MINIREVIEW

Siderophore-mediated cooperation and virulence in *Pseudomonas aeruginosa*

Angus Buckling[1], Freya Harrison[1], Michiel Vos[1], Michael A. Brockhurst[2], Andy Gardner[3,4], Stuart A. West[4] & Ashleigh Griffin[4]

[1]Department of Zoology, University of Oxford, Oxford, UK; [2]School of Biological Sciences, Biosciences Building, University of Liverpool, Liverpool, UK; [3]St John's College, University of Oxford, Oxford, UK; and [4]Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, UK

Correspondence: Angus Buckling, Department of Zoology, University of Oxford, Oxford, OX1 3PS, UK. Tel.: +44 (0) 1865 281305; fax: +44 (0) 1865 310447; e-mail: angus.buckling@zoo.ox.ac.uk

Received 5 April 2007; revised 27 August 2007; accepted 27 August 2007.
First published online October 2007.

DOI:10.1111/j.1574-6941.2007.00388.x

Editor: Jim Prosser

Keywords
kin selection; inclusive fitness; pyoverdin; experimental evolution; mutator.

Abstract

Why should organisms cooperate with each other? Helping close relatives that are likely to share the same genes (kin selection) is one important explanation that is likely to apply across taxa. The production of metabolically costly extracellular iron-scavenging molecules (siderophores) by microorganisms is a cooperative behaviour because it benefits nearby conspecifics. We review experiments focusing on the production of the primary siderophore (pyoverdin) of the opportunistic bacterial pathogen, *Pseudomonas aeruginosa*, which test kin selection theories that seek to explain the evolution of cooperation. First, cooperation is indeed favoured when individuals interact with their close relatives and when there is competition between groups of cooperators and noncooperators, such that the benefit of cooperation can be realized. Second, the relative success of cheats and cooperators is a function of their frequencies within populations. Third, elevated mutation rates can confer a selective disadvantage under conditions when cooperation is beneficial, because high mutation rates reduce how closely bacteria are related to each other. Fourth, cooperative pyoverdin production is also shown to be favoured by kin selection *in vivo* (caterpillars), and results in more virulent infections. Finally, we briefly outline ongoing and future work using this experimental system.

Introduction

Cooperative behaviours – behaviours that benefit other individuals – appear to be ubiquitous throughout the domains of life (Maynard-Smith & Szathmary, 1995; Hamilton, 1996; West *et al.*, 2007a, b). Why should cooperation exist, given that noncooperating cheats are likely to be able to exploit their cooperative neighbours, and therefore have a short-term selective advantage? There are two general explanations for the evolution of cooperation, and they apply to all taxa (Hamilton, 1964; Lehmann, 2006; West *et al.*, 2006, 2007a, b) First, cooperation might provide a direct fitness benefit to the actor. This would be the case if, for example, individuals can choose to cooperate only with those individuals who reciprocate the cooperation; or if the interests of two parties are directly aligned; or if cheating behaviour is punished. Second, cooperation might provide an indirect fitness benefit to the actor, by promoting the transmission of copies of its genes that are carried by its relatives. This latter process is termed kin selection (Maynard-Smith, 1964). Individuals are predicted to behave as if trying to maximize their inclusive fitness, which is their direct and indirect fitness added together (Hamilton, 1964).

Recent years have witnessed a growing interest in understanding the evolution and ecology of cooperation in microorganisms (Crespi, 2001; Velicer, 2003; Sachs *et al.*, 2004; Travisano & Velicer, 2004; Keller & Surette, 2006; Diggle *et al.*, 2007; Foster *et al.*, 2007; West *et al.*, 2007a, b). There are probably three major reasons for this. First, there is something very impressive about apparently simple organisms showing complex social behaviours. Second, microorganisms offer experimentally very tractable systems for studying the ecology and evolution of cooperation in real time and under carefully controlled conditions. Third, microbial cooperation has important implications for human and agricultural disease and industry: cooperating pathogens and industrial contaminants are likely to be bad news for us.

In this review, an overview is given of recent work addressing the evolution and ecology of one particular cooperative trait that appears to be maintained by kin selection: the production of iron-scavenging molecules, siderophores, of the opportunistic pathogen *Pseudomonas aeruginosa* (Visca *et al.*, 2007). Iron, which is essential for metabolism, is frequently not readily available to bacteria because it is primarily in a largely insoluble ferric form and many host species actively withhold iron from infecting bacteria (Ratledge & Dover, 2000). Some mechanism of iron scavenging is therefore crucial for the success of bacteria. Specifically, we focus on the primary siderophore of *P. aeruginosa,* pyoverdin, but it is noted that *P. aeruginosa* produces other types of siderophores, in addition to having other nonsiderophore iron-uptake systems (Visca *et al.*, 2007). A great advantage of studying pyoverdin as a cooperative trait is that it is a fluorescent yellow–green pigment (Meyer *et al.*, 1997). Pyoverdin-negative colonies can therefore be readily distinguished from the wild type by their lack of yellow-green pigmentation (Griffin *et al.*, 2004). Furthermore, the relationship between pyoverdin production and bacterial growth rates has led to the suggestion that pyoverdin production contributes to *P. aeruginosa* virulence, a view supported by the reduced virulence of pyoverdin-minus mutants (Meyer *et al.*, 1996; Harrison *et al.*, 2006). Pyoverdin can also regulate the expression of other virulence factors (Lamont *et al.*, 2002).

Siderophore production is an example of a cooperative trait because it potentially benefits all bacteria (including the producer) within the locality that are capable of taking up the iron–siderophore complex (West & Buckling, 2003). Siderophore production is beneficial as shown by the fact that a wild-type, pyoverdin producer reaches a higher population density than a nonproducer, when the strains are grown in isolation from each other (Griffin *et al.*, 2004). However, siderophore production is metabolically costly: nonproducers outcompete the wild-type when in direct competition (Griffin *et al.*, 2004). Consistent with the above *in vitro* results, pyoverdin-negative mutants have been observed in natural populations of *P. aeruginosa* that have infected the lungs of cystic fibrosis (CF) patients (De Vos *et al.*, 2001; Smith *et al.*, 2006). Note that fitness differences between producers and nonproducers should increase with increasing iron-limitation, because siderophore production is upregulated in response to iron limitation (Ratledge & Dover, 2000). This is what is observed (Griffin *et al.*, 2004).

Kin selection and the scale of competition

For kin selection to favour cooperative behaviours, the degree of genetic relatedness of interacting individuals must be high relative to the population as a whole. Genetic relatedness is a statistical measure of genetic similarity, and refers specifically to the cooperative loci in question (Hamilton, 1964) – however, high relatedness at one locus is likely to show on average a positive correlation with relatedness at other loci across the whole genome (Grafen, 2006). A relatively high relatedness favours the evolution of cooperation, because it means that an individual with a cooperative gene is helping other individuals with the same gene.

In addition to relatedness, selection for cooperation also depends on the scale of competition (Taylor, 1992; Queller, 1994; Frank, 1998; West *et al.*, 2002). Most organisms, including bacteria, exist in metapopulations. That is, the population is made up of loosely connected patches of individuals, and most interactions occur within these patches. If successful patches (i.e. those containing a high frequency of cooperating individuals) contribute more progeny to the whole metapopulation (there is competition between patches; global competition), then cooperation is more likely to be favoured. However, if competition is local, occurring only within patches (i.e. the output of the patch is independent of patch density), then cooperative strategies will not be successful. Even if the relatedness within patches is very high, cooperation will not be favoured by selection when competition is local because helping some kin effectively hinders others (West *et al.*, 2001).

The importance of relatedness and the scale of competition on the evolution of pyoverdin production in *P. aeruginosa* was addressed by competing a wild-type strain (cooperator) with a pyoverdin-negative mutant (cheat) under conditions of high and low relatedness, and local and global competition (Griffin *et al.*, 2004). The arena for these competitions was replicate metapopulations of 12 tubes containing iron-limited media (Fig. 1a). High relatedness was achieved by inoculating single colonies into tubes, and low relatedness by inoculating two colonies into tubes (initially a single cheat and a single cooperator). Cultures were then propagated for 24 h, and plated onto agar to isolate individual colonies. Local competition was created by plating each tube individually, and then choosing random colonies from random plates to inoculate into fresh media. This meant that the productivity of a tube did not influence the likelihood of it contributing clones to the new metapopulation. Global competition was achieved by mixing all the tubes within a metapopulation together before plating, increasing the chance of clones from productive, cooperating cultures contributing to the new metapopulation. The process of growing cultures, plating and selecting clones (a transfer) was carried out six times (*c.* 50 generations).

The results were entirely consistent with theory: high relatedness and global competition favoured cooperation (Fig. 1b). Global, unlike local, competition allowed the more productive cooperative tubes to contribute more to the global pool, and high relatedness prevented cheats from coming into direct contact with, and hence being able to

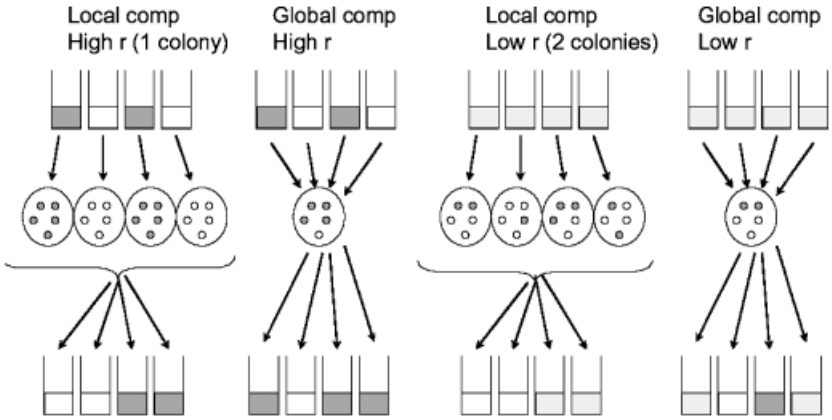

Fig. 1. (a) Experimental design for investigating the impact of relatedness (r) and the scale of competition (local or global) on the evolution of cooperation. Each replicate within each treatment consisted of 12 tubes; four are shown for clarity. High and low relatedness are achieved by inoculating one and two clones, respectively, from agar plates to iron-limited media. Pyoverdin-producing cooperators are shown in dark grey, and pyoverdin-minus cheats in white. From Griffin *et al*. (2004). (b) The evolution of siderophore production in response to relatedness and the scale of competition. The proportion of cooperating individuals who produce pyoverdin siderophores is plotted against time (transfer number; t). The different lines represent relatively high (solid line) and low (dashed line) relatedness. The different symbols represent relatively global (circle) and local (triangle) competition. Each of the four treatments was replicated four times, and SEs are shown for the final time point, data from Griffin *et al*. (2004).

exploit, cooperators. By contrast, under low relatedness conditions, cheats were able to exploit the pyoverdin produced by cooperators, and hence the cheats increased in frequency relative to the cooperators.

The maintenance of cooperation in this study could be interpreted as an example of 'new' group selection (Wilson, 1975; Queller, 2004). Groups of cooperators are more likely to contribute individuals to the next generation than groups of cheats. However, the conditions that allow the operation of group selection are in fact mathematically identical to the conditions that allow the operation of kin selection (see West *et al*., 2006, Box 3, for further discussion and references). Both rely on selection operating between groups of interacting individuals to be stronger than selection operating within interacting groups. In other words, there is greater diversity between than within groups, such that cooperators will be on average more likely to interact with other cooperators, and cheats will be more likely to interact with other cheats. In the context of bacteria, and certainly in the present experiment, this genetic structure is likely to arise because nearby individuals are also likely to be clone mates. It seems most appropriate to describe the force that favours cooperative interactions between close relatives as kin selection. But if interacting individuals are likely to be genetically more similar with respect to cooperative behaviours than non-interacting individuals for reasons other than kinship, group selection would be a more appropriate definition.

Where studied, the majority, but not all, natural isolates of *P. aeruginosa* produce pyoverdin (Meyer *et al*., 1997; De Vos *et al*., 2001; Pirnay *et al*., 2002, 2005). Based on kin selection theory, this suggests that the population structure of *P. aeruginosa* is such that bacteria primarily interact with their clone mates, and that these clonal populations compete with each other. The global population structure of *P. aeruginosa* is broadly consistent with this view. A few clones appear to be at particularly high frequencies (Pirnay *et al*., 2002, 2005; Scott & Pitt, 2004), an epidemic population structure (Maynard-Smith *et al*., 1993), suggesting that competition must occur between clones (i.e. competition is relatively global). Furthermore, given the limited number of dominant clones and the potential for rapid population growth, it is likely that bacteria interact more with their clone mates than other clones. However, more detailed genetic studies through time and at the spatial scale at which bacteria actually share siderophores are required to draw any firm conclusions.

Kin selection and frequency dependence

Another testable prediction that can be made from kin selection theory is that the fitness of cheats should decrease as their frequency, relative to cooperators, decreases (Ross-Gillespie *et al*., 2007). This is expected for two reasons. First, if the population is structured, i.e. made up of spatially distinct subpopulations, then cheats are more likely to be next to cooperators, who they can exploit, when a higher proportion of the population is cooperators (Frank, 1998). Second, a higher proportion of cooperators allows greater levels of population growth, which gives greater opportunity for cheats to exploit cooperators (Ross-Gillespie *et al*., 2007).

Support has been provided for this prediction with both *in vitro* (Ross-Gillespie *et al*., 2007) and *in vivo* (infections of caterpillars) studies (Harrison *et al*., 2006). The mechanism responsible for the result in the *in vitro* work was probably

due entirely to a higher amount of population growth with increasing cooperator frequency, as tubes were continually shaken to destroy the spatial structure. This interpretation was supported by the fact that the amount of population growth was positively correlated with the proportion of cooperators, and that experimentally reducing the time available for growth led to a decrease in the extent of frequency dependence. Both elevated population growth with increasing cooperation as well as spatial heterogeneity is likely to be important for the frequency dependence *in vivo*, but teasing these mechanisms apart is not possible *in vivo*.

Mutators and cooperation

Bacteria with mutation rates 10–1000-fold higher than normal are frequently found in natural populations (LeClerc *et al.*, 1996; Matic *et al.*, 1997). Indeed, one study reported that 20% of *P. aeruginosa* strains colonizing the lungs of patients suffering from CF were 'mutator' genotypes (Oliver *et al.*, 2000). When adaptation is limited by the beneficial mutation rate, as is the case in novel or changeable environments, mutator alleles can hitch-hike with beneficial mutations to reach high frequencies (Leigh, 1970; Taddei *et al.*, 1997; Tanaka *et al.*, 2003). However, mutators may be disadvantaged if population growth depends on cooperative behaviours, because cooperation is more likely to break down in mutator lineages (Frank, 1994). This is because mutators are likely to decrease the relatedness of evolving populations, or, put more simply, are more likely to generate social cheats.

This idea was recently tested by evolving separate populations of wild type and mutator *P. aeruginosa* clones under entirely local competition for *c.* 200 generations in iron-limited media (Harrison & Buckling, 2005). The mutator was isogenic to the wild type, but with *mutS*, a gene involved in the methyl-directed mismatch repair system, knocked out, resulting in a 100-fold increase in mutation rate. Siderophore cheats rapidly evolved in all populations (competition was entirely local; hence, there was no selection for cooperation), but increased in frequency much more rapidly in the mutator lineages. Furthermore, cheats reached fixation in mutator lineages but showed considerable fluctuation in frequency in wild-type populations, suggesting that mutator cheats were fitter than wild-type cheats (Harrison & Buckling, 2005). Note that, as for all described experiments, pyoverdin cheats are still likely to be able to obtain iron via other iron-uptake mechanisms, and hence they can still grow to some extent in iron-limited media.

Having established that mutators are more likely than wild-type to produce cheats, the prediction that mutators should be selected against under conditions when cooperation is favoured (Harrison & Buckling, 2007) was directly tested. Mutator and wild-type genotypes were competed under global competition and either high or low relatedness conditions, as outlined above. As hypothesized, mutators reached significantly lower frequencies under conditions of high compared with low relatedness, conditions that favour more and less cooperation, respectively. Crucially, the frequency of mutators within a metapopulation showed a significant positive correlation with the frequency of pyoverdin cheats (Fig. 2), strongly suggesting that it was indeed the propensity of mutators to generate cheats that was responsible for their relative disadvantage under conditions of high relatedness. Note that in these studies of *de novo* evolution of pyoverdin-cheats, the reduced iron-uptake activity of white colonies was confirmed using a CAS colorific assay (Schwyn & Neilands, 1987).

The impact of mutators on cooperative interactions may help to explain observed distributions of *P. aeruginosa* mutator genotypes. The frequency of mutators tends to be higher in clinical isolates of bacteria than in conspecific environmental populations (LeClerc *et al.*, 1996). It has been suggested that this is because *in vivo* populations face greater fluctuations in selection pressures, as a result of the host immune system and chemotherapy. It is suggested that there may also be weaker selection for bacterial cooperation, and hence weaker selection against mutators, in clinical vs. environmental populations. *Pseudomonas aeruginosa* infections are largely the result of a single infecting clone (Struelens *et al.*, 1993) and infections are generally long term (Harrison, 2007), suggesting that transmission between *P. aeruginosa* infections is very low, and hence competition is largely local. Furthermore, considerable genetic change, and hence the opportunity for cheats to evolve, of these initially clonal infections is observed during the long

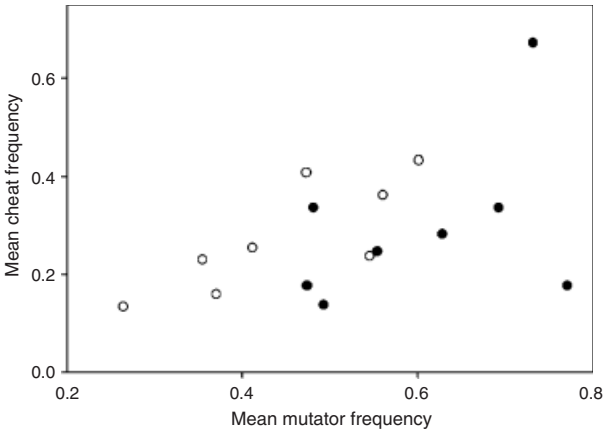

Fig. 2. Mutator genotypes break down cooperation. The relationship between mutator frequency and pyoverdin cheat frequency in experimental metapopulations. Open circles show high relatedness treatments; closed circles show low relatedness treatments, data from Harrison & Buckling (2007).

life span of the infection (Smith *et al.*, 2006; Harrison, 2007). Little is known about the natural ecology of *P. aeruginosa* in soil and water at the scale at which bacteria actually interact. However, it is tentatively suggested that relatedness will be higher than in clinical infections because of short patch longevity, but that competition will be more global because of greater dispersal between bacterial 'patches'.

Relatedness, siderophore production and virulence

Pyoverdin production is linked with virulence (Meyer *et al.*, 1996; Lamont *et al.*, 2002). Assuming that bacteria are iron-limited *in vivo*, it follows that conditions that favour cooperative siderophore production will result in more virulent infections than conditions that do not. As emphasized above, both the relatedness of an infection and the scale of competition are likely to be crucial factors determining the degree of cooperation. Mathematical modelling supports these verbal arguments: high relatedness and global competition are predicted to favour siderophore production, and result in increased virulence (Brown *et al.*, 2002; West & Buckling, 2003).

The importance of public goods cooperation to virulence had not been addressed previously and is in apparent contrast to many other models of parasite evolution (Bremermann & Pickering, 1983; Nowak & May, 1994; Frank, 1996). These models predict that virulence is increased when intrahost parasite relatedness is low, due to increased competition for host resources resulting in more rapid rates of host exploitation. The discrepancy, however, arises comes from the fact that in these models parasites are not producing a cooperative 'public good', such as siderophores, which is required for parasite growth. Cooperation is, however, still important in these models in the form of 'prudence': the careful use of host resources that maximizes the success of the infecting parasite population as a whole, by not killing the host too quickly (West & Buckling, 2003). There is, however, little empirical support for the prediction that low relatedness leads to increased virulence.

The authors wanted to test the hypothesis that virulence decreases with decreasing relatedness in cases where a public good, such as siderophores, is important for bacterial growth. Waxmoth larvae (*Galleria mellonella*) were inoculated with single clones of a wild-type, pyoverdin-producing strain of *P. aeruginosa*, a pyoverdin-minus mutant or both (Harrison *et al.*, 2006). Consistent with previous work (Meyer *et al.*, 1996), it was found that insects were on average killed by cooperator infections *c.* 15% sooner than cheater infections, and that mixed infections resulted in an intermediate time to death (Fig. 3). The growth rate of single- and mixed-clone infections showed the same pattern as for virulence: cooperators grew faster than cheats, and mixed infections showed an intermediate growth rate. These data demonstrate that the presence of siderophore cheats can reduce the growth rate of a bacterial population, and hence reduce virulence.

It was next addressed whether cheats were more likely to be favoured in low- vs. high-relatedness infections. As predicted, cheat populations grow more rapidly in mixed-, as opposed to single-clone, infections, while the opposite pattern is observed for cooperators. Low relatedness infections are therefore more likely to favour the evolution of siderophore cheats than are high relatedness infections (Harrison *et al.*, 2006). Given that the presence of cheats reduces virulence, it follows that if coinfection by multiple genotypes is the norm, then the average infection will be less virulent.

However, in these simple competition experiments between siderophore cheats and cooperators, cheats never had a detectable selective advantage: at best, their fitness was the same as cooperators (Harrison *et al.*, 2006). This is in contrast with *in vitro* work, where cheats were consistently fitter under iron-limited conditions (Griffin *et al.*, 2004). This poses a major problem for the theory: if cheats never have a selective advantage *in vivo*, then virulence will not decrease under low relatedness infections. Possible explanations for the lack of invasion success of the cheats are pleiotropic effects caused by the knockout of the pyoverdin mutant, or that the reduced, rather than zero, pyoverdin production may be the optimal strategy. Therefore, 12 independent populations of *P. aeruginosa* (six founded from the wildtype and six from the mutator mutant) were evolved in caterpillars, under entirely local competition, such that cheating should be favoured. Twenty-four hours after the initial inoculation, caterpillars were squashed in sterile salts, and then a sample was inoculated into new caterpillars. *Pseudomonas aeruginosa* was purified from resident bacteria by ampicillin selection. After five passages, an average of

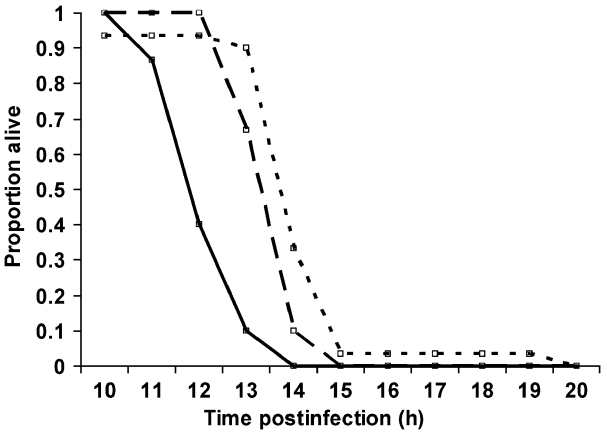

Fig. 3. Survivorship of insects inoculated with pyoverdin cooperators (solid), cheats (short dashes) and mixtures of the two (long dashes), data from Harrison *et al.* (2006).

3.5% (between 0% and 12%) white colonies was detected, indicating that pyoverdin cheats can evolve *de novo* and have a selective advantage *in vivo* (D. Racey, F. Harrison & A. Buckling, in preparation).

Perspective

The work outlined here is only the tip of the iceberg. The pyoverdin system provides an excellent opportunity to expand one's understanding of the evolution of cooperation. First, it is possible to address how a range of ecological conditions [e.g. disturbances (Brockhurst *et al.*, 2007); environmental quality; resource partitioning within species (Brockhurst *et al.*, 2006); competition with other species; parasitism and predation] affect the evolution of cooperation, through their effects on the costs and benefits of cooperation, and relatedness. Second, pyoverdin production and uptake may provide unique insights into the evolution of cooperative acts specifically directed at kin as a mechanism to protect against exploitation by cheats. There appears to be strong diversifying selection acting on pyoverdin-producing and uptake genes (Smith *et al.*, 2005), and not all siderophore types can be taken up by all strains of *P. aeruginosa* (Meyer *et al.*, 1997; Tummler & Cornelis, 2005). Third, experimental studies to date have largely been limited to consideration of social behaviours as fixed traits, i.e. cooperators and cheats. Pyoverdin production, like many social behaviours, is, however, facultative, allowing studies to be carried out that explicitly address the evolution of environment-dependent cooperation. Finally, siderophore production represents only one of the numerous social traits of *P. aeruginosa* and other bacteria, including polymer production for biofilm formation (Webb *et al.*, 2003; Hall-Stoodley *et al.*, 2004), quorum sensing (Diggle *et al.*, 2006, 2007) and spiteful anticompetitor toxins (bacteriocins) (Riley, 1998; Michel-Briand & Baysse, 2002; Parret & De Mot, 2002). Investigating how selection acts on these traits simultaneously will provide a much clearer understanding of the evolution, and implications, of microbial sociality.

Acknowledgements

The authors gratefully acknowledge the Royal Society and NERC (UK) for funding this research.

References

Bremermann HJ & Pickering J (1983) A game-theoretical model of parasite virulence. *J Theor Biol* **100**: 411–426.

Brockhurst MA, Hochberg ME, Bell T & Buckling A (2006) Character displacement promotes cooperation in bacterial biofilms. *Curr Biol* **16**: 2030–2034.

Brockhurst MA, Buckling A & Gardner A (2007) Cooperation peaks at intermediate disturbance. *Curr Biol* **17**: 761–765.

Brown SP, Hochberg ME & Grenfell BT (2002) Does multiple infection select for raised virulence? *Trends Microbiol* **10**: 401–405.

Crespi BJ (2001) The evolution of social behavior in microorganisms. *Trends Ecol Evol* **16**: 178–183.

De Vos D, De Chial M, Cochez C, Jansen S, Tummler B, Meyer JM & Cornelis P (2001) Study of pyoverdine type and production by *Pseudomonas aeruginosa* isolated from cystic fibrosis patients: prevalence of type II pyoverdine isolates and accumulation of pyoverdine-negative mutations. *Arch Microbiol* **175**: 384–388.

Diggle SP, Cornelis P, Williams P & Camara M (2006) 4-Quinolone signalling in *Pseudomonas aeruginosa*: old molecules, new perspectives. *Int J Med Microbiol* **296**: 83–91.

Diggle SP, West SA, Gardner A & Griffin AS (2007) Evolutionary theory of bacterial quorum sensing: when is a signal not a signal? *Phil Trans R Soc Lond B* **362**: 1241–1249.

Foster KR, Parkinson K & Thompson CRL (2007) What can microbial genetics teach sociobiology? *Trends Genet* **23**: 74–80.

Frank SA (1994) Kin selection and virulence in the evolution of protocells and parasites. *Proc R Soc Lond B* **258**: 153–161.

Frank SA (1996) Models of parasite virulence. *Q Rev Biol* **71**: 37–78.

Frank SA (1998) *Foundations of Social Evolution*. Princeton University Press, Princeton, NJ.

Grafen A (2006) Optimisation of inclusive fitness. *J Theor Biol* **238**: 541–563.

Griffin AS, West SA & Buckling A (2004) Cooperation and competition in pathogenic bacteria. *Nature* **430**: 1024–1027.

Hall-Stoodley L, Costerton JW & Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. *Nature Rev Microbiol* **2**: 95–108.

Hamilton WD (1964) The genetical evolution of social behaviour, I & II. *J Theor Biol* **7**: 1–52.

Hamilton WD (1996) *Narrow Roads of Gene Land: I Evolution of Social Behaviour*. W. H. Freeman, Oxford.

Harrison EF & Buckling A (2005) Hypermutability impedes cooperation in pathogenic bacteria. *Curr Biol* **15**: 1968–1971.

Harrison EF & Buckling A (2007) High relatedness selects against hypermutability in bacterial metapopulations. *Proc R Soc Lond B* **274**: 1341–1347.

Harrison F (2007) Microbial ecology of the cystic fibrosis lung. *Microbiology* **153**: 917–923.

Harrison F, Browning LE, Vos M & Buckling A (2006) Cooperation and virulence in acute *Pseudomonas aeruginosa* infections. *BMC Biol* **4**: 21.

Keller L & Surette MG (2006) Communication in bacteria: an ecological and evolutionary perspective. *Nat Rev Microbiol* **4**: 249–258.

Lamont IL, Beare PA, Ochsner U, Vasil AI & Vasil ML (2002) Siderophore-mediated signaling regulates virulence factor production in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* **99**: 7072–7077.

LeClerc JE, Li BG, Payne WL & Cebula TA (1996) High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science* **274**: 1208–1211.

Lehmann LKL (2006) The evolution of cooperation and altruism. A general framework and classification of models. *J Evol Biol* **19**: 1365–1378.

Leigh JEG (1970) Natural selection and mutability. *Am Nat* **104**: 301–305.

Matic I, Radman M, Taddei F, Picard B, Doit C, Bingen E, Denamur E & Elion J (1997) Highly variable mutation rates in commensal and pathogenic *Escherichia coli*. *Science* **277**: 1833–1834.

Maynard-Smith J (1964) Group selection and kin selection. *Nature* **201**: 1145–1147.

Maynard-Smith J & Szathmary E (1995) *The Major Transitions in Evolution*. W.H. Freeman, Oxford.

Maynard-Smith JNH, Smith M, Rourke O & Spratt BG (1993) How clonal are bacteria. *Proc Natl Acad Sci USA* **90**: 4384–4388.

Meyer JM, Neely A, Stintzi A, Georges C & Holder IA (1996) Pyoverdin is essential for virulence of *Pseudomonas aeruginosa*. *Infect Immun* **64**: 518–523.

Meyer JM, Stintzi A, DeVos D, Cornelis P, Tappe R, Taraz K & Budzikiewicz H (1997) Use of siderophores to type pseudomonads: the three *Pseudomonas aeruginosa* pyoverdine systems. *Microbiology* **143**: 35–43.

Michel-Briand Y & Baysse C (2002) The pyocins of *Pseudomonas aeruginosa*. *Biochimie* **84**: 499–510.

Nowak MA & May RM (1994) Superinfection and the evolution of parasite virulence. *Proc R Soc Lond B* **255**: 81–89.

Oliver A, Canton R, Campo P, Baquero F & Blazquez J (2000) High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* **288**: 1251–1253.

Parret AHA & De Mot R (2002) Bacteria killing their own kind: novel bacteriocins of *Pseudomonas* and other *Gammaproteobacteria*. *Trends Microbiol* **10**: 107–112.

Pirnay JP, Vos DD, Mossialos D, Vanderkelen A, Cornelis P & Zizi M (2002) Analysis of the *Pseudomonas aeruginosa* oprD gene from clinical and environmental isolates. *Environ Microbiol* **4**: 872–882.

Pirnay JP, Matthijs S, Colak H, Chablain P, Bilocq F, Van Eldere J, De Vos D, Zizi M, Triest L & Cornelis P (2005) Global *Pseudomonas aeruginosa* biodiversity as reflected in a Belgian river. *Environ Microbiol* **7**: 969–980.

Queller DC (1994) Genetic relatedness in viscous populations. *Evol Ecol* **8**: 70–73.

Queller DC (2004) Social evolution: kinship is relative. *Nature* **430**: 975–976.

Ratledge C & Dover LG (2000) Iron metabolism in pathogenic bacteria. *Ann Rev Microbiol* **54**: 881–941.

Riley MA (1998) Molecular mechanisms of bacteriocin evolution. *Ann Rev Genet* **32**: 255–278.

Ross-Gillespie A, Gardner A, West SA & Griffin AS (2007) Frequency dependence and cooperation: theory and a test with bacteria. *Am Nat* **170**: 331–342.

Sachs JL, Mueller UG, Wilcox TP & Bull JJ (2004) The evolution of cooperation. *Q Rev Biol* **79**: 135–160.

Schwyn B & Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. *Analyt Biochem* **160**: 47–56.

Scott FW & Pitt TL 2004 Identification and characterization of transmissible *Pseudomonas aeruginosa* strains in cystic fibrosis patients in England and Wales. *J Med Microbiol* **53**: 609–615.

Smith EE, Sims EH, Spencer DH, Kaul R & Olson MV (2005) Evidence for diversifying selection at the pyoverdine locus of *Pseudomonas aeruginosa*. *J Bacteriol* **187**: 2138–2147.

Smith EE, Buckley DG, Wu ZN *et al*. (2006) Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. *Proc Natl Acad Sci USA* **103**: 8487–8492.

Struelens MJ, Schwam V, Deplano A & Baran D (1993) Genome macrorestriction analysis of diversity and variability of *Pseudomonas aeruginosa* strains infecting cystic-fibrosis patients. *J Clin Microbiol* **31**: 2320–2326.

Taddei F, Radman M, Maynard-Smith J, Toupance B, Gouyon PH & Godelle B (1997) Role of mutator alleles in adaptive evolution. *Nature* **387**: 700–702.

Tanaka MM, Bergstrom CT & Levin BR (2003) The evolution of mutator genes in bacterial populations: the roles of environmental change and timing. *Genetics* **164**: 843–854.

Taylor PD (1992) Altruism in viscous populations – an inclusive fitness model. *Evol Ecol* **6**: 352–356.

Travisano M & Velicer GJ (2004) Strategies of microbial cheater control. *Trends Microbiol* **12**: 72–78.

Tummler B & Cornelis P (2005) Pyoverdine receptor: a case of positive Darwinian selection in *Pseudomonas aeruginosa*. *J Bact* **187**: 3289–3292.

Velicer GJ (2003) Social strife in the microbial world. *Trends Microbiol* **11**: 330–337.

Visca P, Imperi F & Lamont IL (2007) Pyoverdine siderophores: from biogenesis to biosignificance. *Trends Microbiol* **15**: 22–30.

Webb JS, Givskov M & Kjelleberg S (2003) Bacterial biofilms: prokaryotic adventures in multicellularity. *Curr Opin Microbiol* **6**: 578–585.

West SA & Buckling A (2003) Cooperation, virulence and siderophore production in bacterial parasites. *Proc R Soc Lond B* **270**: 37–44.

West SA, Murray MG, Machado CA, Griffin AS & Herre EA (2001) Testing Hamilton's rule with competition between relatives. *Nature* **409**: 510–513.

West SA, Pen I & Griffin AS (2002) Cooperation and competition between relatives. *Science* **269**: 72–75.

West SA, Griffin AS, Gardner A & Diggle S (2006) Social evolution theory for microbes. *Nat Rev Microbiol* **4**: 597–607.

West SA, Diggle S, Gardner A, Buckling A & Griffin AS (2007a) The social lives of microbes. *Ann Rev Ecol Evol Syst*, in press.

West SA, Griffin AS & Gardner A (2007b) Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. *J Evol Biol* **20**: 415–432.

Wilson DS (1975) A theory of group selection. *Proc Natl Acad Sci USA* **72**: 143–146.