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The Evolution of Cooperation: Insights from Experimental Populations of *Pseudomonas fluorescens*

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requirements of the degree of
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0.1 ABSTRACT

The field of experimental evolution is burgeoning under the power of microbial systems. Our ability to manipulate experimental design for use with microbes is only limited by our imagination. This thesis is a study that uses *Pseudomonas fluorescens*, a soil dwelling bacterium, as an experimental tool for understanding evolutionary processes. The evolution of cooperation has been a thorny issue for many years, because it initially seems to contradict the intrinsically selfish concepts established in Darwin's theory of evolution by natural selection. Advances in microbiology and the ability to test important evolutionary theories using microbes, provides an exciting opportunity for those working in the field of experimental evolution.

This thesis uses *P. fluorescens* to investigate four aspects of the evolution of cooperative behaviour organised into four results chapters (Chapters 2-5). The first describes the genotypic and phenotypic diversity of 26 independently derived 'wrinkly spreader' genotypes in order to analyse the genetic and phenotypic variation among morphotypes. Mutations were identified in 25 of the 26 wrinkly spreaders including a new locus *mws* and three new genes of known loci *wspE*, *awsR* and *awsO*. This new genetic information provided additional insight into the molecular causes of the wrinkly spreader phenotype. Multivariate analysis of the phenotypic traits revealed that *wspF* mutants were phenotypically distinct from other morphotypes at a level below the ecological niche. The second chapter extended existing studies on the evolution of wrinkly spreader genotypes within the well-known Haystack model for evolution in group-structured populations, by studying the population dynamics of cooperative genotypes with and without group structure, in a multi-level selection one framework. It was shown that the time spent in a haystack affects the fitness of cooperators, because the longer group-generation treatment conformed to the predictions of the Haystack model, while the shorter group-generation treatment did not. The third chapter was an investigation into how the fitness of the emergent group-level phenotype formed by cooperating wrinkly spreader cells was dependent on the density of wrinkly spreader cells. Contrary to prediction, no density dependence was observed when calculated in a multi-level selection one framework, but rather it was determined that the emergent fitness was dependent on time, implicating a role for a development-like process. The final

results chapter of this thesis incorporated the hypothesised role for a development-like process into a novel theoretical model for the evolution of multicellularity in which fitness would be determined in a multi-level selection two framework. Novel apparatus and experimental design were developed to determine if it were possible to observe a response to a selective regime that selected simultaneously at the level of the individual cell and the level of the group of cells. A significant response was shown after only six group-generation cycles.

In summary, this thesis exploits *P. fluorescens* as an experimental tool to gain insight into complex ecological and evolutionary phenomena such as cooperation, biofilm formation and the evolution of multicellularity, and provides insight into the molecular causes of the cooperation among wrinkly spreader genotypes.

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0.4 GLOSSARY AND TERMS

Actor: The focal individual that performs a behaviour (West *et al.*, 2007b).

Adaptation: A process of genetic change of a population, owing to natural selection, whereby the average state of a character becomes improved with reference to a specific function, or whereby a population is thought to have become better suited to some feature of its environment (Futuyma, 1998).

Adaptive Radiation: The evolution of ecological diversity within a rapidly multiplying lineage characterised by phenotypic divergence and speciation due to the availability of environments, resources and resource competition (Schluter, 2000).

Altruism: A behaviour that is costly to the actor and beneficial to the recipient(s) where these costs and benefits are defined by the consequences on the lifetime fitness of the actor and the recipient, *i.e.* –/+ (West *et al.*, 2007b).

Cheater: An individual that does not cooperate (or contributes less than its 'fair share'), and gains the benefit of others cooperating (Velicer, 2003; West *et al.*, 2007b).

Cheating: Obtaining benefits from a collectively produced public good that are disproportionately large relative to a cheater's own contribution to that good (Velicer, 2003).

Coevolution: Evolution in two or more species in which the evolutionary changes of each species influence the evolution of the other species (Ridley, 2004).

Cooperation: Any action *selected to enhance* the fitness of others at a relative cost to the acting individual (West *et al.*, 2006). Cooperation includes all behaviours that are altruistic (–/+) and some mutually beneficial (+/+) behaviours.

Cooperator: An individual that provides a benefit that increases the fitness of another individual (other individuals) at a relative cost to itself (West *et al.*, 2006).

Defector: An individual that does not cooperate, but may or may not benefit from the cooperative benefits of others. Therefore, biologically not all defectors are cheats (Velicer, 2003).

Deme: A group of individuals that readily intermix during some point in their life cycle, giving any two an equal probability of becoming neighbours (Wilson, 1977).

Direct fitness: The component of fitness gained through the impact of an individual's behaviour on the production of [their own] offspring (Hamilton, 1964a; West *et al.*, 2007b).

Emergence: The appearance of emergent properties. *See also* Emergent property.

Emergent character: An emergent property in which any character of groups originating from non-additive interaction among lower-level units that do not exist at the lower level (Gould, 2002 p.657).

Emergent fitness: Any trait that characterises or influences the differential rate of proliferation of groups in interaction with the environment (Gould, 2002 p.659).

Emergent property: Any property arising from an interaction among individuals that is 'not otherwise attainable' (Corning, 2002), because the effect is qualitatively or quantitatively 'greater than the sum of the parts' (Gould, 2002).

Exaptation: A character previously evolved for one reason (whether or not as an adaptation), and then coopted for utility in another role (Gould and Vrba, 1982).

Fitness: The average number of offspring produced by individuals with a certain genotype relative to the number produced by individuals with other genotypes (Ridley, 2004).

Focal individual (FI): The focal individual is the individual for whom fitness is evaluated in mathematical calculation and is also the conceptual individual for the description of the model.

Gene flow: The movement of genes into, or through, a population by interbreeding or by migration and interbreeding (Ridley, 2004).

Genetic drift: Random changes in gene frequencies in a population (Ridley, 2004).

Group-generation: In MLS 1, the period of time between successive formations of groups, *i.e.* the period of time spent within a group. In MLS 2, the period of time between the reproduction of successive group offspring.

Group selection: The process of genetic change caused by the differential proliferation and extinction of groups of organisms (Wright, 1945; Wynne-Edwards, 1962; Maynard Smith, 1964; Williams, 1966; Lewontin, 1970; Wade, 1977). *See also* trait-group selection.

Kin selection: The process of selection by which traits are favoured because of their beneficial effects on the fitness of relatives (West *et al.*, 2007b).

Inclusive fitness: The combination of an individual's direct fitness plus the indirect fitness of that individual's effect on all other individuals (Grafen, 1984).

Indirect fitness: The component of fitness gained from aiding the reproduction of related individuals (Hamilton, 1964a; West *et al.*, 2007b).

Individual: A physiologically discrete organism (Buss, 1987) that satisfies Lewontin's conditions for a unit of selection (Lewontin, 1970). *See also* Unit of selection.

Individual Selection: Selection that favours the spread of a trait through a population based only on the number of offspring left by that individual (Grafen, 1984).

Individuality: The properties of an individual. *See also* Individual.

Interdemic group selection: Selection between groups where group membership is defined by interactions between individuals and not by all individuals in the vicinity (Wilson, D.S., 1975).

Iterated Prisoner's Dilemma: A successively repeated version of the Prisoner's Dilemma.

Local group: A subset of the population that interact with one another; the local group may vary from the perspective of different behaviours or traits (West *et al.*, 2007b).

Malthusian parameter: The ratio of final to initial population density after bacterial growth (Lenski *et al.*, 1991).

Malthusian ratio: The relative fitness of a strain compared to a competitor calculated by taking the ratio of Malthusian parameters (Lenski *et al.*, 1991).

Multi-level selection 1: Multi-level selection 1 is said to occur whenever an individual's expected viability, mating success, and/or fertility cannot be accounted for solely on the basis of that individual's phenotype, but rather additional information is required about properties of the group or groups of which the individual is a member (Heisler and Damuth, 1987).

Multi-level selection 2: Multi-level selection 2 occurs whenever any group properties co-vary with group-level fitness, implying that the proportions of different kinds of gorups will change in the population (and noting that group characters may change as a result of lower-level selection among the individuals that the groups comprise) (Heisler and Damuth, 1987).

Multicellularity: The property of an individual that spends part of its life cycle as an entity consisting of more than a single cell (Michod and Roze, 1997; Michod and Roze, 2001; Michod *et al.*, 2005).

Mutual benefit: A behaviour which is beneficial to both the actor and the recipient, *i.e.* +/+ (West *et al.*, 2007b).

Mutualism: Cooperation between species (West *et al.*, 2007b).

Natural selection: The differential reproductive success of evolutionary individuals based on the fitnesses of their traits in interaction with the environment (Gould, 2002).

Phenotype: Any observable physical manifestation of an organism, such as its morphology, development, biochemical or physiological properties, function or behaviour.

Prisoner's Dilemma (PD): A popular non-zero sum game theory approach to analysing cooperation in which cooperation by two individuals generates the highest average payoff, but unilateral defection gives the greatest individual advantage regardless of the choice of the other prisoner.

Public good: Any fitness-enhancing resource that is accessible to multiple individuals within a local group (Velicer, 2003).

Recipient: Any individual receiving the benefit of a cooperative behaviour (West *et al.*, 2007b). Recipients may be cooperators or defectors.

Trait-group selection: *See* interdemic group selection.

Trait-groups: Populations enclosed in areas smaller than the boundaries of the deme (Wilson 1975).

Unit of selection: Any physical entity in nature that has variation, reproduction, and heritability (Lewontin, 1970).

Weak altruism: any action that provides a benefit to others that leads to a decrease in the fitness of the focal individual, relative to the other members of its group (Wilson, D.S., 1975; Wilson, 1977; West *et al.*, 2007b).

Wild-type: The normal or ancestral form of members of a species, as distinct from derived mutant forms (Ridley, 2004).

0.5 ABBREVIATIONS

aa: Amino acid

ANOSIM: Analysis of similarity

ANOVA: Analysis of variance

BLAST: Basic local alignment search tool

c-di-GMP: Cyclic-di-guanosine monophosphate

CDD: Conserved domain database

CR: Congo red

DGC: Di-guanylate cyclase

DMF: Dimethyl Formamide

EPS: Exopolysaccharide

FI: Focal individual

GLS: Group level selection

IPD: Iterated Prisoner's Dilemma

IWS: Independent wrinkly spreader isolates

KB: King's medium B (King *et al.*, 1954)

LB: Lysogeny broth (Bertani, 1951; Bertani, 2004)

LSWS: Large spreading wrinkly spreader

MLS 1: Multi-level selection 1 (Heisler and Damuth, 1987; Damuth and Heisler, 1988)

MLS 2: Multi-level selection 2 (Heisler and Damuth, 1987; Damuth and Heisler, 1988)

MPD: Modified Petri dish

NCBI: National Centre for Biotechnology Information

NF: Nitrofurontoin

OD: Optical density

PDE: Phosphodiesterase

PTFE: Poly-tetrafluoroethene

REC: Signal receiver domain

SM: Smooth colony morphotype of *P. fluorescens* (Rainey and Travisano, 1998)

SNP: Single nucleotide polymorphism

SRC: Selection rate constant (Lenski *et al.*, 1991)

TMHHM: Transmembrane hidden Markov model

WS: Wrinkly spreader colony morphotype of *P.fluorescens* (Rainey and Travisano, 1998)

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