

Scientific Report on the ESF Workshop
"Long- Term Experimental Evolution with Microbes"

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from the Centre National de la Recherche Scientifique (CNRS)

Convenors: M Blot (Grenoble) and P Rainey (Oxford)

1) Executive Summary

Evolution remained descriptive until recently, but it became experimental with the possibility to grow living organisms for long periods and to compete the evolved individuals against their ancestor. This new approach of evolutionary biology is easier with microbes because their generation time is short, their genome small and sequenced for more than 70 of them. Moreover, molecular and genetic tools are available to act on their genomes,

The ESF workshop has convened many specialists of experimental evolution who conduct their research in Europe. There were grossly two types of talks: on one side, groups who were able to demonstrate that genes or genetic rearrangements allow a bacterial population to cope with an environmental constraint. On the other side, groups showed that the mutation abilities have a positive effect on adaptiveness, and that wild populations have mutators promoting beneficial mutations. Altogether, this led to the conclusion that experimental evolution with microbes is very important to the basic and the applied research in the post-genome era. During the meeting, a few questions were addressed on the contribution of a european network in experimental evolution to this new discipline but also to other research supported by ESF. Chapter 4 details the assessments of the results and suggests that this new group of European researchers represent a large part of the international community. Since links already exists to another ESF Workshop (convenor J Balandreau) and to an EU Framework (MECBAD, convenor K. Smalla), the integration of this group into a larger program should be taken into consideration. This is to rapidly use the knowledge and the concepts of experimental evolution in neighbouring fields of microbiology, biotechnology and public health, but also to highlight to national agencies, through ESF, on the potential of this research..

Although the main goal of the participants is to understand the population, the genetic and the molecular mechanisms of evolution in bacteria, their research has many outcomes in areas where micro-organisms are important. The main result was that bacteria have a large potential to evolve rapidly their genome to adapt a new constraint. However, this possibility is not taken into consideration in the biotechnologies when bacteria are asked to provide a new molecule with a yield compatible with economical rules. Thus, up-scaling is often a disaster. In the same line of thoughts, the inescapable adaptation of pathogens to a new antibiotic is never analysed *a priori* in a long term experimental design, and antibiotics will have shorter and shorter lifespan.

The community of participants to this workshop, together with other groups, have shown the genomic potential of bacteria, the molecular mechanisms of adaptation and the effect of a rapid evolution on populations or communities. Because these groups have acquired the methods for high through output (genomics or proteomics) they have now the ability to study rapidly a new phenomenon, and to predict its evolution. None of the participants were surprised to discover that human pathogens keep a similar ratio of mutators as laboratory strains placed under a selection. These bacteria thus retain the ability to cope with sophisticated antibiotherapies.

Due to a diversity in the level of the observations (from the phenotype to the genome architecture) but a common use of the methods in experimental evolution, the participants have understood their need to collaborate and exchange researchers who might learn concepts and techniques. The still large diversity of model organisms remains the unique barrier for extended collaborations, which could be improved by suggestions in the frame of international funding.

2) Scientific content of the event

The main result that was underlined during the workshop is that bacteria can adapt genetically very rapidly to almost any constraint. This was illustrated in all talks, with different bacteria, different environmental pressures, and most importantly both in laboratory experiments with model bacteria and in naturally occurring pathogens. Different levels of observation were presented, and they appeared very complementary. Moreover the participants could discuss how the contribution of another group, at another level of observation, might improve their own research. Obviously, this will have an output in the future.

The first level is the phenotype, for which a few groups showed that a population under a constraint would shift to another phenotype as a consequence of natural selection. With few exceptions, these groups were not yet able to pinpoint the genes responsible for the effect because the phenotype was diffused, and thus most likely multigenic. The second level was genetic in the sense that the impact of different alleles could be shown on the life history traits of bacteria; this includes also the evolution of ageing in a differentiating bacterium, and also the role of DNA repair mutants known as mutators. It is now clear that mutability (also understandable as the ability to generate genetic diversity) is the first parameter driving the evolutionability of a genome. The last level is genomic and focuses on the evolutionary dynamics of the genome architecture during an adaptation process. At this level genomic rearrangements allow the optimisation of, for instance, gene expression and adaptation is based on the reshuffling of an existing genome.

One important issue of the meeting is a new concept which emerged: the populations of bacteria have not only their real genome (the expressed or expressible set of genes), but also a virtual genome made of mutations improving the fitness of their carrier. The virtual genome has to be seen at the level of a population, and it is made of the evolutionary potential residing in new genomic combinations. Indeed, all communications at the workshop can be understood with the virtual genome view. Because bacterial populations are large (often the inverse of the mutation rate), numerous mutants are continuously generated until one shows better abilities and replaces its ancestor. During conditions of adversity, we heard that bacteria could modify their mutation rates, but also select for mutators. This was illustrated both with model bacteria, natural pathogens and mathematical models. Thus, the *ad hoc*

mutant has more chance to arise, although most other mutants will be deleterious. This is obviously an important discovery in other fields of microbiology, one example being the development of antibiotic resistances.

It was clear from all communications that the participants were usually contributing different fields and the workshop was a first opportunity to convene a community who now knows its existence, and who will benefit from a new group of collaborations.

It is important to recall the weight of the plenary lecture by Prof. Werner Arber. Besides of discovering restriction enzymes (Nobel price in Physiology and Medicine 1979), he has always thought the biological mechanisms in bacteria as the consequence of evolutionary processes, and his predictions for genomic plasticity as a life style of micro-organisms are appealing for further developments in this research.

3) Final Programme

Thursday 13 September 2001

- 20h45 **Welcome**
Michel Blot (Grenoble) and Paul Rainey (Oxford)
- 20h50 **Introduction to the European Science Foundation (ESF)
and to the ESF Standing Committee for Life and
Environmental Sciences (LESC)**
Jacques Balandreau (ESF/LESC member, Lyon)
- 21h00 **Molecular evolution of micro-organisms**
Werner Arber (Basel)

Friday 14 September 2001

- 08h45 **New technologies in experimental evolution**
Austin Burt (Ascot)
- 09h15 **Evolution in laboratory populations of *Saccharomyces
cerevisiae*: identification of fitness enhancing
mutations**
Julian Adams (Ann Arbor)
- 09h45 **Metal stress selects for bacteria with reduced catabolic
versatility**
Dirk Wenderoth (Braunschweig)
- 10h15 *Coffee break*
- 10h45 **Can cheaters prosper again and again ? Prolonged
competition between wild-type *Myxococcus xanthus*
and derivative cheats**
Gregory Velicer (Tübingen)
- 11h15 **Unravelling the complexities of phenotypic evolution in
experimental populations of *Pseudomonas fluorescens***
Paul Rainey (Oxford)
- 11h45 **Mutation and selection in the yeast *Saccharomyces
cerevisiae***
Ryszard Korona (Krakow)
- 12h15 *Lunch*
- 16h00 **Experimental studies of the role of mutation in
microbial evolution**
Arjan De Visser (Wageningen)
- 16h30 **Role of mismatch repair genes in bacterial evolution**
Ivan Matic (Paris)
- 17h00 **Adaptive hypermutation of *Pseudomonas* in Chronic
Lung Infections**
Fernando Baquero (Madrid)
- 17h30 **The Integron: a natural genetic engineering device**
Didier Mazel (Paris)

- 18h00 *Pause*
- 18h15 **Inferring the evolutionary dynamics in growing *E. coli* cultures by molecular markers**
Christian Schlotterer (Wien)
- 18h45 **The fate of diversity in heterogeneous environments: insights from experimental evolution**
Rees Kassen (Oxford)
- 19h30 *Dinner*
- 20h30 **Divergent and parallel evolution in a structured and starved environment**
Denis Faure (Gif sur Yvette)
- 21h00 **Organisation of the *tuf* genes influences bacterial fitness**
Diarmaid Hughes (Uppsala)
- Saturday 15 September 2001
- 08h45 **Nothing in microbiology makes sense except in the light of clonal evolution**
Michel Tibayrenc (Montpellier)
- 09h15 **50 million years of evolution with Aphid Endosymbionts**
Siv Andersson (Uppsala)
- 09h45 **Antagonistic host-parasite coevolution and host diversity**
Angus Buckling (Oxford)
- 10h15 *Coffee break*
- 10h45 **Dynamics of genome architecture in *Rhizobium***
Patrick Mavingui (Geneva)
- 11h15 **Genomics and proteomics in the genus *Ralstonia* and their large plasmids: a potential for evolution studies**
Max Mergeay (Mol)
- 11h45 **Exploring the role of symbionine (GroEL) in buffering deleterious mutational effects during vertical transmission of bacteria**
Santiago Elena (Valencia)
- 12h30 *Lunch*
- 15h00 **Pathogenicity islands - impact on the evolution of microbes**
Ulrich Dobrindt (Würzburg)
- 15h30 **Macrophage-adapted mutants of *Salmonella typhimurium***
Mikael Rhen (Stockholm)

- 16h00 **Analysis of population subdivision in *Helicobacter pylori* using structure**
Daniel Falush (Berlin)
- 16h30 **A senescent bacterium**
Martin Ackerman (Basel)
- 17h00 **Directed evolution of the genetic code in bacteria**
Philippe Marlière (Evry)
- 17h30 *Pause*
- 18h00 **Bacterial mutagenesis induced by germinating rice seeds**
Jacques Balandreau (Lyon)
- 18h30 **Discussion and Prospectives**
Jacques Balandreau Michel Blot and Paul Rainey

4) Assessment of results. Contribution to the future direction of the field

The primary aim of the meeting was to bring together of a broad range of like-minded scientists, with interests ranging from the applied to the theoretical. This was clearly achieved (see lists of participants). Rarely has such a diverse collection of scientists met to discuss a common set of interests and ideas. Through listening and discussion participants have gone back to their respective laboratories with a broader understanding of the significance of their own work and possibilities for potential application of their ideas.

Particularly important for all participants was the recognition that experimental evolution with microbes has much to contribute toward both fundamental and applied science within the EU. One of the major strengths of this field is its interdisciplinary nature and it is widely recognized that studies that traverse boundaries make real progress. Those of use working with microbial systems are better placed than most to facilitate exciting science. At present the EU contains many scientists that contribute to the general area of experimental evolution with microbes, but lack of a “common voice” limits the ability of this team of scientists to influence funding opportunities. An important result of the meeting was recognizing the importance of being proactive at science policy levels.

In line with the desire of all participants to increase funding opportunities for fundamental and applied aspects of experimental evolution, consideration was given to possible routes by which this might be achieved. It was recognized that we already do well on an individual basis, but it was agreed that more could be achieved through collaboration. One immediate outcome of the meeting was the formation of a new network of groups for collaborative science. This will not only benefit the individual groups, but will also help facilitate the efforts of the entire group of interested scientists.

Without exception all participants expressed a strong desire to continue to meet on a regular basis. This was seen as critical to the further development of the field, which was probably at too young a stage to spearhead directly into the sphere of EU Framework-influence. Nevertheless, this was an agreed long-term goal. In order to bring this about the group discussed the idea of collective representations to both ESF and the EU. The intention is that the Chair and Co-chair visit ESF in the near future. One immediate wish is that ESF will continue to support meetings of this

kind through the EURESCO scheme. There was also agreement that a bid ought to be made for a EUROCORE grant to support exchange visits between labs. The input of the ESF representative (J Balandreau) was important to suggest that Experimental Evolution with Microbes would easily be integrated into a larger program dedicated to other aspects of Microbiology. All participants agreed on this possibility.

The final result of the meeting was a discussion in which the group laid out a possible funding scenario. This was intentionally large-scale in vision, with a view to providing an indication of the kind of problems to which the science of experimental evolution could be applied. What follows is hypothetical, but conceptually feasible.

Concepts for a future programme

Aims

- To exploit knowledge of ecological and evolutionary principles and processes to generate improved products (enzymes / organisms / cell types / populations, etc) for use in biotechnology / medicine / agriculture.
- To exploit developments in biochemistry and genetics to unravel the causes of improvement.
- To exploit this knowledge in biotechnology / medicine / agriculture
- To exploit knowledge of ecological and evolutionary principles and processes to generate improved products (enzymes / organisms / cell types / populations, etc) for use in biotechnology / medicine / agriculture.
- To exploit developments in biochemistry and genetics to unravel the causes of improvement.
- To exploit this knowledge in biotechnology / medicine / agriculture

Deliverables

- Novel strains, enzymes, populations etc.
- Enhanced ability to genetically engineer enzymes / antimicrobials / polymers / strains, cell types / populations, etc
- Predictability: elucidation of rules of adaptive evolution

Leverage

- Non GMO improvements – “green”
- Emergent infectious disease
- Antibiotic resistance
- Industrial partnerships
- Use of investment made in genomics and functional genomics

5) Statistical information on participants

There were 28 participants at the workshop who attended the complete program, with one exception. There were 8 participants with positions equivalent to professors, and 18 researchers. The sex ratio was extreme, since there were no women participating to the workshop. This was of course not an intention, and it should be taken into account in future meetings of this community as a factor of equilibrium.

The average age of the participants was low, as a sign of the young discipline they represent. By purpose, this exploratory workshop was not opened to students, because the aim was to establish a connection between scientists that should include further developments. However One PhD student from Grenoble was present to help for the organisation. Most participants were from small junior groups. Although very dynamic, these groups are weak and depend much on research policies to which they have not yet a strong influence. For this reason, the support of the ESF Exploratory Workshop was highly appreciated, and the possibility to ask the national agencies involved in ESF to initiate a EUROCORE program (maybe with other neighbouring projects) is essential.

The participants were from a wide range of countries: Austria : 1; Belgium :1; Netherland: 1; Poland :1; Sweden : 2; Spain :2; Switzerland :3; United Kingdom :4; Germany : 4; France : 8 (including a PhD student for organisation and the ESF representative), as well as a US researcher on sabbatical in France. With a few exceptions of invited speakers who could not join nor send representatives, most research groups directly involved in the topic of the workshop were present. This gave a broad overview of the current progress in the field which are made in Europe. Since the time of the workshop, a number of scientists across Europe (broad sense) declared their interest to be involved in furthe development. This includes some participants to the EU network MECBAD (mobile elements contributing to bacterial adaption and diversity). Thus the possibility to extend the number of participants in a larger community is real.

6) Final List of participants

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