

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.

Evolution of the Genotype-Phenotype Map

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

Master of Science

In

Genetics

at Massey University, Albany, New Zealand

Michael Barnett

2016

Abstract

The relationship between genotype and phenotype, the genotype-phenotype map (GPM), not only describes the genetic and molecular underpinnings of phenotypes, but also determines their variational properties. That is, it determines how genetic variation maps to phenotypic variation. Because of this, the phenotypic consequence of a random mutation may be highly constrained by properties of the GPM. Motivated by the challenge of understanding the GPM and its effect on the course of evolutionary change I here use a bacterial model to investigate how the GPM itself evolved throughout a previously conducted experiment that selected for lineages adept at cycling between the gain and loss of a simple phenotype. The Wrinkly Spreader (WS) morphotype of *Pseudomonas fluorescens* SBW25 is distinguished from the ancestral type by overproduction of an extracellular cellulose polymer that gives it a wrinkled colony morphology and allows it to colonise the liquid surface of a broth-filled vial, a niche unavailable to the ancestral type. The genes underpinning WS have been previously identified allowing the GPM to be characterized. This formed the basis by which I could compare the GPM of those WS derived from the selection experiment and so determine what changes had occurred throughout the extensive cycling of gain and loss of WS. Suppressor analysis of the derived WS types revealed in some cases a striking difference from the ancestral WS state, including one example of a significant re-wiring of regulatory connections and an expansion of the network of genes underpinning WS. In another case a novel association with a gene encoding a fatty acid desaturase was revealed with possible implications for an unusual switching mechanism. In some derived WS the GPM remained apparently unchanged but these WS were also implicated in switching strategies. By repeatedly re-evolving the same phenotype the GPM is required to find new viable configurations and I show in this thesis that the capacity to do so is vast.

Acknowledgments

Many thanks to my supervisor Professor Paul Rainey for giving me a crazy project that made me think and my co-supervisor Dr Philippe Remigi for his guidance. Also the ever-reliable Yunhao for technical assistance when Philippe wasn't around.

To my mum, dad and brother, thank you.

Also to the two cats in my life, Charles and Milly.

Table of Contents

0.1 Abstract	II
0.2 Acknowledgments	III
0.3 Table of Contents	IV
0.4 Figures	VII
0.5 Tables	VII
0.6 Abbreviations	VIII
1: Introduction	1
1.1 Genetic architecture constrains evolution	1
1.2 The wrinkly spreader phenotype	5
1.2.1 Alternative pathways	6
1.3 The evolution of multicellularity	7
1.4 Research objectives	9
2: Methods and Materials	
2.1 Materials	10
2.1.1 Strains list	10
2.1.2 Plasmids	11
2.1.3 Primers	11
2.1.4 Antibiotics, reagents and enzymes	12
2.1.5 Media and culture conditions	12
2.1.6 Electrophoresis materials	13
2.1.7 Photography and microscopy materials	13
2.2 Methods	13
2.2.1 Cellulose assay	13

2.2.2 Transposon mutagenesis	13
2.2.3 Cre-mediated excision of transposon	14
2.2.4 Tri-parental conjugation	14
2.2.5 Production of electro-competent cells	14
2.2.6 Polymerase chain reaction	15
2.2.7 Arbitrary primed PCR	15
2.2.8 Enzymatic purification	16
2.2.9 Strand overlap extension	16
2.2.10 Extraction and purification	17
2.2.11 Cloning and transformation	17
2.2.12 Allelic exchange	18
2.2.13 Sanger sequencing	18
2.2.14 Identifying gene orthologs, synteny and domain architecture	19
2.2.15 Additional life cycle generations	19

3: Results

3.0.1 Selection of candidate lines from the life cycle experiment	20
3.0.2 Cellulose assay	21
3.1 Interpreting the mutations	22
3.1.1 Overview of mutations	23
3.2 Suppressor Analysis	24
3.2.1 Line 17	25
3.2.1.1 The suppressor loci indicate no change from ancestral WS	26
3.2.2 Line 43	27
3.2.2.1 The suppressor loci indicate no change from ancestral WS	28
3.2.2.2 Suppressor analysis of line 43+3	29

3.2.3 Line 54	30
3.2.3.1 A previously unseen association between <i>aws</i> and a predicted fatty acid desaturase	31
3.2.3.2 The role of pflu0184	31
3.2.3.3 <i>fadA</i>	32
3.2.3.4 The role of <i>fadA</i> in line 54	33
3.2.3.5 Fatty acid desaturases	33
3.2.3.6 pflu5420	34
3.2.3.7 pflu1555	35
3.2.3.8 Mutational causes of altered GPM in line 54	35
3.2.4 Line 57	35
3.2.4.1 Line 57 is underpinned by a complex architecture featuring a known negative regulator of <i>wss</i>	36
3.3 Additional life cycle generations	38
4: Discussion	41
4.1 Evolution of the GPM: a redundancy of pathways	41
4.2 Possible switching mechanisms	44
4.3 Possible switching mechanisms: line 43	45
4.4 Evolution of the GPM: scaffolding	45
4.5 Evolution of the GPM: line 57	45
4.6 Concluding discussion	47
Bibliography	50
Appendix	57

Figures

1.1 A potentially beneficial mutation is unrealised due to an associated deleterious effect	2
1.2 Modularity and pleiotropy. Diagram representing two simples GPMs	3
2.1 AP-PCR amplification of transposon-chromosome junction	16
3.1 Cellulose matrix stained with calcofluor and visualized at 60x magnification	21
3.2 Morphological features of line 17	25
3.3 Morphological features of line 43	27
3.4 Morphological features of line 54	30
3.5 Distribution of transposon inserts affecting <i>fadA</i>	32
3.6 Morphological features of line 57	35
3.7 The fate of line 43 throughout three extra generations of the LCE	39
3.8 The fate of line 54 throughout three extra generations of the LCE	39
3.9 The fate of line 57 throughout three extra generations of the LCE	40

Tables

2.1 Designation and genetic properties of the bacterial strains used in this study	10
2.2 Plasmids used in this study	11
2.3 Primers used in this study	11
3.1 Mutations present in each line as revealed by whole genome sequencing	23
3.2 The distribution of transposon insertions suppressing the WS phenotype in line 17	25
3.3 The distribution of transposon insertions suppressing the WS phenotype in line 43	28
3.4 Suppression loci of line 43+3	29
3.5 The distribution of transposon insertions suppressing the WS phenotype in line 54	30
3.6 The distribution of transposon insertions suppressing the WS phenotype in line 57	35

Abbreviations

GPM: genotype-phenotype map

WS: Wrinkly spreader

SM: Smooth morphology

DGC: diguanylate cyclase

PDE: phosphodiesterase

RE: Re-evolution experiment