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**The role of chance and history in the
evolution of a novel trait in *Pseudomonas
fluorescens* SBW25**

A thesis submitted in partial fulfilment of the requirements for the
degree of
Doctor of Philosophy
in
Evolutionary Genetics

At Massey University, Albany Campus, New Zealand

Sylke Nestmann

2013

Abstract

The processes that lead to the evolution of novel adaptive traits are poorly understood. It is not clear how the combination of natural selection and random variation determine evolutionary pathways, specifically how evolutionary history affects the outcome of evolution. In a previous experiment in which populations of the bacterium *Pseudomonas fluorescens* were subjected to repeated evolutionary reversals (the *Reverse-Evolution Experiment* (REE)) stochastically switching genotypes evolved *de novo* in two out of 12 replicate lineages. Whole genome sequencing revealed not only causative mutations in *carB* (referred to as *carB**) and *rpoD* but also the entire evolutionary pathways comprised of eight additional mutations. It was hypothesized that evolutionary history played a major role in switcher evolution.

This was tested by ‘reviving’ four REE lineages and the performance of ‘replay evolution’ experiments. Whereas the repeated evolution of switcher genotypes was observed in three of the four lineages, the likelihood of switcher occurrence varied and depended on the genotype. By artificially introducing the original switcher mutation *carB** into genotypes from one lineage, potential fitness benefits of the switcher mutation at each point in evolutionary time were assessed. The introduction of *carB** into the ancestral genotype of SBW25 created a switcher with higher fitness than the ancestor, indicating that evolutionary history is not necessarily required to give rise to an evolutionary successful switcher. This idea was tested further during a real-time evolution experiment using the same genotypes as founder populations. The capacity to evolve a switcher, based on the competitive fitness of *carB**, was only partly reflected in the ability to re-evolve switchers, which is most likely due to the availability of alternative evolutionary pathways, the number of competitors, and the fitness of the founder genotype. In addition, epistasis contributed to an increased capacity to produce a switcher based on *carB**, resulting in an increased likelihood to evolve this novel trait in genotypes from the later evolutionary time points.

Evolutionary history plays an important role in the evolution of switching. Even though switchers evolved repeatedly from different genotypes, the likelihood that this path was taken depended on genetic constraints and ecological factors. These factors and their contribution to the evolutionary outcome were dependent on the genetic composition and changed throughout evolutionary history.

Acknowledgements

This research project would not have been possible without the support of many people. First and foremost, I wish to thank my supervisor, Professor Paul Rainey for his support, his continuous faith and help throughout my project. I also want to thank my co-supervisor Dr. Austen Ganley for encouragement and discussions during the research. I would like to thank the New Zealand Institute for Advanced Study for funding the project and for the financial support to attend overseas conferences.

I would like to acknowledge former Rainey lab members in particular Dr. Hubertus Beaumont for his extensive work on the Reverse-Evolution Experiment and Dr. Jenna Gallie for her studies on the switcher lineages. Dr. Monica Gerth, thank you for teaching me many methods and tricks in the laboratory. Thanks to Dr. Gayle Ferguson for helping with the material and methods chapter of my thesis. My special thanks are extended to all the Rainey lab members for their help and kindness and for being so weird and entertaining at the same time. Thanks to Katrina Rainey for improving my thesis and for her valuable comments.

Special thanks goes to Dr. Katrin Hammerschmidt for her treasured assistance with statistic problems and for her help with the planning of experiments. You have been a great source of inspiration, hope and motivation.

I am grateful to my parents who supported me throughout the entire time and encouraged me to start this project and to move to New Zealand. Without my parents my PhD would not have been possible. Thanks to my flatmates, Jazz, Stef, Nielvin, Andrew and Sat for being my New Zealand family, for the *flinners* and for cheering me up. Finally, I would like to thank Katrin and Peter for being great friends. Fishing, extensive cooking sessions, delicious food and *Tatort* nights are things that I really enjoy in life and I am glad that I could share this with the two of you. Thanks for looking after me.

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