# Painting by numbers

The animal world is full of the most astonishing patterns. Many are internal, but enough are carried on the surface, available for all to see, to satisfy any naturalist for a number of lifetimes. There are the spots of leopards, the stripes of zebras and tigers, the zig-zags of snakes, the immensely complex patterns on the wings of a butterfly and even the patches of Friesian cows, to name but a few. The mechanisms that create these patterns have long fascinated biologists. Many cases, espcially those in large animals, are thought to be produced by the action of a system first proposed by the British mathematician Alan Turing in a brilliant paper of 1953 (see links: this paper remains high in my personal affection because reading it as a first-year Natural Sciences undergraduate is what made me want to specialize in developmental biology, rather than follow the career in radio astronomy for which I had previously been aiming).

Regular readers of this blog and its predecessor will know that the lab had a strong interest in applying the techniques of synthetic biology to challenges in animal development. When I first wrote about these ideas in a formal publication, in 2008 (see links), I stressed the potential constructive power of combining synthetic biology with the applied side of developmental biology, tissue engineering. Since that time, those of us taking the ideas forward have come to realize that synthetic biology can also be a powerful means of testing our basic understanding of development.. What is more, the ability that synthetic biology gives us to engineer 'developmental' systems from scratch can allow us to explore methods for solving a developmental problem that seem *different* from the solutions hit on in natural evolution. Assessing their strengths and weaknesses can perhaps be a way of gaining more insight into the balance, in evolutionary choices, between chance and necessity (to steal from the words of Jacques Monod).

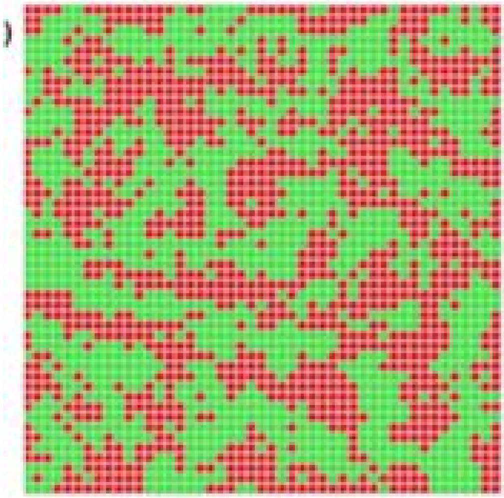
The lab is immensely fortunate that, some years ago, the ideas we were discussing attracted an truly talented post-doctoral fellow, Dr. Elise Cachat, to join us. I have described her construction of a library of synthetic biological modules to be used for developmental purposes in an earlier post. Since then, she has been using two of these modules to create a system that causes cells in culture to organize themselves, on command, into patches, spots or stripes in two or even three dimensions. The mechanism is not based on naturally evolved coat patterning: it is instead, as far as we know, a 'road not taken' in evolution. The easiest way to explain how it works is to tell the real story of its conception (the published paper, like almost all published papers, presents facts and ideas in a logical order and glosses over the actual messy chain of thought).

Ultimately, I suppose I should thank my car. The ageing Citroën I use as my every-day car hails from the same country as Elise but, alas, does not share her penchant for being neat and tidy. It is one of those vehicles that eschews springs and instead floats on high pressure nitrogen, in an arrangement made possible – on a good day – by an insanely complex arrangement of hydraulic valves and pipes that form a sort of analogue computer that whirs and clicks to itself and, I am almost sure, mutters French oaths when the roads are rough. After a few years, this system becomes prone to springing leaks, not from its high-pressure side which is made of very solid metal but from a selection of cheap plastic tubes used as low pressure returns of spent fluid. By the time the cars have passed their first quarter century, they assure their further longevity by a kind of rust-defeating, self-oiling process in which weeping hydraulic fluid ends up coating their entire undersides. The effect of all of this is a kind of 'green motoring', in the sense that the car has a habit of leaving small spots of lurid green LHM fluid wherever it is been parked. When a drip falls on to damp ground, the result is a richly patterned mosaic of oil and water that scatters low light in a rainbow-like way.

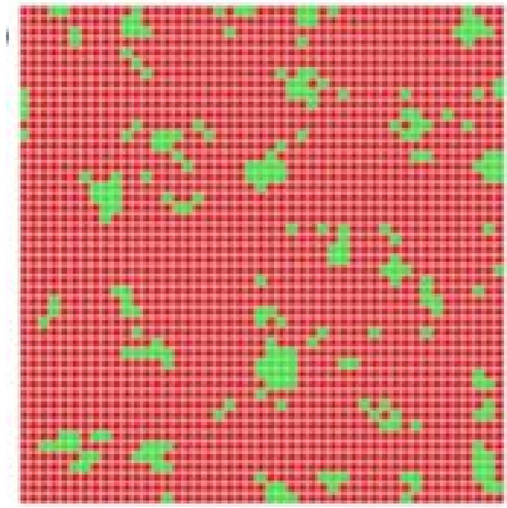
One time that I was working under the car, replacing yet another split pipe because the car park decoration was getting rather embarrassing, I got a great view of sunlight reflecting off one of these patterned puddles and started to wonder about the physics that created it. Given the chance, oil and water separate completely. The reason for this is that the free energy of the system is much lower if as many of the water molecules as possible are surrounded by other water molecules, with which they can form strong hydrogen bonds,  instead of water being faced with oil molecules with which it can interact only weakly. The mixture therefore tries to minimize the are of water-oil interface. On a thin surface, though, there is a problem: the water molecules will still tend to stick together and exclude the oil but, when this has been happening for a while, each patch of water is surrounded by the oil it has excluded and no more water can join without crossing the oil. The result is therefore a complex swirly pattern of water and oil.

Many years ago, in the 1960s, Malcolm Steinberg showed that two populations of living cells, one sticky to its own kind and other less so, will if given the chance separate like water and oil. A string of subsequent papers extending into the 21$^{st}$ century showed that this really is to do with adhesion.

It occurred to me, lying under my car with LHM fluid gently dripping into my hair, that if we confined a mix of very adhesive and less adhesive cells to a surface, they might have the same problem in sorting out as oil and water on a puddle, and make spectacular patterns. After fixing the pipe, having a shower that used about half a bottle of shampoo, and making a really big pot of tea, I settled down at my computer and wrote a simple cellular-automaton type simulation of the idea as a sanity check. It suggested that the system should indeed make patterns (see below). The next day I turned up early to the lab to wave pictures like the one below in front of the ever-patient Elise. *At least let me get my coat off!*, was her first, and utterly reasonable, response.



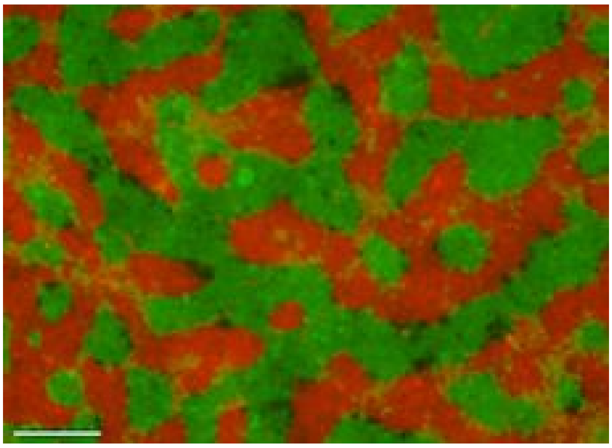
50/50 mix, AA>BB>AB



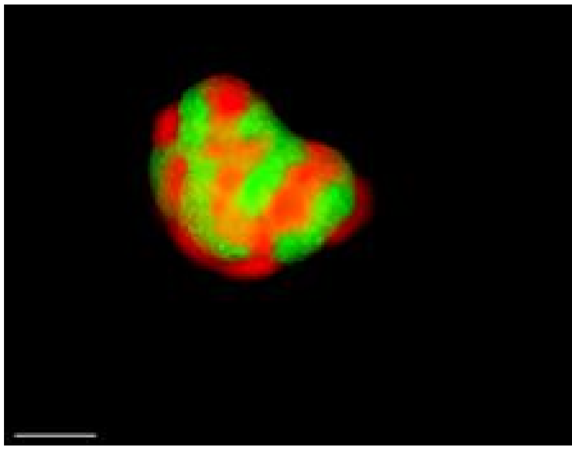
90/10 mix, AA>BB>AB

*A computer prediction of patches or spots, depending on the ratios of cells.*

Elise had already made cells of different adhesiveness that, additionally, glowed different colours so the extension to making patterns was relatively straightforward. She quickly proved that, with the adhesion systems switched off, no patterns were seen (the mix of the cells was statistically random) but, when the adhesion was switched on, they made patterns. What is more, the patterns could be spots or patches depending on the ratios of cells, just as the computer simulations said they should. A real surprise was that, while small 3-dimensional clumps of cells could sort completely instead of making a pattern, large 3-D clumps made rich patterns instead. The arrangements of the cells were not critical – what mattered most were two numbers, the ratio of adhesiveness and the starting ratios of the cells (sticky to non-sticky). We were not painting by numbers, though – the cells were doing this for themselves.



*An image of a self-organized pattern made by real human cells (growing in a dish), engineered with Elise's adhesion modules.*



*Cells of the same type now making 3-dimensional patterns. The red/green colours come from fluorescent proteins that have been engineered into the cells along with the adhesion systems.*

Is this directly useful? Probably not. But is has pushed forward the range of things that synthetic biology can do, and it has drawn attention to a patterning system that can work but that evolution seems not to exploit, at least not to pattern simple surfaces. We are now making the patterns even richer, and thinking about using them to do something that might actually be properly useful in regenerative medicine. More on this if, and when, we get it to work.

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**Links:**

- The publication itself: http://www.nature.com/articles/srep20664
- Turing's 1953 paper: http://www.ncbi.nlm.nih.gov/pubmed/2185858 (this is not the actual paper but leads you to the full reference: you may need a real library, the kind with real paper books, to find the article).
- My 2008 proposal for synthetic morphology: http://onlinelibrary.wiley.com/doi/10.1111/j.1469-7580.2008.00896.x/epdf