The Genetics of *Pseudomonas fluorescens* SBW25: Adaptation to a Spatially Structured Environment.

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ABSTRACT

Experimental microbial populations provide powerful models for testing the most challenging problems in evolutionary biology. In the midst of the genome sequencing revolution microbial evolutionary genetics has flourished; promising high-resolution explanations for the underlying causes of evolutionary phenomena. This thesis describes four investigations into the adaptation of *Pseudomonas fluorescesns* SBW25 to a spatially structured environment. The first builds upon a large body of experimental work characterising the genetic and phenotypic causes of the ability of divergent Wrinkley Spreader (WS) types to colonise the air-liquid interface in spatially structured microcosms. The *mws* and *aws* genetic loci are described, which together with the previously described *wsp* locus, account for the location of the causal mutation for all known WS genotypes. It was found that if these loci were deleted from the *P. fluorescens* genome, it could still evolve the WS phenotype via a previously undiscovered locus (*sws*). This study provides the first explicit evidence that genetic biases can influence the outcome of evolution. The second study used a novel method to sample WS genotypes without the biasing effects of natural selection; the distribution of the fitness effects of these genotypes was measured and analysed from a unique perspective. The distribution of fitness effects of new mutations is found to best fit the normal distribution, facilitating the extension of the mutational landscape model of adaptation to include all possible adaptive walks. The third study investigates the underlying causes of genetic biases on evolution; many WS genotypes are obtained at different time points during colonisation of the air-liquid interface (including WS obtained without selection) and the causal mutations of many of these mutants determined. Together these results allowed the elucidation of the relative effects of natural selection, genetic architecture and mutation rate on evolutionary outcomes. The final study considers the WS mat as the product of cooperative interactions, and uses a group selection experiment to investigate the potential of WS mats to evolve group level adaptations. A novel strategy is developed to overcome cheating types, considered the main barrier to the evolution of group level complexity. Furthermore, WS groups evolved specialised cell types, the first example of a *de novo* evolution of a division of labour, a hallmark of complexity.

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ABBREVIATIONS

WS – Wrinkly spreader

SM- Smooth

DGC- Di-Guanylate Cyclase

PDE- Phosphodiesterase

LSWS- Large Spreading Wrinkly Spreader

AWS- Alternative Wrinkly Spreader

MWS- Mike's Wrinkly Spreader

SWS- Slow Wrinkly Spreader

DFE- Distribution of Fitness Effects

EVT- Extreme Value Theory

CLT- Central Limit Theorem

MSC- Mutation Selection Cassette