

Experimental evolution in Lisbon

# Flexible Bugs

The rate at which beneficial mutations accumulate in genomes is difficult to quantify. However, Isabel Gordo's group has recently succeeded in doing so for *E. coli* and found a rate 1,000 times higher than previously estimated.

Genomics, proteomics, transcriptomics and many more strange 'omics'. Microarrays, real-time PCR, sequencing robots, databases and cooperative international projects. Do you think that modern biology has become an excessively technical and descriptive science? Do you miss the golden age of genetics, when a couple of boffins in a dusty lab required only the brains they were born with and some Petri dishes to tackle life's deepest mysteries? Have clever and elegant experiments, as pioneered by those classical geneticists that we all studied at university, disappeared forever?

Well, maybe. Or perhaps we should remove our rose-tinted spectacles and accept that hindsight is a wonderful, if sometimes deceptive, thing. Consider also that there are still diamonds to be found amongst



Old question, young researchers: Lilia Perfeito (l.) and Isabel Gordo.

the mediocrity of modern biology. Focus on the few cases of good back-to-basics science that rise to the surface of an otherwise murky pool.

## Digging out a diamond

The work of Perfeito *et al.* (*Science* 317: 813) belongs to this last category of good back-to-basics scientific thinking, but also incorporates a good dash of modern technological expertise and descriptiveness. The introduction to their recent paper, *Adaptive mutations in bacteria: high rate and small effects*, states that, "the rate at which new

mutations arise in natural populations and their fitness effects are of key importance in evolutionary genetics". They go on to explain that, although beneficial mutation is considered the driving force of adaptation, theoretical models have always lacked good estimations of its actual significance in populations. The rate at which beneficial mutations accumulate is difficult to quantify. They are expected to be rare, with most of them exerting little effect upon fitness and experiencing a low probability of fixation because of stochastic loss and competition (clonal interference).

## Persistence, will and group spirit

Trying to overcome these difficulties, Perfeito *et al.* designed an experiment to measure both the rate at which beneficial mutations are generated in the bacterial genome and the effects that they have on bacterial fitness. As expected, they observed that clonal interference increases in populations with very large effective sizes. But by reducing a population's effective size, they got a surprising result: in *Escherichia coli*, the rate of beneficial mutations was 1,000 times higher than previous estimates.

The research was done by four scientists working at the Instituto Gulbenkian de Ciência (IGC) in Lisbon (Portugal): Lilia Perfeito, Lisete Fernandes, Catarina Mota and Isabel Gordo. Isabel Gordo is a young group leader at only 34. She obtained a first degree in physics and then started on the Gulbenkian PhD programme in biology and medicine. After taking a year of advanced courses she wrote her thesis at the University of Edinburgh, under the supervision of Professor Brian Charlesworth, a well-



known evolutionary geneticist. In 2002, after a spell as an assistant researcher at Oxford University, she moved back to the Instituto Gulbenkian de Ciência, where she took a principal investigator (PI) position.

Gordo devised the project soon after finishing her thesis. Lisete Fernandes, a molecular geneticist leading another research group at the Instituto Gulbenkian, discussed the details and helped her to develop her ideas. In 2003, they obtained funding from the Portuguese Fundação para a Ciência e a Tecnologia. Lilia Perfeito joined the lab as a PhD student in 2004 and the project subsequently became the main focus of her thesis. In 2005, technician Catarina Mota joined the group to assist with experiments. "The initial period of the whole project was a bit hard" says Gordo, "since we had to carefully set up the conditions of the experiment to be able to collect and analyze the data. I was personally very excited, since in the beginning my experience in the field was not a huge one. But the persistence, the will and the group spirit that accompanied all the people in this work made it happen."

## In need of data

The first stages of the project were a steep learning curve for Gordo, since she had little previous experience of experimental research. Until then, she had been doing theoretical research on evolutionary genetics. Together with Charlesworth, and with other collaborators later on, she produced analytical approximations, predictive models and calculations. These studies focused mainly on the evolution of asexual haploid populations and the processes by which they accumulate deleterious mutations in an irreversible manner (Muller's ratchet). But Gordo needed some accurate experimental data. "Since I started my PhD it became more and more evident for me the importance of estimating basic parameters that govern the strength of the different evolutionary forces, namely the rate and fitness effects of mutations. In 2001, a paper came out [Imhof and Schlotterer, 2001,

*Proc Natl Acad Sci USA* 98: 1113] that I read carefully and that inspired my future work. Soon after this event I decided to start performing experiments which would allow me to study adaptation of bacteria to novel environments. From the initial experiments it became evident that the rate of production of adaptive mutations was very high and so we decided to try to estimate it the best we could."

### Enormous potential for adaptation

In the Imhof and Schlotterer experimental model that inspired Gordo, an *E. coli* strain is transformed with a plasmid containing an exogenous microsatellite locus, and cultured by serial transfer in 5ml of liquid media. The high instability of microsatellites rapidly generates different alleles in the dividing cells. Later, in successive generations, the original genomically homogeneous populations evolve by the accumulation of deleterious, neutral and beneficial mutations. Cells incorporating beneficial mutations increase their fitness and their competitive ability. Thus, beneficial mutation means that some clones (and their correspondent microsatellite alleles) are more abundant than others. The DNA of populations in successive generations may then be extracted, its microsatellite alleles separated in a polyacrylamide gel and their respective abundance measured by hybridization. Doing so provides a graphic illustration of how bacterial clones in a liquid culture compete down the generations.

Perfeito *et al.* adopted this basic protocol and introduced a significant new variable: a population's effective size. They allowed bacterial populations with differing effective sizes to evolve for 1,000 generations and followed their microsatellite al-

lelic distribution at periodic intervals. Their comparison of mutation rates led the authors to conclude that "1 in 150 newly arising mutations is beneficial and 1 in 10 fitness-affecting mutations increases the fitness of the individual carrying it". In other words, that, "an enterobacterium has an enormous potential for adaptation".

As usual, interesting results spark new inquiries. "There are a couple of questions that arose and that we are trying to answer" says Gordo. "One of these has to do with understanding what type of genetic changes have occurred during the adaptive process, which genes have changed and how. Another question relates to understanding the similarities and differences in the adaptive process in more natural environments and in the test tube. In this respect it was a pleasure to read a recent paper in *PNAS* where the authors have followed the evolutionary process of *Staphylococcus aureus* by whole-genome sequencing in a human host. They also observed an outstanding production of potentially adaptive mutations [*Proc. Natl. Acad. Sci. USA* 101: 9786].

### Techno-biology tackles old questions

Well, this *PNAS* paper is at least one example of how modern techno-biology can offer interesting results. "My own feeling is that the 'omics' era may well be excellent for highlighting patterns of biological complexity that we are yet to discover. But in science observation is only the first step; generating hypotheses and testing them in order to support or reject them is what is important. The most important questions, in my research field at least, were asked a long time ago, and some simple experiments might be all it takes to tackle them."

RAFAEL FLORÉS

## One fine day in the lab...

by Leonid Schneider

