

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

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

A thesis presented in partial fulfilment of the  
requirements for the degree of

Doctor of Philosophy  
In  
Genetics

at Massey University, Auckland Campus.

Michael Joseph McDonald

2009

## 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 fluorescens* 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 *mms* and *ams* genetic loci are described, which together with the previously described *msp* 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 (*sms*). 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.

## ACKNOWLEDGMENTS

Like all things worth having a Ph.D takes a lot of time and work, however, I can say I have enjoyed nearly every minute of it. First I would like to thank Paul for taking me on as a student. On paper I was far from a sure bet and I am not sure how I got past his method for screening potential students. However that happened I am always grateful that I have been able to benefit from his mentorship and gain the new perspective of the world that comes with the study of Evolution.

Secondly, my co-supervisor Xue Xian deserves thanks for his always-sound advice and also for passing on his excellent lab techniques; I soon learnt the way to make it work was do exactly as he did.

The Rainey lab seems to attract excellent post docs. Among the many things taught to me I will remember these lessons: Bertus, for thinking deeply and outside the square; Tim, for perceptive analysis and introducing me to the big problems in evolutionary biology; Dominik, for expounding the history of evolutionary theory and Christian, for demonstrating the benefits of careful experiment design.

Fellow Ph.D students Jenna and Pete, who apart from providing a good source of commiseration, cooperation and information also set high standards for me to keep up with. Also Annabel, Ellen and Andy for technical and other help.

Thanks to my Parents, Bryan and Carol; without their support doing a PhD, getting married and having kids over the same period of time would have been impossible. Also Dad for teaching me how to examine a problem logically, a tool I use everyday.

Finally to my wife Wen-Pei, whose calming influence allowed me to focus my attention on a worth-while endeavour for the first time in my life. Also, by giving me two children before the age of 28, I became the fittest person (evolutionarily speaking) in the lab.

## TABLE OF CONTENTS

0.1 ABSTRACT	II
0.2 ACKNOWLEDGEMENTS	III
0.3 TABLE OF CONTENTS	IV
0.4 FIGURES	viii
0.5 TABLES	viii
0.6 ABBREVIATIONS	ix

1	INTRODUCTION	1
1.1	Darwin's Insight	2
1.1.1	The modern synthesis	2
1.2	Experimental evolution	5
1.3	Microbial models of evolution	6
1.3.1	The <i>E. coli</i> long term evolution experiment	6
1.4	<i>P. fluorescens</i> SBW25, a model system	8
1.4.1	Cooperation and conflict in WS mats	9
1.4.2	Causes of the WS phenotype	11
1.4.2.1	The <i>msp</i> locus	13
1.4.2.2	DGC proteins and c-di-GMP	14
1.4.3	The genetic causes of WS	15
1.4.3.1	The <i>mspF</i> locus	15
1.4.3.2	The <i>ams</i> operon	16
1.5	The genetical theory of evolution	17
1.5.1	The distribution of fitness effects of new mutations	20
1.5.2	The mutational landscape of adaptation	22
1.5.2.1	The mutational landscape and fitness landscapes	24
1.5.2.2	Extensions and tests of the mutational landscape	25
1.5.3	Genetic architecture and the genotype-phenotype map	26
1.5.4	Genetic architecture and constraints on evolution	29
1.6	Summary	30
1.7	Research objectives	31
1.8	Bibliography	32

## 2 GENETIC CONSTRAINTS GUIDE EVOLUTIONARY TRAJECTORIES IN A PARALLEL ADAPTIVE RADIATION

2.1	ABSTRACT	38
2.2	INTRODUCTION	39
2.3	MATERIALS AND METHODS	42
2.3.1	Bacterial strains, growth conditions and manipulation	42
2.3.2	Molecular biology techniques	43
2.3.3	Construction of deletion mutants and allelic replacements	43
2.3.4	Transposon mutagenesis analysis	44
2.3.5	Fitness of genotypes	45

2.4	Results	46
2.4.1	Comprehensive suppressor analysis of LSWS	46
2.4.2	Aws: a <i>wsp</i> independent route to WS	47
2.4.2.1	Mutations in <i>awsX</i> are necessary and sufficient for AWS	49
2.4.2.2	<i>AwsX</i> is a negative regulator	52
2.4.3	Mws: a <i>wsp</i> and <i>aws</i> independent route to WS	53
2.4.3.1	Predicting the mutational cause of the MWS	54
2.4.3.2	Mutations in <i>mwsR</i> are necessary and sufficient for MWS	55
2.4.3.3	The EAL domain negatively regulates <i>MwsR</i> activity	55
2.4.4	Fitness of AWS and MWS	56
2.4.5	The relative contribution of <i>wsp</i> , <i>aws</i> and <i>mws</i> to WS variation	59
2.4.6	SWS: a <i>wsp</i> , <i>aws</i> and <i>mws</i> independent route to WS	60
2.4.7	The mutational origins of the independent WS genotypes	61
2.5	Discussion	62
2.5.1	Parallel genetic evolution due to genetic constraints	65
2.5.2	Mutational target size	67
2.5.3	Conclusion	70
2.6	Bibliography	72
3	THE DISTRIBUTION OF FITNESS EFFECTS OF NEW MUTATIONS	
3.1	Introduction	77
3.2	Results	80
3.2.1	Gathering an unbiased sample of WS mutations	80
3.2.1.1	Argument that WS will not experience selection	81
3.2.1.2	Experiments to exclude bias in the collection of WS	82
3.2.2	Fitness of the 100 WS	85
3.2.3	Determining the uniqueness of clones within the 100 WS	89
3.3	Discussion	91
3.3.1	The normal distribution and modular systems	91
3.3.2	The normal distribution and the fitness landscape	93
3.3.3	Empirical support for the generality of the normal $f(x)$	95
3.3.4	Concluding comments	96
3.4	Bibliography	97
4	THE CAUSES AND CONSEQUENCES OF THE BIASED PRODUCTION OF VARIATION	
4.1	Introduction	100
4.2	Results	104
4.2.1	Experimental dissection of an adaptive radiation	104
4.2.2	<i>wspF</i> can produce the widest range of WS genotypes	108
4.2.3	The AWS and $WS_T$ alleles are produced at a higher rate	111
4.2.3.1	Comparing the observed to expected rate of mutation	114
4.2.4	Elevated mutation rates in <i>mwsR</i>	115
4.2.4.1	Comparison of observed to expected <i>mwsR</i> mutations	119
4.3	Discussion	120
4.3.1	The causes of the genetically biased production of variation	120
4.3.2	Mutation influences evolutionary trajectories randomly	122

4.3.3	The day zero <i>mmsR</i> WS	123
4.3.4	Characteristics of mutable DNA sequence	124
4.3.4.1	The birth of a contingency locus	127
4.3.4.2	Mutable or fragile	129
4.3.5	Concluding comments	129
4.4	Bibliography	131
5	THE EVOLUTION OF COMPLEXITY IN WS MATS	
5.1	Introduction	133
5.1.1	A model for the evolution of a germ-soma separation	135
5.1.2	Group selection	136
5.1.3	The evolution of bacterial multicellularity	137
5.2	Results	138
5.2.1	Group selection for stronger WS mats	138
5.2.2	Diminishing mat strength correlates with more cheats	140
5.2.3	Conflict mediation facilitates evolution of the group	143
5.2.4	Differentiated cell types within WS mats	145
5.2.5	The WS <sub>1</sub> and WS <sub>2</sub> have specialised functions	147
5.3	Discussion	148
5.3.1	The failure of group selection due to cheats	149
5.3.2	Artificial conflict mediation rescued the evolution of the group	151
5.3.3	The evolution of a division of labour	152
5.3.4	Prokaryotic and eukaryotic potential to evolve multicellularity	153
5.3.5	Concluding comments	155
5.4	Bibliography	156
6	CONCLUDING DISCUSSION	
6.1	Introduction	159
6.2	DGC protein networks as a model of evolvability	160
6.3	The relative role of genetic structure on evolution: mutational distance	161
6.4	The predictability of evolution	163
6.5	Final comments	165
6.6	Bibliography	166

7	MATERIAL AND METHODS	
7.1	Materials	169
7.1.1	Media and growth conditions	169
7.1.2	Bacterial strains	169
7.1.3	Plasmids and transposons	170
7.1.4	Primers	170
7.1.5	Antibiotics and markers	171
7.2	Methods	173
7.2.1	DNA preparation	173
7.2.2	Polymerase Chain Reaction (PCR)	173
7.2.3	Electrophoresis	174
7.2.4	DNA extraction	174
7.2.5	DNA sequencing	174
7.2.6	Allelic replacement	175
7.2.7	Transformation	175
7.2.8	Restriction enzyme cleavage	175
7.2.9	Bi-parental conjugation	176
7.2.10	Tri-parental conjugation	176
7.2.11	Transposon mutagenesis	177
7.2.12	Fitness assays	177
7.2.13	Artemis	178
7.2.14	Group selection experiment	178
7.2.15	Reciprocal invasion assays	179
7.2.16	Assay for mat persistence at the broth surface	179
8	APPENDICES	
8.2	Chapter two	181
8.2.1	Optimisation of transposon mutagenesis	181
8.3	Chapter three	182
8.3.1	Theoretical and sample quantiles for fitted curves	182
8.3.2	Maximum likelihood table for static and shaken measurements	183
8.4	Chapter four	184
8.4.1	The birthday problem program	184
8.4.2	Mutations discovered in chapter four	184

## FIGURES

1.1 Colony morphology and niche specificity in WS microcosms	10
1.2 The <i>ms</i> operon.	12
1.3 Model of the <i>wsp</i> chemosensory pathway	14
1.4 Fisher's model of adaptation	18
1.5 Fisher's fitness effects of new mutations	20
1.6 The distribution of fitness effects	23
1.7 The genotype to phenotype map	27
2.1 Relative fitness of WS genotypes	58
3.1 Regression of colony diameter and WS fitness.	84
3.2 Distribution of WS fitness effects measured in static microcosms	85
3.3 Regression of shaking against static fitness for 26 independent WS	88
3.4 Distribution of WS fitness effects in the shaken microcosm	89
4.1 The experimental dissection of the WS adaptive radiation	105
4.2 Fitness measurements for all unique WS mutations.	107
4.3 Two possible explanations for the distributions of fitness effects	109
4.4 Mutations in <i>awsX</i>	112
4.5 Day zero <i>msR</i> mutations	118
4.6 Strand slippage mechanism	125
4.7 Theoretical steps towards the evolution of a contingency locus	128
5.1 Fitness trajectories of group selected lines	139
5.2 Relationship of mat strength and the proportion of cheating types	141
5.3 Proportion of cheats in the group selected lines	142
5.4 Conflict mediation in the group selected lines	145
5.5 Mat strength WS <sub>1</sub> , WS <sub>2</sub> and combined mats	146
5.6 Visual comparison of WS <sub>1</sub> , WS <sub>2</sub> and combined mats	148
8.1 Theoretical and sample quantiles for goodness of fit: static	198
8.2 Theoretical and sample quantiles for goodness of fit: shaken	199

## TABLES

2.1 The mutational causes of WS	51
3.1 The mutations found by sequencing 20 of the 100 WS genotypes	96
4.1 The proportion of <i>awsX/wspF</i> mutations found at each time point	106
5.1 Initial and final mean mat strengths with and without conflict mediation	144
7.1.2 Bacterial strains	169

7.1.3 Plasmids and transposons	170
7.1.4 Primers	170
8.1 Maximum likelihood table for static and shaken fitness measurements	183
8.2 Mutations discovered in chapter four	185

## 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