley who claimed that this virus was an infectious protein.

Between 1936 and 1940 Pirie was in Cambridge and his collaborator Bawden was at Rothamsted. Bryan speculated that purified viral RNA would have degraded and lost infectivity *in transit* and that separation had prevented this pair from making the huge discovery that viral nucleic acids are infectious. Had Bawden and Pirie been in the same institution they might have taken Stanley's place at Stockholm and anticipated the later discovery of Avery and Macleod that nucleic acids are the material of heredity. I like this story because it illustrates how plants can be informative about biology in general. I make this point in a lecture entitled "Of Peas and People or Maize and Men[1]" together with many other examples of key discoveries in biology that are based on findings from plants.

Figure 4 Yellow chlorosis caused by non coding RNA. I could have been a contender if I had followed up our research on this disease

My initial interest in viruses took me into infectious disease and disease resistance. I was interested in this topic because disease is often caused by perturbation of genetic regulation in the host. There is also an important practical dimension because the certainties of life are not restricted to Franklin's death and taxes: infectious disease is also a constant threat because pathogens

[1] Monod justified his work on bacteria saying that "what is true for E. coli is also true for elephants". I thought that plants provide a better alliteration.

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readily adapt to the host's defence strategies.

One of my first findings with viruses was that RNA could cause disease even if it does not encode a protein. We identified non-coding viral RNAs that trigger a spectacular yellow mosaic on tobacco plants (Figure 4) or that caused tomato seedlings to keel over and die. These findings were not compatible with the standard disease paradigm involving virus-encoded proteins and we inferred that the viral RNA was somehow preventing expression of a host gene. The idea was right but we did not have the technology to prove it and, unfortunately, we dropped the project. That was a very bad decision - for reasons explained below.

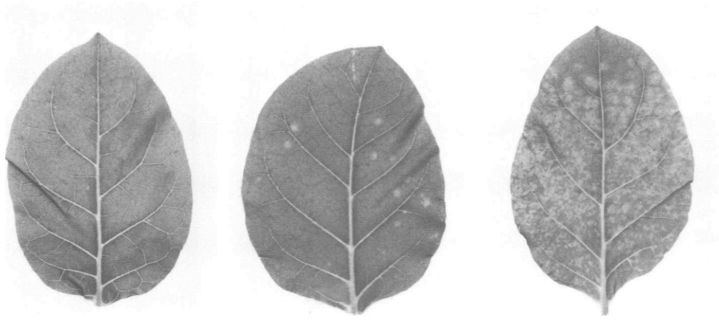
A very pretty shell

In parallel with these experiments I was also exploring the emerging technology of genetic modification (GM) for disease resistant crops. We produced some of the first disease resistant GM plants and, although they were not grown in the field, they did support my successful application to join the newly established Sainsbury Laboratory in Norwich.

The Laboratory was an experiment by David Sainsbury who had been persuaded by one of his advisors that plant pathology is important and interesting. David's aim was to set up a well-resourced facility in which the researchers were free to follow their scientific nose. There was no requirement to stock the shelves of his supermarket but, if there was a chance to do something useful with our research findings, we had a responsibility to follow up.

It was a fantastic opportunity although positions in the Sainsbury Laboratory were on five-year contracts and I had to give up my tenure at the PBI in the Scientific Civil Service. I now had to reapply for my job after each contract period but I was happy because the facility and resources were so good. If I could not justify my continued employment in that setting then clearly I should go and do something else. At least we could buy a bigger house in Norwich than in Cambridge for our three - soon to be four - children.

Figure 5 It doesn't look much but this was a big result. All leaves are from virus-inoculated plants but the two on the left are GM and they failed to develop the disease. The virus, like most plant viruses, has a boring name and is called potato virus X.



Some of our first experiments in the Sainsbury Laboratory explored the concept of parasite-derived resistance in which a gene is transferred from a parasite into the host – a type of "genetic immunisation". This approach worked well in *Escherichia coli* and I wanted to test it in plants.

Working with plants can be frustrating because each step takes such a long time. To test parasite-derived resistance, for example, we had first to transfer genes from a virus into tobacco cells and then we had to regenerate mature plants. That took about six months. Then we had to produce seedling progeny of those plants and test them for the presence of the viral transgene. It was almost a year after starting the project before we could test the plants for resistance.

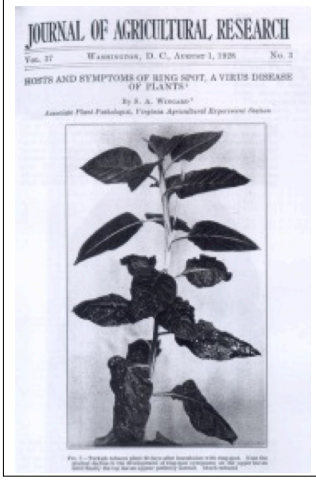
Fortunately the virus test was quick because symptoms emerge within a few days of the inoculation and, even in our initial experiments, the results were very clear: the plants were resistant against the virus (Figure 5). In some of the lines the resistance was very strong so that even the most concentrated inoculum would not cause disease.

To get such a definite result was exciting but there was something strange: the immunity was strongest in plants in which the newly acquired viral transgene was switched off. I did not understand how there could be resistance from a gene that is not expressed but, eventually, I realised that the process causing the virus resistance also silenced the transgene.

We referred to this process as 'RNA silencing' and, using a combination of molecular biology and genetics, we described aspects of its mechanism in a fair amount of detail. Probably our biggest discovery was a new type of RNA known as small interfering RNA, although we took too long to find it. It turns out that small interfering RNA caused the symptoms due to non-coding RNA (Figure 4) in our early experiments. With a bit more persistence, we could have been ten years ahead of ourselves.

Our work on RNA silencing converged satisfyingly with various other animal groups, including that of Trinity title B Fellow Hannon who was then at Cold Spring Harbor. The animal researchers were interested in a process that also involves small interfering RNA and we were clearly all on the same RNA bandwagon looking at a set of homologous processes. I am not quite sure whether the collective discovery of these RNA-based mechanisms is a Kuhnian paradigm shift but, if not, it has to be at least a nudge in the gene expression field and certainly a prettier shell or smoother pebble.

Figure 6: An important result from 1927 showing how plants recover from virus disease. The recovered leaves are resistant to secondary infection and we helped to explain how in 1997.


JOURNAL OF AGRICULTURAL RESEARCH

Vol. 37 Washington, D. C., August 1, 1928 No. 3

HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS[1]

By S. A. Wingard[2]

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We might have been feeling quite pleased with ourselves at that point but the natural world is always good protection against hubris. So it was with our 'clever' biotechnology against viruses because we eventually found that small interfering RNA is part of a natural defence system in plants against virus disease. Bryan Harrison helped me appreciate this point when he showed me a paper published in 1927 (Figure 6) about a mysterious immune system in plants. We thought that RNA silencing could explain this early work and so we set up a series of

experiments to test that idea. I am embarrassed that these were the last experiments, more than twenty years ago, that I carried out myself. Ever since then my role has been as a supervisor of the students, postdocs and technicians who put in such long hours in the glasshouse and at the laboratory bench. I hope my suggestions about their work have been useful.

The real world is less accommodating than academia

The disease resistance in our GM plants was based on RNA silencing and, at least in principle, we could refine the approach to protect any crop against any virus. Problem-solving in the real world, however, requires more than good ideas. We also have to persuade people (and be sure) that our solution does not introduce complications that are worse than the original problem.

With GM crops our persuasion has failed spectacularly over the last 30 years. Of global farmland only about 13% is planted with GM soybeans and maize and they have just two different GM traits – herbicide tolerance and insect resistance. There are many other GM traits in the research pipeline including virus resistance but, with so many people who mistrust the technology, it is likely that most of these innovations will never be used.

Intellectual property is one of the most problematic aspects of GM crops because the patents on enabling technologies and genes prompt general discomfort about ownership of food production by large companies. Perhaps GM would have been more palatable if, rather than patents, it had been subject to the International Convention for the Protection of New Varieties of Plants drawn up by Philip Allott *et al* in 1961? This forward-thinking Convention is similar in some respects to the open source approach of the computing industry and it could have provided some rights for inventors without stifling further rounds of innovation.

Additional to the problems with IP it is likely that the poor acceptance of GM is because crops and agriculture, especially in regions of intensive cultivation, do not have a good track record for the environment, economy and society. I am optimistic that we will find a good solution, however, because the prolonged deliberation about and opposition to GM has stimulated deeper thinking about new technology in crops and agriculture.

One of my strongest hopes is for reconciliation of organic agriculture and biotechnology. Many biotechnologists, like myself, want GM to be used in more sustainable agriculture that does not necessarily underpin oligopoly of large companies. It seems to me that there is common ground with organic farmers and scope for industrial agriculture to learn from the organic sector.

Ever since I came to Cambridge in 2007 I been trying to create an environment in which crop science research will flourish and I helped my Department form an alliance with the National Institute for Agricultural Botany (NIAB). An outcome of this alliance is the Cambridge Centre for Crop Science (3CS) that will provide a research home for a Professor of Crop Science endowed by a generous alumnus of Trinity. The new laboratory will be on the NIAB Huntingdon Road site and it is funded by the UK Research Partnership Infrastructure Fund.

Science has driven the revolution in healthcare and 3CS could contribute to a parallel improvement in the crops of the future for food and industry. One of the most important advances in life science is the 'next generation' DNA sequencing technology developed by Trinity Fellow Shankar Balasubramanian. I expect that this next generation sequencing will feature strongly in the 3CS research programme together with advances in computing, imaging and chemical analysis.

I like to think that these powerful research tools coupled to open-minded thinking lead us to better technologies for sustainable and sufficient crop production.

Continuing to avoid the obvious

In my group's research we continue to explore virus resistance. In recent years we have collaborated with Kenyans trying to find a solution to maize lethal necrosis disease that is devastating the maize crop in East and Central Africa (Figure 7). I am also hoping to start working on a cocoa virus responsible for swollen shoot disease that is a challenge for smallholder growers in West Africa. At present the only solution to this virus is to remove the infected plants. The farmer then loses income until the replacement tree is productive.

Our main line of research, however, is with epigenetics and tomato. Epigenetics is a rather fashionable topic that is important in developmental biology and cancer. It concerns a layer of information in genomes in the pattern of methyl groups on the C residues of the DNA. This pattern of DNA methylation is heritable: it is copied when cells divide and it is important because it influences gene expression – the starting point of my scientific career. A motif in the sequence of A, C, G, T may promote expression of a gene if the C residues are unmethylated and it may suppress it if they have methyl groups attached.

I am interested in epigenetics because it is likely to explain hybrid vigour – the extraordinary over-performance of hybrids over the better of the two parents – and it is likely to influence crop plant breeding. I cannot claim, however, that I

Figure 7: An serious virus disease in Kenya. At present there is no easy way to stop this disease.

started work on eipigenetics because I had insight that it would be important. It was a smoother pebble from our earlier work on parasite-derived resistance in which I noticed that small interfering RNA correlated with the methylation status of the corresponding DNA. This was a puzzling observation because everything else that we knew about the small interfering RNA involved interactions at the RNA level.

At first we did not know whether the DNA methylation was a consequence of the small interfering RNA or *vice versa*. The answer, rather pleasingly, is both: small interfering RNA promotes DNA methylation and, conversely, DNA methylation triggers the biogenesis of small interfering RNA. This system creates a positive feedback that explains, in part, the heritability of epigenetic effects. In more recent work we have linked this process with a phenomenon that was first described in the 1950s by maize geneticists in which inheritance does not follow Mendel's laws. They referred to this process as paramutation.

The current challenge in my lab is find out to what extent these epigenetic effects, including paramutation, have an effect on natural populations and how they can influence the course of evolution. I would also like to explore their potential application in agriculture. One possible technology would lead to epigenetic modification that would improve the crop for the grower or consumer but in which the DNA sequence would be identical to the unmodified progenitor.

At present there is no regulatory framework for risk assessment of such epigenetically modified crops and so my initial approach will be to submit an application to the same body that regulates GM crops. I will also try to engage the general public so that they can understand what we are trying to achieve and why. I hope we will do a better job this time round than we have done since the 1980s with GM crops.

Trinity botanists

Since 1724 there have been fifteen Professors of Botany in Cambridge and I am the first of them to be a Fellow of Trinity. In fact there have been only a few Trinity botanists with any position in Cambridge. John Ray is the best known (1627 – 1705) and Richard Walker (1679 – 1724) (Figure 8) founded the first botanic garden in Cambridge. Otherwise there have been just a few others including Francis Darwin (1848 – 1925) and most of them developed their careers

Figure 8, A rare but not necessarily beautiful species. Trinity botanists Ray and Walker (left and right respectively).

and reputation away from the College. Stephen Hales (1677 – 1761) was a Fellow of Corpus Christi but we may claim him because he carried out experiments on water transport in plants in Vigani's laboratory. John Bradfield could also be counted as a botanist manqué because he started his research career investigating carbonic anhydrase in plants although he was in the Zoology Department. Elliot Meyerowitz was with us briefly as a botanical Title F Fellow in 2012 and 2013.

Perhaps there have been so few plant scientists in Trinity because the Fellowship electors think that "Botany is monotony – the study of plants I leave to my

aunts[2]"? I did not do a very good job defending the opposite view in an undergraduate essay and I hope this text is more convincing. Perhaps the electors will admit a few more botanists over the next few hundred years. If any candidates consult me then I can tell them that this is an excellent academic home for their neglected subject, even if they are only beachcombers.

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[2] This misguided statement might be from TH Huxley who allegedly supported Darwin's award of a medal from the Royal Society on the understanding that it was not recognition for his botanical work.