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Getting Wrinkly Spreaders to demonstrate evolution in schools

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ABSTRACT (49/50 words)

Understanding evolution is crucial to modern biology, but most teachers would assume that practical demonstrations of evolution in school laboratories are unfeasible. But perhaps they haven't heard of 'evolution in a test-tube' and how Wrinkly Spreaders can form the basis for both practical demonstrations of bacterial evolution and further work.

MAIN TEXT (1,306 / 1,200 words)

In the absence of a good fossil collection or a nearby natural history museum, the teaching of evolution in schools is largely an exercise in which experimental laboratory-based work is unfeasible (as generally it would be expected to greatly exceed normal school hours). Nonetheless, providing students with a strong understanding of evolution and its broader relevance is essential for the development of a well-rounded biological education. Indeed, the importance of evolutionary theory was famously presented by Theodosius Dobzhansky in 1964 as “nothing makes sense in biology except in the light of evolution” [1] (see also [2, 3]). Furthermore, demonstrations of the reality of evolution are also required to combat the insidious penetration of science education by religious fundamentalists and the proponents of intelligent design (e.g. the Dover Panda trial in the USA [4]).

Recent attempts to introduce evolution in science classes have focussed on developing ‘thought-provoking’ discussion-based exercises (e.g. [5]) for dulled students who would rather watch David Attenborough interact with extinct animals in CGI-augmented natural history programmes (or more probably the other Attenborough in ‘Jurassic Park’). However, I advocate the use of more exciting bacterial microcosms, and in particular the Wrinkly Spreaders, as means of investigating evolution in secondary schools through practical demonstrations involving basic microbiological techniques. We first presented this as ‘Evolution in a Test-tube : The Rise of the Wrinkly Spreaders’ in [6] and it should probably be accompanied by a very slow progression of minor chords ending with an unresolved diminished 7th (this is a more sophisticated version of leaving out a coffee cup until it develops a surface biofilm, but without the attendant health and safety constraints).

Simple artificial environments or microcosms have long been used to investigate aspects of microbial ecology, originating from the early work of Sergei Winogradsky and others in the late 19th and early 20th centuries (reviewed in [7]). More recently they have been used for experimental evolution studies, where the rapid growth of bacteria, large population sizes, and the ease of isolating mutants and storing strains indefinitely at -80°C makes them ideal for investigating various evolutionary processes (reviewed in [8, 9, 10]). In particular, adaptive radiation can be readily demonstrated by quantifying diversification (radiation) by the appearance of mutants in growing populations and determining fitness of mutants compared to the ancestral strain (adaptation).

In the peculiar case of *Pseudomonas fluorescens* SBW25, a non-pathogenic environmental bacterium, radiation in static liquid microcosms results in the rise of a class of adaptive mutants known as the Wrinkly Spreaders (first described by Paul Rainey and Michael Travisano [11] and recently reviewed in [12]). Whilst I happily acknowledge the work of others who have investigated bacterial evolution using different systems (e.g. the long-term *Escherichia coli* experiments initiated by Richard Lenski, see [13]), none have produced adaptive mutants as spectacular as the Wrinkly Spreader in so little time: typically they appear within three days in static microcosms and may represent ~30% of the population by the fifth day [6].

The Wrinkly Spreaders are readily distinguished from the ancestral *Pf.* SBW25 by virtue of their wrinkled colony morphology on agar plates and an altered ecological preference as demonstrated by the formation of a robust biofilm at the air-liquid interface (see Figure 1). In static microcosms, the fitness (*W*) advantage of the Wrinkly Spreader is ~2.5 greater than the non-biofilm–forming ancestral *Pf.* SBW25, though in shaken microcosms where biofilms cannot form, the Wrinkly Spreader has no fitness advantage (*W* ~1) [6]. The value of producing a biofilm is that it allows bacteria to intercept O_2 diffusing into the liquid column from the air above, with those in the highly-oxygenated, < 200 μm top layer growing faster than that possible lower down where O_2 levels are ≤ 0.05% of that found at the surface [14]. Remarkably, it is the early ancestral colonists that establish the O_2 gradient that then provides the selective pressure and ecological reward that drives the evolution of the Wrinkly Spreader in this simple environment [14].

Substantial research has been published investigating the underlying molecular biology of the Wrinkly Spreader, providing a satisfying mechanistic explanation linking mutation, ecological preference and fitness (for an introduction to this subject and links to the primary literature, see the review by [12]). In the archetypal Wrinkly Spreader, a single DNA base change (A → T) results in an alteration of an amino acid (Ser → Arg) in the methylesterase (WspF) subunit of the Wsp complex. This and similar chemosensory complexes receive environmental signals and allow the bacteria to respond to changing conditions by modulating the levels of cyclic-di-GMP, an internal second messenger that plays a central role in the regulation of motility, virulence and biofilm formation in many bacteria through a complex signalling network integrating environmental signals and controlling riboswitches, transcription factors and enzyme activities. In *Pf.* SBW25, the mutation in WspF results in increased levels of cyclic-di-GMP, leading to the over-expression of cellulose and an attachment factor essential for the Wrinkly Spreader phenotype, whilst differences in wrinkleality between individual Wrinkly Spreaders is probably due to differences in the underlying mutations that increase cyclic-di-GMP levels.

I recognise a tendency for those who are not biochemists or molecular biologists to skip paragraphs such as the preceding one, but the value of this mechanistic explanation is that it provides extensions into molecular biology, ecology, experimental design and statistics for further discussion and project work (see Figure 2). Although the 'evolution in a test-tube' experiments are quantitative and can be rigorously tested with statistics, this type of work has a very obvious visual component, allowing a more qualitative approach based on observations and photography to be taken where appropriate. It is even possible to link the rise of the Wrinkly Spreaders with famous literature, as the Red Queen (a character in Lewis Carroll's 'Through the Looking-Glass') is also an evolutionary hypothesis which proposes that organisms must constantly evolve to survive when pitted against ever-evolving competitors in an ever-changing environment. I hesitate to purloin Dobzhansky, but things may make more sense (or fun) in biology when illustrated by Wrinkly Spreaders.

Just as the ancestral *Pf.* SBW25 made the adaptive leap in static microcosms to the Wrinkly Spreader, teachers also need to make some effort to bring this research into secondary schools in order to demonstrate evolution. In a recent survey of UK teachers, equipment access and confidence in techniques were found to be significant limitations in using practical microbiology in schools [15]. However, 'evolution in a test tube' does not depend on expensive equipment or complex techniques: it requires an initial inoculum of *Pf.* SBW25 which is available on request, basic equipment to produce sterile media and for incubation, and skills including sterile technique, serial dilutions and plating-out (as described in [6]), and would be suitable for Scottish Highers or English A or AS-level students (i.e. the last two years of secondary school). We also use

Wrinkly Spreaders in BSc undergraduate laboratories as a means to acquire laboratory confidence and basic skills, to generate and analyse replicate data, and to provide a narrative linking mutation, phenotype and fitness (in these students are expected to access and comment on the primary research literature in their final reports).

Like many other researchers involved with STEMNET (see Box 1), I am willing to support teachers with practical help and expert advice. Collectively, we need to make sure that the biologists of the future will have a solid understanding of evolution. Like many science disciplines, it is not always easy to provide practical demonstrations or research-based projects for students, but in this instance, it seems that by bringing Wrinkly Spreaders into schools, our students of today can be involved directly in experimental evolution and further enthused by the subject. Who knows, but in a future dominated by synthetic biology and biotechnology, some of these might produce Super Wrinkly Spreaders or indeed, something really quite useful.

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Figures

Figure 1. Adaptive radiation of *Pseudomonas fluorescens* SBW25 populations in experimental static microcosms gives rise to the Wrinkly Spreader. **(A)** Wrinkly Spreader colonies (with an irregular circumference) are easily distinguished on agar plates from ancestral *Pf.* SBW25 which produces smooth rounded colonies. **(B)** Ancestral *Pf.* SBW25 colonises the liquid column of static microcosms (left) while the Wrinkly Spreader (right) produces a biofilm at the air-liquid interface, demonstrating an altered niche-preference. **(C)** Cellulose fibres form the matrix of the Wrinkly Spreader biofilm and can be visualised by fluorescent microscopy (at this low magnification individual bacteria are not detectable).

Figure 2. Running an ‘evolution in a test-tube’ laboratory session in schools will allow many further extensions into evolution and ecology, molecular biology, experimental design, science communication and awareness.

Box 1. The STEM Network in the UK

- STEMNET (the Science, Technology, Engineering and Mathematics Network) creates opportunities to inspire young people in STEM.
- Works with schools, colleges and STEM employers, to enable young people of all backgrounds and abilities to meet inspiring role models, understand real world applications of STEM subjects and experience hands-on STEM activities that motivate, inspire and bring learning and career opportunities to life.
- STEMNET delivers three core national programmes, including STEM Ambassadors who volunteer their time and support to promote STEM

subjects to young learners in a vast range of original, creative, practical and engaging ways, STEM Clubs to boost enjoyment and learning across STEM outside of the classroom, and the Schools STEM Advisory Network.

- Visit www.stemnet.org.uk to find your UK Regional Contact.