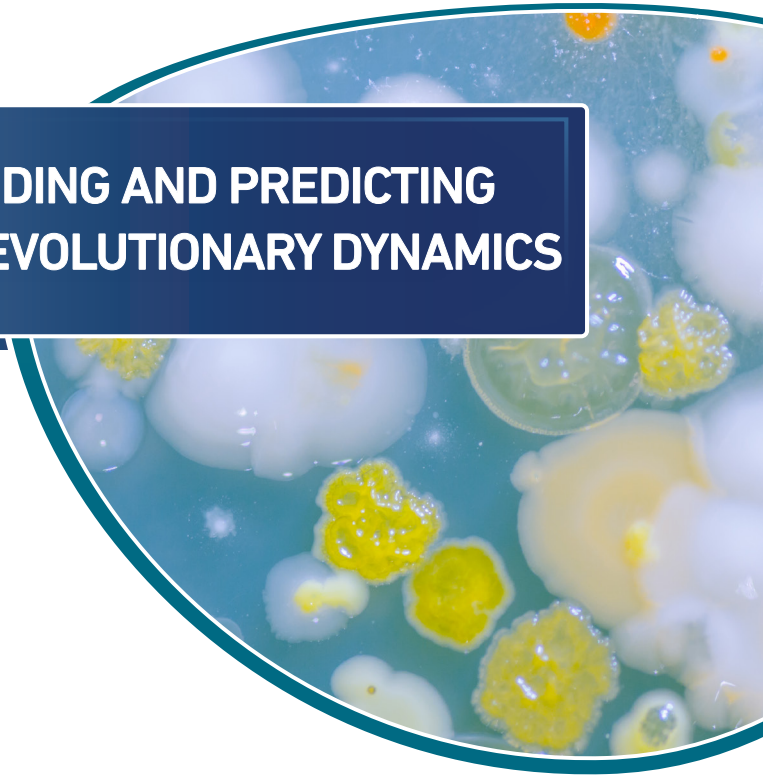
MICROBIOLOGY SOCIETY

UNDERSTANDING AND PREDICTING MICROBIAL EVOLUTIONARY DYNAMICS



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Poster book

Pangenome evolution in *Escherichia coli* is sequence type, not phylogroup, specific

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Abstract

The *Escherichia coli* species contains a diverse set of sequence types and there remain important questions regarding differences in genetic content within this population that need to be addressed. Pangenomes are useful vehicles for studying gene content within sequence types. Here, we present an inter-pangenome study - a novel approach to analysing closely related pangenomes. We analyse 21 *E. coli* sequence type pangenomes constructed from one of the biggest collections of *E. coli* genomes used to date, using comparative pangenomics, to identify variance in both pangenome structure and content. We present functional breakdowns of sequence type core genomes and identify sequence types that are enriched in metabolism, transcription and cell membrane biogenesis genes. We also uncover metabolism genes that have variable core classification depending on which allele is present. Our comparative pangenomics approach allows for detailed exploration of sequence type pangenomes within the context of the species. We show that pangenome evolution is independent of phylogenetic signal at the phylogroup level, which may be a consequence of distinct sequence type-specific driving factors relating to ecology and pathogenic phenotype.

2

An Experimental Model System to Explore the Evolutionary Consequences of CRISPR-Cas Acquisition to a Naïve Host

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Abstract

CRISPR-Cas defence systems are akin to an adaptive immune system, allowing the acquisition of resistance against invading foreign DNA, including phages. While CRISPR-Cas systems facilitate heritable acquired immunity, they also interact with the inflow of beneficial horizontally acquired genes and incur metabolic growth costs. Thus, although widespread, CRISPR-Cas systems are not ubiquitous across bacteria, and the ecological and evolutionary drivers that govern their prevalence and maintenance are currently unknown. Phylogenetic evidence shows that bacterial anti-viral defence systems are frequently exchanged via horizontal gene transfer (HGT). A high rate of HGT provides a potential mechanism to maintain CRISPR-Cas systems at low levels within populations, dispersing the fitness costs of maintaining CRISPR-Cas systems between hosts. However, the adaptive forces that select for the maintenance of, and adaptation to, horizontally acquired CRISPR-Cas systems remains understudied. We have modelled an HGT event by creating a synthetic, modular, minimal version of the CRISPR-Cas system from the opportunistic pathogen Pseudomonas aeruginosa and integrating it into the genome of the common soil bacterium Pseudomonas fluorescens, which lacks CRISPR-Cas. We use an experimental evolution approach to explore how the acquisition of a CRISPR-Cas system affects the co-evolutionary dynamics between P. fluorescens (SBW25) and its phage Φ2 – a well-studied model for rapid, reciprocal, antagonist arms race dynamics. This work offers a model system to test the long term, dynamic evolutionary consequences of CRISPR-Cas HGT events into naïve hosts, potentially allowing us to predict what abiotic and biotic selection factors are required for successful acquisition of new anti-viral systems.

3

Genomic approaches to identifying social genes

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Abstract

Bacteria cooperate and communicate to perform a stunning array of social behaviours that benefit the local group of cells. Many of these behaviours, such as the secretion of toxins and nutrient-scavenging molecules, are highly relevant in the way bacteria cause disease. Although we know a lot about how these behaviours evolve in model organisms in the lab, we know relatively little about their evolutionary dynamics in nature.

We can fill this knowledge-gap using approaches such as molecular population genetics that allow us to detect how social behaviours are evolving using DNA sequence data. However, to do this it is crucial that we can identify social genes in genomes. In the last two years, five distinct methods for identifying social genes from genome data have been proposed. Here, we critically assess these contrasting methods, highlighting the strengths and weaknesses of each, and making suggestions on which research questions are best addressed by each method.

We hope that our critical analysis of the methods available can provide a solid foundation for this emerging field. The ability to accurately identify social genes in genomes opens up the study of social evolution in bacteria to a much broader range of taxa, and allows us to look for evidence of kin-selection outside the lab, investigate when happens in genomes as sociality evolves (or breaks-down), and test theories about whether social traits are maintained by pleiotropy or horizontal gene transfer.

4

Can the use of high density transposon libraries be used to study and predict evolution in order to understand stress responses ?

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Abstract

aboratory-based evolution has become a widely used method to explore fundamental questions about evolution as a process and is also a powerful tool to study the link between genotype and phenotype. We are interested in understanding short and long term adaptations of bacteria to stressful environments, and to study this we have evolved five populations of E. coli MG1655 in a dynamic pH environment, by iterative growth and daily dilution in unbuffered LB, starting at pH 4.5. This was done over five months, keeping a frozen fossil record of intermediate populations during this process. Whole genome sequencing of the evolved populations and of individual clones from each population revealed many striking similarities in the evolutionary trajectories in the evolved strains; for example, mutations in arcA are common and may cause loss or alteration of function. We are interested in exploring the impact of multiple different parameters on evolutionary trajectories, but as evolution experiments take a long time, we are currently investigating whether experiments, using a high density transposon libraries and Transposon Directed Insertion-site Sequencing (TraDIS), can partially replicate evolution experiments in a relatively short time frame. Since TraDIS provides a measure of relative contributions to fitness of each gene (by comparison of read counts after growth in two conditions), in principle it should be possible to use TraDIS to identify genes whose loss of function provides a fitness benefit. Here we compare the outcome of these two techniques and present our latest results.

5

Bacteriophage exposure leads to a successive productivity boost in Variovorax sp. populations

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Abstract

Bacteriophage ("phage") are hypothesised to be the drivers of bacterial populations, cited as maintaining diversity through direct infection and costs of resistance. The negative effects of phage have made these viruses attractive as biocontrol agents in both agriculture and medicine. However, the effects of phage are not consistent across all strains and species. Additionally, the long-term effects of bacteriophage on host fitness are not fully understood, especially following resistance evolution and/or phage extinction. Here, we report experimental evolution between a Variovorax sp. and a novel bacteriophage VAH_51 over 80 generations. After 20 generations, bacteriophage went extinct following 100% emergence of phage resistance. After 40 generations, Variovorax populations that had been exposed to phage were significantly more productive (greater equilibrium density) than unexposed populations. Further experiments suggested this productivity boost was not mediated by phage-resistance driving greater adaptability or intrinsically higher growth rates. Instead, phage exposure appears to generate highly variable growth rates among clones that have a higher average growth rate. Full genome sequencing suggested the mechanisms of resistance that may have partially stimulated metabolic shifts in bacterial populations towards greater productivity. These results highlight the potential benefits conferred to bacterial populations following phage exposure which may be mediated by eco-evolutionary responses. As such, predicting how phages affect bacterial populations should not be limited to short-term assays testing phage efficacy (in bacterial killing) or costs of resistance.

6

Role of evolution on ecological resilience against antibiotic disturbance

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Abstract

Biological communities regularly face abiotic stress that can change their stability and ultimately shift communities into alternative ecological state. Role of evolution in this process is however less studied. Antibiotics are societally highly relevant abiotic stress factor / selection force that microbial communities face. Understanding for example how gut microbiome reacts and recovers to and from antibiotic therapy is highly relevant for human health. At the same time, substantial amount of effort has been invested in studying how antibiotic resistance (AMR) evolves in experimental settings, focusing on effect of antibiotics on single species evolution. Bacteria that become resistant to antibiotics however are embedded in complex microbial communities. In order to understand the AMR problem, we need to understand how community level interactions, e.g. resource competition, predation etc. potentially alter the outcome of evolution and at the same time, we need to understand the role of evolution on ecological dynamics, e.g. resilience of the community. For testing these questions in laboratory settings, we have developed a multispecies bacterial model community. The community consists of 23 gram-negative bacterial strains—representing various taxa—that are able to coexist in complex medium. I will present results from experiments testing different aspects of interplay between ecology and evolution in determining the community assembly and dynamics under antibiotic stress. Key insights include for example results indicating the importance of initial genetic variability on ecological community dynamics and that the spatial source sink metacommunity dynamics can be important how community level AMR evolution develops.

7

Host microbiota shape the evolution of 'proactive invader' pathogens.

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Abstract

'Proactive invaders' are opportunistic pathogens which change the within-host environment to their advantage, at the expense of resident microbiota. The extent to which this type of exploitation can play a role in pathogen virulence evolution is unclear. We show that killing by the opportunistic pathogen *Staphylococcus aureus* is increased in the presence of native microbiota strains in the *Caenorrhabditis elegans* nematode gut. Subsequently, we experimentally evolved *S. aureus* through successive *C. elegans* host generations with and without a microbiota, to determine the evolutionary trajectory of pathogen virulence under both conditions. We found that pathogens evolved in gnotobiotic hosts display the highest level of host-killing. We explore potential genomic targets of selection underpinning virulence in the pathogen. Moreover, metagenome sequencing of microbiota co-evolved with the pathogen shows that the infection strategy used by *S. aureus* reduces microbiota diversity over evolutionary time. Further work is underway to pick apart the interactions between pathogen and microbiota, to better understand the evolution of *S. aureus* as a proactive invader in infectious disease.

8

The evolutionary fate of duplicate tRNA genes in *Pseudomonas fluorescens* SBW25

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Abstract

The transfer RNA (tRNA) content of cells affects the efficiency of protein synthesis. Previously, our laboratory studied adaption of *Pseudomonas fluorescens* SBW25 to novel translational demands: two slow growing mutants – each lacking one or more tRNA genes – recovered fitness during 14-day or 21-day serial transfer experiments through duplicating large sections (up to 1Mb) of the genome. The compensatory element of each large duplication was a single, tiny (~80bp) tRNA gene. While the large duplications are adaptive, they are also highly unstable (i.e., are lost at extremely high rates). Here, we use empirical and theoretical approaches to investigate the long-term evolutionary fate of the large duplications, and the new tRNA genes that they contain. The initial serial transfer evolution experiments were extended to 100 days (~700 generations). Variability in colony morphology rapidly emerged and subsequently abated in each evolving lineage, indicating complex evolutionary dynamics. Whole genome re-sequencing of several day 14 isolates revealed the presence of a novel type of compensatory mutation: a stable SNP in the promoter of a tRNA gene, that – similarly to duplications – serves to increase tRNA expression. Currently, we are delving into the evolutionary dynamics of our populations by (i) selecting isolates for whole genome re-sequencing, and (ii) using mathematical simulations to investigate key factors influencing the fate of the two mutational classes. Our results will provide insight into whether large duplications can be refined to persist over longer time scales, illuminating the degree they contribute to the long term evolution of bacterial tRNA gene sets.

9

Contrasting responses of diverse *Pseudomonas aeruginosa* strains to oxidative stress in an environment mimicking cystic fibrosis lung sputum

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Abstract

During chronic infection, *Pseudomonas aeruginosa* can rapidly adapt to the within-host environment. The cystic fibrosis (CF) airway provides a range of potential selective forces to drive such adaptation, including novel resources, such as abundant iron and amino acids, and stressors, such as reactive oxygen species from immune responses. In this study, we experimentally tested the ecological responses of three diverse *P. aeruginosa* strains to oxidative stress in a defined medium mimicking the CF lung. Surprisingly, we found that LESB58, an important clinical strain isolated from a CF chronic infection, is extremely susceptible to oxidative stress, whilst PA14, a highly virulent clinical strain, is more susceptible to oxidative stress than PAO1, a commonly used lab strain. Next, we conducted an evolution experiment to test whether PAO1 and PA14 were able to adapt to elevated oxidative stress in a synthetic CF environment. Whereas all PAO1 lineages survived, 8 out of 9 replicate PA14 lineages were driven extinct under oxidative stress within 12 daily serial transfers and failed to adapt to oxidative stress. Together these findings show that clinical strains adapted to CF lung conditions are not necessarily highly tolerant of oxidative stress. Further, low tolerance to oxidative stress may in turn constrain the ability of strains to adapt to elevated oxidative stress in future. Future work will seek to uncover the mechanisms behind the adaptability of different *P. aeruginosa* strains to oxidative stress.

10

Host-specific determinants of *Legionella pneumophila* virulence passaged with single hosts and in alternation

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Abstract

Legionella pneumophila are endosymbiotic bacteria able to infect, and reproduce in, various protist hosts. Upon entry in human lungs, they are able to infect lung macrophages, causing Legionnaires' disease (LD), an atypical pneumonia, using the same mechanisms despite the two hosts being separated by a billion years of evolution. In this study, we passaged *L. pneumophila* in two different hosts - *Acanthamoeba castellanii* and the human macrophage-like cells U937 - separately and in alternation, twice a week for a year. Among the 42 fixed mutations in the 18 lineages, two pairs of mutations, one pair with mutations in the 30S ribosomal proteins S12 (RpsL) and S4 (RpsD), the other pair including a different RpsL mutation and a mutation in the chaperonin GroES, were present in almost all lineages, but mutually exclusive. Each of these pairs, either alone or in combination with further mutations, provided an increased growth rate in both hosts. The gene coding for LerC, a key regulator of protein effector expression, was independently mutated in 6 lineages grown in presence of the U937 cells. This mutation provides a slight gain in growth rate in these hosts compared to the ancestor. Although the selective forces acting in this system are presumably too complex to simply estimate fitness through maximum growth rate, we conclude that the loss of the regulator LerC probably improves the growth of *L. pneumophila* in human-derived cells. This is a first step in further investigating determinants of host specificity in *L. pneumophila*.

11

Rewiring bacterial regulatory networks with *de novo* regulators from random sequence

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Abstract

In bacteria, genes are often regulated at multiple levels by combinations of different RNAs, proteins, and small molecules. While many studies have characterized the components and mechanisms involved in the regulation of various bacterial pathways, the origins and evolution of existing regulatory networks remain largely unexplored. To investigate how regulatory interactions might arise *de novo* from nonfunctional sequence, we probed in two different screens how random DNA sequences might rewire existing regulatory circuits to enable select *Escherichia coli* strains to survive under non-permissive conditions. In the first screen, we isolated three small proteins that rescue the growth of a Δ*serB* auxotroph by upregulating *hisB* expression via interactions with the *his* operon mRNA. In the second screen, we selected a novel noncoding RNA that allows a strain carrying a *lacZ* reporter regulated by the *thiM* thiamine pyrophosphate riboswitch to grow on lactose under conditions in which the riboswitch inhibits *lacZ* expression. Unexpectedly, the same RNA was also found to independently increase expression of nitrogen starvation regulator *glnK*. We are currently investigating the mechanisms underlying these changes in gene expression. Until recently, data demonstrating the *de novo* emergence of novel functions have mostly been limited to bioinformatics studies. This work provides *in vivo* experimental evidence that new functions, including novel regulatory interactions, can indeed originate *de novo* from completely random DNA. Our findings offer insight into the constraints governing the formation and (re)arrangement of bacterial regulatory networks, with implications for predicting adaptation to new ecological niches and designing synthetic genetic circuits.

12

Sub-lethal antibiotic treatment leads to the emergence of stronger resistance in well mixed environments

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Abstract

Vast antibiotic concentration gradients exist in the environment and within humans undergoing treatment. Previous studies focus primarily on antibiotic concentrations well above lethal levels, leaving dynamics of populations present in sub-lethal concentrations a missing piece in our knowledge of resistance evolution. We aimed to holistically investigate the role of sub-lethal antibiotic concentrations in the evolution of antibiotic resistance in the context of different environment structures and elucidate underlying molecular mechanisms.

We exposed E. coli to various sub-lethal ciprofloxacin concentrations in either swim agar or well mixed liquid culture environments over 3 days, monitoring resistance development with minimum inhibitory concentration assays. We combined single-cell microfluidics with time-lapse microscopy and whole genome sequencing to find both sub-lethal antibiotic concentrations, and environment structure, have significant impact in the evolution of antibiotic resistance mechanisms. Well mixed cultures showed significant mutations in genes associated with transcriptional repression and DNA gyrase, key controllers of resistance. Swim agar cultured mutants also showed mutations in transcriptional repression along with genes involved in regulating efflux pumps. We then link these mutations to phenotypic heterogeneity in cell growth with different resistant mutants having different growth dynamics during and after a fresh antibiotic challenge.

These results highlight for the first time the importance that different growth environments have in driving the evolution of resistance. We show the different resistance mechanisms that evolve under different antibiotic concentrations and environment structures, both of which are vital if we are to identify the drivers of this microbial evolution to tackle and overcome antibiotic resistance.

13

Assessing the impact of fever temperatures on bacteria-phage interactions

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Abstract

In an era of increasing antibiotic resistance, a promising alternative is the application of bacteriophages, viruses that target and kill bacterial pathogens. Phages collected from the environment and then used therapeutically in humans and livestock will experience broad temperature ranges, including from infection-induced fevers. Whilst it has been established that phage have a narrower thermal range than their bacterial hosts, the extent to which phage treatment efficacy is maintained, promoted, or restricted across body temperatures (and phage types) is unclear. My PhD thesis will investigate the impact of temperature variation on phage infectivity and bacterial resistance to phage across ecological and evolutionary time. We will use the opportunistic pathogen *Pseudomonas aeruginosa* and a diversity of lytic phages across a range of typical mammalian body temperatures, including low and high-grade fevers. We will determine whether some phage types are better than others during fevers and will additionally use experimental evolution to 'train' phages to improve their performance across temperatures. This work will improve our understanding of how phage activity varies with infection-induced fevers while informing the development and application of phages to treat bacterial infections in both medicine and agriculture.

14

Mutators drive evolution of multi-resistance to antibiotics

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Abstract

Combination drug treatments are an approach used to counter the evolution of resistance--the guiding principle being that they can prevent multiple independent resistance mutations from arising sequentially in the same genome. Here, we show that bacterial populations with 'mutators', organisms defective in DNA repair, can evolve multi-resistance under conditions where purely wild-type populations cannot. We exposed experimental populations of *Escherichia coli* to rising concentrations of single-drug and combination antibiotic treatments. Introducing mutators at low-to-intermediate frequencies permitted the evolution of multi-resistance. Using whole-genome sequencing, we detected a remarkable amount of genomic diversity in resistance-determining mutations, multi-drug efflux pumps, and mutation-rate altering genes. Notably, the evolution of multi-resistance did not require direct selection for both resistance mutations, as the elevated mutation rates allowed it to evolve under single-drug and combination treatments alike. Using stochastic eco-evolutionary simulations, we show that the mutator allele was sufficient to explain multi-resistance evolution through acquiring independent resistance mechanisms in the canonical target of each drug. Multi-resistance arose in both single- and multi-drug treatments. This emerged because the mutator allele swept to fixation through hitch-hiking with single-drug resistance, which subsequently allowed additional resistance mutations to arise sequentially. Ultimately, our results suggest that the utility of combination therapy may be limited when mutators are present, and selection for multi-resistance may have unintended consequences for future antibiotic therapies.

15

Large-scale evolutionary flexibility around the replication terminus of *Pseudomonas fluorescens* SBW25 chromosome: An experimental study

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Abstract

Bacterial genomes are typically organized according to gene utility; essential genes tend to occur near the origin of replication, while niche-specific genes cluster around the replication terminus. This organization is presumed to enhance evolvability, and hence survival in changing environments. A prominent example is provided by pseudomonads, a diverse bacterial taxon in which the evolutionary flexibility of the terminus region has been inferred by comparing the genome sequences of different strains. We present direct observations of the proposed flexible region undergoing spontaneous, large-scale rearrangements – including duplication and deletion events – across various laboratory evolution experiments with *Pseudomonas fluorescens* SBW25. Here we focus on a mutant isolated from an SBW25 culture subjected to six months of nutrient limitation. Whole genome resequencing revealed, among other mutations, a deletion of 213,799 bp encompassing 173 genes around the replication terminus (Δbig). Despite its large size, reconstructing Δbig in the ancestor led to no detectable effect on growth or fitness in a range of fresh, agitated media. However, in static cultures, Δbig reduced the fitness of SBW25. Δbig also exhibited enhanced survival under highly acidic conditions. Overall, we report a spontaneous deletion of a section of the computationally defined variable region in a *Pseudomonas* genome with detectable fitness effects in a complex, structured environment. These results support the preferential arrangement of most niche-specific, accessory genes around the replication terminus, and the resulting flexibility of this region during adaptation to novel niches.

16

Species interactions constrain adaptation and preserve ecological stability in an experimental microbial community

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Abstract

Species loss within a microbial community can increase resource availability and spur adaptive evolution. Environmental shifts that cause species loss or fluctuations in community composition are expected to become more common, so it is important to understand the evolutionary forces that shape the stability and function of the emergent community. Here we study experimental cultures of a simple, ecologically stable community of Saccharomyces cerevisiae and Lactobacillus plantarum, in order to understand how the presence or absence of a species impacts coexistence over evolutionary timescales. We found that evolution in coculture led to drastically altered evolutionary outcomes for L. plantarum, but not S. cerevisiae. Both monoculture- and co-culture-evolved L. plantarum evolved dozens of mutations over 925 generations of evolution, but only L. plantarum that had evolved in isolation from S. cerevisiae lost the capacity to coexist with S. cerevisiae. We find that the evolutionary loss of ecological stability corresponds with fitness differences between monoculture-evolved L. plantarum and S. cerevisiae and genetic changes that repeatedly evolve across the replicate populations of L. plantarum. This work shows how coevolution within a community can prevent destabilising evolution in individual species, thereby preserving ecological diversity and stability, despite rapid adaptation.

17

Does plasmid transfer stabilize cooperation in bacteria?

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Abstract

Bacteria are highly social. Much of this sociality occurs through the production of cooperative 'public goods'. It has been suggested that horizontal gene transfer, particularly via plasmids, could stabilize cooperation in bacteria. Transfer of a cooperative gene could turn non-cooperative 'cheats' into cooperators, preventing cheats from invading and destabilizing cooperation. We tested this with a comparative genomics analysis across 51 diverse bacterial species. In contrast to the predictions of the hypothesis, we found that genes for extracellular proteins, which are likely to act as cooperative 'public goods', were not more likely to be carried on either: (1) plasmids compared to chromosomes; or (2) plasmids that transfer at higher rates. Our results were supported by theoretical modelling which showed that, while horizontal gene transfer can help cooperative genes initially invade a population, it has less influence on the longer-term maintenance of cooperation. Instead, we found that genes for extracellular proteins were more likely to be on plasmids when they coded for pathogenic virulence traits, in pathogenic bacteria with a broad host-range.

18

Maintenance of a conjugative plasmid in a focal species is cost-dependent and determined by community context

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Abstract

As promiscuous mobile genetic elements, plasmids are key to exchanging genetic material between species and shaping bacterial pangenomes. Often plasmids disseminate antimicrobial resistance genes and virulence factors, and it is therefore critical to predict and reduce their spread within microbial communities. The cost of plasmid carriage is a key metric which can be used to predict their ecological fate.

Here, we investigate cost and maintenance of IncP1 conjugative plasmid pKJK5 to focal strain *Variovorax* sp. The presence of certain growth partners increased plasmid cost to this host, and accordingly embedding *Variovorax* in a community context led to rapid loss of pKJK5 compared with culturing this strain in monoculture.

We generalized these findings by tracking maintenance of pKJK5 in all constituents of a synthetic 5-species community and found community-dependent plasmid loss in a second species, as well as loss of a second plasmid lacking payload genes.

We propose that the destabilizing effect of interspecific competition on plasmid maintenance may have an impact on plasmid abundance in clinical and natural environments, and that this effect may be leveraged to cure plasmids from focal strains by addition of growth partners.

19

Directed Evolution of Microbes on Plastic Polymers

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Abstract

Plastic pollution is a global environmental issue causing great threat to the Earth's ecosystem. Prolonged decomposition times have meant plastics have polluted the environment, with 5-13 metric tonnes estimated to enter the ocean annually, with no significant observation of environmental biodegradation. A multiphasic approach will be required to tackle global plastic pollution, but one promising research area involves the exploitation of enzymes from naturally occurring plastic-degrading microorganisms. This requires the discovery, characterisation, and *in vitro* engineering of novel plastic-degrading enzymes to confer an enhanced ability suitable for industrial use. An example of this, and the subject of this research, is the bacterium *Ideonella sakaiensis*, which is able to degrade polyethylene terephthalate (PET) using its enzyme PETase. *I. sakaiensis* was used within a directed evolution experiment on amorphous PET film as a sole carbon source. Over a time course of 100 days; culture based, HPLC, and SEM analysis tests were conducted. Changes in the genome were monitored using DNA extraction and Nanopore sequencing at four timepoints. Genomes from each time point were assembled and compared to the reference genome for any variations that may confer improved ability for plastic degradation. The aim is to elucidate how these microorganisms have evolved enhanced degradation pathways in their genome. This in turn, may inform the engineering of enzymes *in vitro*, or the discovery of further plastic-degrading enzymes and microorganisms. Future goals include applying this approach to other pollution sources, investigating novel pathways through culturing, screening, whole-genome sequencing, and gene expression through RNA sequencing.

20

The influence of temperate phages on the spread of conjugative plasmids – friend or foe?

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Abstract

Bacteriophages, the viruses of bacteria, are omnipresent in nature and shape microbial evolution, for example by interfering with the transfer of other mobile genetic elements like conjugative plasmids. Yet, studies on plasmid conjugation generally neglect the presence of phages. These interactions are likely to be particularly complex for temperate phages, which can choose between killing the bacterial host and integrating their DNA into the host genome, thereby protecting the bacterial cell from further phage infection. We studied the influence of temperate phages on the dynamics of conjugative plasmids by combining experiments and mathematical modelling.

We found that phage λ could substantially limit the spread of the multi-drug resistant plasmid RP4 through an *Escherichia coli* population. This inhibitory effect was strongly dependent on environmental conditions and bacterial genetic background. Our empirically parameterized model suggests that there are complex interactions between plasmids and phages in sequential infections, which dictate the numbers of cells acquiring the plasmid and the phage. Further, varying phage and plasmid infection parameters over empirically realistic ranges revealed that plasmids can overcome the negative phage impact through high conjugation rates. Overall, temperate phages introduce an additional death rate for plasmid carriers, the magnitude of which is determined in non-trivial ways by the environment, the phage and the plasmid.

21

The influence of plant hosts and genetic symbionts on rhizobial population structure

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Abstract

Rhizobia are symbionts of legume plants, exchanging nitrogen for carbon within a specialist intracellular symbiosis. Despite the intimacy of this interaction the genes which underlie this function are exclusively carried on mobile genetic elements. Rhizobia populations typically carry multiple variants of these symbiosis genes encoding interactions with different plant hosts. For example, in the species Rhizobium leguminosarum different symbiosis plasmids facilitate interactions with either clovers or vetches, plants which exist in sympatry. Mixed legume communities therefore create a heterogeneous landscape with areas of local adaptation/maladaptation acting on the plasmid replicon, but not necessarily on the bacterial chromosome. In this study I investigate the role of plant host imposed selection on the structure of rhizobial populations across the multipartite genome. Natural rhizobial populations were sampled across a distance gradient from both the vetch and clover rhizosphere. Using whole genome sequencing I show that the population structure of symbiosis genes and their plasmids but not the chromosome is driven by plant host. Thus horizontal gene transfer allows diverse rhizobial communities to persist despite heavy selection acting on functional traits.

22

Conditional evolution of biocide tolerance in nosocomial pathogens

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Abstract

Biocides are widely used to control and prevent healthcare-associated infections. The emergence of bacterial resistance to biocides and cross-resistance to antibiotics is a major concern. Understanding how nosocomial pathogens respond to biocidal agents is key to improve infection prevention and control.

A biofilm evolution model was used to study the evolutionary changes that occur in *Staphylococcus aureus* and *Enterococcus faecalis* with biocide exposure. Biofilm and planktonic lineages were exposed to sub-lethal concentrations of Chlorhexidine digluconate (CHG) and Octenidine dihydrochloride (OCT) in parallel for ≈250 generations.

Both pathogens were able to adapt significantly above the MIC of both biocides with planktonic lineages able to survive higher concentrations of CHG and OCT before growth was inhibited. Lower biofilm formation was seen in *E. faecalis* mutants after being stressed with CHG than with OCT. Similarly, for *S. aureus*, CHX exposure was consistently linked with low biofilm biomass. Evolved isolates had no major fitness deficit and low level changes to susceptibility to various other antimicrobials were observed after biocide exposure.

Sequencing of mutants identified various mutations including in genes associated with phospholipid synthesis. SNPs in *S. aureus* fatty acid kinase (fakA), a negative regulator of biofilm formation, were repeatedly isolated from independent lineages strongly suggesting a role in biocide tolerance.

This data shows important pathogens can adapt tolerance to two common biocides but that this has collateral impacts on biofilm formation, colony morphology and fitness. Genotyping revealed changes to phospholipid synthesis, which is consistent with the mechanism of both biocides.

23

Evolution of Translation Termination and Programmed Ribosomal Frameshifting Sequence in *Pseudomonas fluorescens* SBW25

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Abstract

Translation termination in bacteria requires the recognition of stop codons by release factors PrfA and PrfB. Most genes have a single stop codon, located at the 3' terminus, but the *prfB* gene has an additional, intergenic stop codon. This stop codon works as a negative regulator of PrfB expression; high PrfB levels lead to self-recognition of the intergenic STOP codon (and halting of PrfB expression), while low PrfB levels lead to a programmed ribosomal frameshift (PRF) (and resuming of PrfB expression). Our goal is to study the role of the PRF sequence in the regulation of PrfB expression. To determine the role of the PRF sequence in PrfB expression, we intend to mutate the PRF sequence by engineering, in the model bacterium *Pseudomonas fluorescens* SBW25. These manipulations are expected to result in *prfB* genes that encode full, functional PrfB proteins, but with altered PRF regulation. We expect that these changes will causes defects in global translation, and hence lead to loss of fitness (as compared with the wildtype). Next, we plan to perform experimental evolution to observe if, and how, loss of PRF can be compensated. We intend to characterize our evolved mutants to determine the effects of evolution on fitness (competition experiments), PrfB expression (*e.g.* with translational fusions), and general translation (*e.g.* with RNA-seq). Overall, we expect our work to provide insight into the evolutionary flexibility of PRF in *prfB*, and the downstream effects on translation termination.

24

Some like it hot (or not); Ancestral thermal niche limits bacterial adaptation to new growth media

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Abstract

Bacterial species are difficult to define; nevertheless, we often see bacterial genotypes cluster into phylogenetic "clades" with distinct ecologies. In some Gram-positive groups, such as *Bacillus* and *Streptomyces*, clades are distinguished by thermal niche. Determining why thermal adaptation is associated with clade may improve our understanding of how clades remain coherent. We conducted a selection experiment to test whether thermal niche constrains evolvability in *Bacillus* species. Since thermal niche is associated with few allelic differences and one cold shock protein gene, we hypothesized that thermal niche is plastic and predicted that *Bacillus* species would show greater fitness increases under a novel temperature regime. We evolved replicated lineages descended from a mesophilic or psychrotolerant *Bacillus cereus* species under "mesophilic" and "psychrotolerant" temperature regimes for over 700 generations. We determined fitness changes in evolved lineages using competition assays and conducted whole-genome sequencing to identify genetic variants associated with different treatments. Contrary to predictions, we found that *Bacillus* lineages did not show consistent fitness increases in novel temperature regimes, but showed large fitness increases under ancestral temperature conditions. Genomic analysis suggested that all treatments experienced similar levels of genetic change, so variation in mutation supply was not responsible for differences in fitness gain. Thermal niche may be a complex polygenic trait; this means that thermal phenotype may constrain adaptation to new environmental conditions and would explain its association with speciation in the *Bacillus cereus* group. Gene knockout/knock-in experiments will determine whether acquisition of a definitive cold shock protein can overcome thermal niche constraint.

25

Density-associated mutation rate plasticity in individual *Escherichia coli* cells

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Abstract

De novo mutations are the basis of evolutionary innovation. They are also fundamental to one of the greatest emerging medical problems, antimicrobial resistance (AMR). Deepening our understanding of conditions that favour emergence of AMR will enable us to develop sustainable strategies against AMR in clinically relevant pathogens.

Using bulk culture assays, we discovered that microbial populations at lower cell-densities have up to 23-fold higher mutation rates. This density-associated mutation-rate-plasticity (DAMP), where "lonely" cells mutate faster, is a genome-wide process present in all domains of life (PLoS Biol.,2017,e2002731). In *Escherichia coli* DAMP depends on the quorum-sensing gene *luxS* and on a mutation-avoidance gene *mutT* (MutT removes oxidised nucleotides). When E. coli grows anaerobically, or lack all four genes encoding hydrogen-peroxide-removing enzymes (*katG*, *katE*, and *ahpCF*), DAMP is absent.

To understand how the dynamic interplay between internal cellular processes and external micro-environments drives DAMP, we quantify DNA mismatches in single cells growing in a community. Combining photoactivated localisation microscopy with total-internal-reflection fluorescence and microfluidics enables us to track single-molecules of proteins involved in repairing and avoiding mismatches. This allows us to visualise mismatches and determine how their distribution among cells depend on micro-environments generated by abiotic factors and neighbouring cells.

Studying mutations both in bulk cultures and individual cells is crucial for the fundamental understanding of spontaneous de novo mutations, including those that confer AMR, which will render the evolutionary process more predictable and aid developing more sustainable AMR-mitigation strategies.

26

Chance and Necessity: Evolution guided antibiotic improvement and discovery

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Abstract

Antimicrobial resistance is a great threat to global human health and we need to develop novel approaches to discover new and enhance production of existing antibiotics. Two-thirds of our antimicrobial drugs are specialised metabolites produced by the genus *Streptomyces*, and the traditional process for the development of industrial antibiotic production has been through iterative random mutagenesis followed by the selection of strains with improved production characteristics – this process is undirected and time consuming. The mutations within these strains that drive increased industrial performance are poorly understood. To address this, we have been using a dual approach to understand evolution of antibiotic production in *Streptomyces* – a long term evolution experiments of adaptation in *Streptomyces* and a study of an authentic, industrially improved lineage (forced evolution; FE) of *Streptomyces*. These studies have shown how that adaptation in the LTEE and in FE results in streamlining of primary metabolism and the degradation of competing specialised metabolic pathways. Reconditioning of strains with collaterally damaged primary metabolic pathways enabled increased antibiotic titres to be achieved in some industrial strains. Growth of strains in conditions that were not permissive of development resulted in rapid loss of sporulation, with extensive negative epistasis in antibiotic production. Genomics and transcriptomics of evolved strains revealed extensive chromosomal plasticity and transcriptional re-wiring. Understanding the wider role of genetic interactions and how strains may have adapted in culture will enable the process of strain improvement to be accelerated in the future through informed strain engineering.

27

Conflict in the pangenome: characterizing the molecular basis of plasmid fitness costs and compensation across divergent species of bacteria

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Abstract

Horizontal gene transfer is an essential component of bacterial evolution and ecology, facilitating the spread of traits including AMR. However, incoming genes can introduce genomic conflict. The basis of such conflict can lie in specific gene-gene interactions, but the mechanistic basis, and whether such conflicts are host specific, is not clear.

Several naturally-occurring mercury resistance 'pQBR' plasmids impose significant costs to *Pseudomonas fluorescens* SBW25, and previous work indicates the principal source is hypothetical DUF262 domain-containing chromosomal protein PFLU4242. PFLU4242-pQBR interactions are therefore an exemplary model of pangenome conflict, but PFLU4242's function is unknown, as is whether pQBR-PFLU4242 conflict is genetic background specific.

We analysed PFLU4242 homologue distribution across diverse species, and show it is part of the accessory genome distributed via HGT, but does not appear closely associated with known mobile genetic elements. Given apparent genomic co-localisation of PFLU4242-like proteins with known 'defence island' elements, we hypothesise that PFLU4242-like proteins are a genome defence mechanism.

To understand PFLU4242's physiological activity and ecological function, our current work explores whether diverse DUF262 homologues perform a similar function by expressing variants from diverse species in *P. fluorescens* SBW25ΔPFLU4242. By expressing PFLU4242 and a naturally-arising inactive mutant in other species, we also ask whether PFLU4242-pQBR conflict is specific to *P. fluorescens* SBW25, or might operate across the *Pseudomonas* pangenome.

Our data provides better understanding of plasmid/host dynamics and explores the poorly understood trade-off between openness to HGT and genome defence: a key mechanism determining genome content, adaptive capacity, and pangenome structure.

28

Comparative analysis of Myxococcus and Streptomyces genomes and predatory activities

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Abstract

Actinobacteria and Myxobacteria are soil-dwelling organisms. Inspite the fact that Actinobacteria and Myxobacteria belong to Gram-positives and Gram-negative , both groups are similar in their huge genome sizes expanding more than 10Kb and a high GC content and their ability to differentiate with cell cycles including the formation of spores. In this paper, we conducted a comparative genomic analysis and predatory profiles of novel Streptomyces with Myxobacteria, with the objective to further categorise their antibiotic profiles.

Soil dwelling bacteria were isolated from woodland regions using standard microbiology techniques. 16S sequencing confirmed the isolation of a novel Streptomyces sp. with identity closest to 95.64%. The whole genome sequences were obtained and annotated using a prokaryotic genome annotation pipeline called Prokka. Pan-genomic analysis shows that with a reduction in the BlastP cut-off value from 90 to 60, there is an increase in the number of core genomes, as new genomes are added, thereby suggesting an increase in the openness of the pan-genome. However there is a difference in the openness of the pan-genome of Myxobacteria isolates. At minimum BlastP percentage identity of 90%, there are more than 2000 core genomes and increase as more gnomes are added, suggesting the openness of the pan-genome and a difference in comparison to the Actinobacteria groups. The predatory profiles of Actinobacteria when compared to Myxococcus xanthus also demonstrates a marked difference in predation over plant pathogens including Pantoea agglomerans, Xanthomonas campestris, Pectobacterium atrosepticum, Rhizobium radiobacter , Pseudomonas syringae and E.coli

29

Bacterial-fungal co-infections and their role in the evolution of antimicrobial resistance

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Abstract

Microorganisms routinely exist in multispecies communities that exchange metabolites, sense each other's presence, and exchange genetic material, including resistance genes. Clinical data show that most patients are simultaneously infected by multiple species. Despite growing evidence that infections that involve multispecies communities are i) more difficult to treat and ii) a source of antimicrobial resistance, microbial pathogens have so far typically been studied in isolation. Hence, we investigated the interactions within a clinically relevant co-infection community, consisting of a multi-drug resistant bacterial (Pseudomonas aeruginosa) and an opportunistic fungal (Candida albicans) pathogen. Pseudomonas aeruginosa (PA) is frequently co-isolated with Candida albicans (CA) and increased antibiotic tolerance of PA has been observed in PA-CA multispecies biofilms. In addition, both pathogens are known to occupy multiple infection niches in the human body, including the lungs and urinary tract.

Here, we have established a co-infection community model and probed how the proteomic make-up of PA and CA shifts in multispecies growth. This experiment revealed that expression of biochemical warfare, but also general metabolic processes are modulated in both species upon co-culturing. In addition, vast metabolic adaptations become apparent when culturing these pathogens in different metabolite environments that mimic lung and urinary tract infection sites. We further show that these changes shape the pathogens' response to various antimicrobial agents. Different metabolite environments exhibit strong selective pressures and predetermine evolutionary dynamics, leading to varying degrees of effectiveness of antimicrobial agents. Ultimately, our findings show that multispecies interactions and metabolite dynamics are key contributors to antimicrobial treatment evasion.

30

Evolutionary innovation through transcription factor promiscuity in microbes is constrained by pre-existing gene regulatory network architecture

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Abstract

The survival of a population during environmental shifts depends on whether the rate of phenotypic adaptation keeps up with the rate of changing conditions. A common way to achieve this is via change to gene regulatory network (GRN) connections conferring novel interactions on transcription factors. To understand the success of rapidly adapting organisms, we therefore need to determine the rules that create and constrain opportunities for GRN innovation. Here, using an experimental microbial model system we construct a maladapted GRN, through deletion of a master transcription factor, and challenge evolution to fix this network. We identify three key properties - high activation, high expression, and pre-existing low-level affinity for novel target genes – that facilitate transcription factor innovation via gain of functional promiscuity. Ease of acquiring these properties is constrained by pre-existing GRN architecture, which was overcome in our experimental system by both targeted and global network alterations. This work reveals the key properties that determine transcription factor evolvability, and as such, the evolution of GRNs.

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Chloramphenicol reduces evolution of resistance to phage ΦX174 in *Escherichia coli* C

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Abstract

Bacteriophages infect bacteria by attaching to molecules at the bacterial outer membrane, often lipopolysaccharides (LPS). Bacteria can become phage-resistant by acquiring mutations that modify the LPS and prevent phage adsorption. Such LPS modifications can also affect antibiotic resistance. Here, we investigate how phage resistance influences antibiotic susceptibility using 33 *Escherichia coli* C mutants resistant to ΦX174, an LPS-targeting phage. These mutants have many different predicted LPS structures which can be categorised into: “rough” and “deep-rough”. Here we show that chloramphenicol completely inhibits growth of deep rough mutants at a chloramphenicol concentration where the wildtype grows uninhibitedly. In gentamicin however, mutants grow largely unaffected by LPS differences. Thus, we predicted that adding sub-inhibitory levels of chloramphenicol to phage treatment should not only reduce the emergence of resistant mutants, but also bias the emergent mutants against deep-rough LPS phenotypes, compared to applying phage alone. In contrast, sub-inhibitory levels of gentamicin should not affect either the emergence of resistance or the emergent LPS phenotypes. Our experimental results are largely in agreement with our predictions and hence show that LPS-targeting phages can be used in conjunction with certain antibiotics at sub-inhibitory levels to reduce the evolution of resistance or drive it in a preferred direction.

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How to Find a Mutational Hotspot that Facilitates Highly Predictable Evolution

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Abstract

Mutational biases are caused by molecular features across the genome that cause errors and introduce mistakes, making certain genomic positions more likely to mutate than others. In some cases, bias can operate at one specific genomic location with enough potency to form a mutational hotspot. When operating on an adaptive position, mutational hotspots can make evolution remarkedly predictable. We have identified one such hotspot in the soil bacterium *Pseudomonas fluorescens* SBW25, in which immotile variant populations adapt via an identical single nucleotide polymorphism (*ntrB* A289C) in ≥95% cases when recovering motility. In order to elucidate the genomic features that allow for such a potent mutational hotspot to form, we experimentally introduced a suite of mutation bias-affecting genomic augmentations and measured their effect on parallel evolution. We show that the hotspot is highly sensitive to local nucleotide sequence, with just six synonymous variations around the hotspot position breaking the hotspot in SBW25 (≥95% to 0%) and building it in a homologous but natively hotspot-lacking strain, Pf0-2x (0% to 80%). We then demonstrate that the hotspot is similarly sensitive to its broader genomic context, as translocating the hotspot closer to the replication origin reduces its potency and inverting the hotspot sequence onto the leading strand removes the bias entirely. Finally, we show that mutator variants reduce hotspot potency not by reducing its mutation rate, but by raising the rates of rival adaptive variants. Together, these findings provide a framework for utilizing genomic features to identify hotspot positions capable of enforcing near-deterministic evolution.

33

Maintenance of plasmid-mediated heterozygosity contributes to the evolution of multiple drug resistance

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Abstract

Bacterial plasmids and other extra-chromosomal DNA elements may carry genes that affect the fitness of their hosts, in particular antibiotic resistance genes. Because plasmids often exist in multiple copies, different plasmid copies can carry distinct alleles, allowing for heterozygosity not possible for loci on the typically-haploid bacterial chromosome. Plasmid-mediated heterozygosity can increase the fitness of host bacteria in scenarios of heterozygote advantage, including the evolution of multidrug resistance, a serious problem in the clinical context. However, plasmid-mediated heterozygosity is also subject to constant loss due to random segregation of plasmids on cell division. We present mathematical models of the establishment in a bacterial population of a novel allele located on a plasmid which has a fitness advantage when present together with plasmids carrying the wild-type allele. We derive the minimum threshold on the selective advantage of heterozygotes required to overcome segregative loss and make establishment possible; this threshold decreases with increasing copy number of the plasmid. We further show that the possibility of the fusion of plasmids into multimers increases the probability of successful establishment, as multiple alleles on plasmid multimers are less subject to stochastic loss. These results contribute to understanding the evolution of bacterial populations in complex selective environments.

34

Unique nature: How does genetic diversity affect Eco-evolutionary dynamics?

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Abstract

It is clear, that ecological and evolutionary processes occur on similar time scales and interact with one another. However, we do not fully understand how eco-evolutionary dynamics operate in ecological communities (e.g. in the presence of interspecific and intraspecific diversity). For example, species diversity may suppress within-species adaptive evolution (if niche space is occupied by another species) or enhance evolutionary diversification (diversity begets diversity). Here, we set out to understand how eco-evolutionary dynamics in experimental microbial ecosystems differ depending on the genetic diversity included at the onset of the experiment. We are isolating clones of five focal bacterial species maintained alongside 18 other bacterial species in a 70-week-long community selection experiment under antibiotic and predation stress. Then we will phenotype clones from each focal species for predator defence and antibiotic resistance at 210 and 406 days. Measuring the traits at intraspecific resolution for these species will provide a granular perspective on the evolution of trait diversity in these communities. In a second step, we will use isolated clones to construct communities with different clonal and trait diversity. Precisely, clonal diversity of 3, 6 or 12 clones at the start will be fully crossed with narrow, wide, or full trait space occupation. Our prediction is that maximizing clonal diversity will only influence eco-evolutionary dynamics and increase the resilience of a community to environmental change at a high level of trait diversity.

35

Methodological inaccuracy inflates the size of the accessory genes in *Mycobacterium tuberculosis*

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Abstract

Critical evaluation of accessory gene content is necessary to understand the evolution of pathogenic microorganisms such as *Mycobacterium tuberculosis* (Mtb). In this study, we investigated the effect of genome annotation and different parameters of pangenome construction on the resulting accessory gene content of Mtb Lineage 1.

Forty-four complete genomes sequenced with the PacBio or Nanopore sequencing were analysed in this study. The quality of assemblies was assessed using BUSCO, and assemblies were annotated using Bakta and PGAP. Panaroo was used to construct the pangenome using the strict, moderate and sensitive cleaning modes as well as remove paralogs option.

We found that the number of accessory genes varied from 61 to 147 with PGAP and Bakta outputs respectively and annotation tool had a significant impact on the resulting accessory genome size ($p < 000.1$). Hypothetical proteins represented most of the accessory genome content, and only 32 genes with known functions were common between the two sets. Cleaning modes did not affect the number of the accessory genes, possibly due to the high quality of assemblies and lower annotation errors. Merging paralogs removed PE/PPE gene families, which are important in pathogenicity and host interaction of Mtb, and further reduced the accessory genes to 16 and 90 (PGAP and Bakta respectively). The small number of accessory genes from PGAP is consistent with the clonal evolution and a closed pangenome of Mtb, and therefore PGAP may represent a better annotation tool for Mtb pangenome studies.

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Repeated alcohol exposure selects for alcohol-tolerant *Staphylococcus aureus* and drives the development of VISA

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Abstract

Alcohol is a common germicide used both by healthcare facilities to disinfect instruments and surfaces and by the broader community for hand hygiene. Alcohol is effective at killing 99.99% of bacteria – however, little is known about the 0.01% of bacteria that survive the treatment. To study the effect of repeated alcohol exposure on bacteria, we conducted an evolution experiment using the opportunistic pathogen *Staphylococcus aureus*. We found that *S. aureus* becomes 10 to 1,000 times more tolerant to alcohol within three weeks of repeated exposure. Whole genome sequencing revealed that alcohol tolerance develops due to mutations in genes involved in cell wall modeling. Many of the mutated genes were previously shown to increase the thickness of the bacterial cell wall, a trait that has been associated with the development of vancomycin-intermediate *S. aureus* (VISA). We have confirmed that the alcohol-tolerant mutants exhibit reduced susceptibility to vancomycin. We suspect that repeated exposure to alcohol-based disinfectants and antiseptics may contribute to the development of VISA and failed antimicrobial therapy.

37

Localized coevolution between microbial predator and prey alters community-wide gene expression and ecosystem function

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Abstract

Closely interacting microbial species pairs (e.g., predator and prey) can become coadapted via reciprocal natural selection. A fundamental challenge in evolutionary ecology is to untangle how coevolution in small species groups affects and is affected by biotic interactions in diverse communities. We conducted an experiment with a synthetic 30-species bacterial community where we experimentally manipulated the coevolutionary history of a ciliate predator and one bacterial prey species from the community. Altering the coevolutionary history of the focal prey species had little effect on community structure or carrying capacity in the presence or absence of the coevolved predator. However, community metabolic potential (represented by per-cell ATP concentration) was significantly higher in the presence of both the coevolved focal predator and prey. This ecosystem-level response was mirrored by community-wide transcriptional shifts that resulted in the differential regulation of nutrient acquisition and surface colonization pathways across multiple bacterial species. Our findings show that the disruption of localized coevolution between species pairs can reverberate through community-wide transcriptional networks even while community composition remains largely unchanged. We propose that these altered expression patterns may signal forthcoming evolutionary and ecological change.

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The effect of copper on bacterial virulence

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Abstract

Anthropogenic metal pollution is known to significantly impact the composition and function of microbial populations. However, the effect of metals on bacterial virulence remains largely undetermined. Here, we experimentally test whether metal stress can alter the virulence of bacterial communities. We first do this in populations of the opportunistic pathogen *Pseudomonas aeruginosa*, by incubating them with environmentally relevant copper concentrations. Secondly, we exposed three wastewater influent communities to the same copper concentrations to test their effect at the community level. We then quantified virulence using the *Galleria mellonella* insect infection model. In *P. aeruginosa* populations, we found copper to select for increased production of pyoverdine – a siderophore associated with both metal detoxification and virulence. Consequently, virulence was positively associated with copper concentration, such that populations incubated with high copper were the most virulent, and those incubated with low copper showed intermediate virulence. However, at the community level, we found high copper to reduce the virulence of the three communities and low copper to have little effect. This was because high copper greatly altered community composition, favouring slower growing – and hence less virulent – taxa. These results demonstrate that copper pollution can have significant implications for bacterial virulence, through both selection for detoxification mechanisms and species sorting.

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No Time to Die: Rapid Conversion to Resistance in Vancomycin-variable *Enterococcus faecium*

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Abstract

Enterococcus faecium is a commensal of the human gastrointestinal tract but also an important opportunistic pathogen. Resistance to the glycopeptide antibiotic vancomycin is increasingly common in this species. A subset of genotypically resistant *E. faecium* strains are phenotypically susceptible leading to their designation as vancomycin-variable enterococci (VVE). In some cases, these VVE can convert to a resistant phenotype when exposed to low concentrations of vancomycin. Here we set out to identify novel mechanisms responsible for the conversion of VVE isolates to a resistant phenotype. Vancomycin-variable *E. faecium* strains were exposed to 8 µg/ml vancomycin to select for isolates which had converted to a resistant phenotype. The genomes of wildtype VVE and evolved resistant isolates were then sequenced using short and long-read technology to identify mutations and rearrangements which could convert the previously susceptible isolates to a resistant phenotype. A VVE strain was found to have a presumptive loss-of-function mutation in the *vanR* gene abolishing expression of the rest of the vancomycin resistance operon. In the resistant isolate the plasmid carrying the vancomycin resistance genes had integrated into the chromosome such that the *vanHAX* genes were located immediately downstream of a ribosomal RNA gene operon. PCR on cDNA identified a transcriptional fusion between the ribosomal genes and the vancomycin resistance genes. A target site duplication suggested that this insertion was mediated by an IS*1251* element. Our results suggest that clinical treatment of VVE isolates with vancomycin is likely to convert the isolate to a resistant phenotype, leading to treatment failure.

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Bows and swords: why bacteria carry short and long-range weapons

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Abstract

Bacteria carry a diverse arsenal of weaponry for fighting competitors, including antibiotics, bacteriocins, tailocins, contact-dependent inhibition and type VI secretion systems. Why bacteria have evolved such a wide array of weapon systems remains a mystery. Here we develop an agent-based model to compare short-range weapons that require cell-cell contact, with long-range weapons that rely on diffusion. Our models predict that contact weapons are useful when an attacking strain is outnumbered, facilitating invasion and establishment. By contrast, ranged weapons tend to only be effective when attackers are abundant. We test our predictions with the opportunistic pathogen Pseudomonas aeruginosa, which naturally carries multiple weapons, including contact-dependent inhibition (CDI) and diffusing tailocins. As predicted, CDI functions better at low frequency, while tailocins require high frequency or cell density. Head-to-head competitions between the two weapon types further support our predictions: a tailocin attacker only defeats CDI when it is numerically dominant, but then we find can be devastating. Finally, we show that the two weapons work well together when one strain employs both simultaneously. We conclude that short and long-range weapons serve different functions, enabling bacteria to fight effectively both as individuals and as a group against their competitors.

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Coevolutionary analysis of bacteria-phage interactions identifies potential receptor targets for phage infection

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Abstract

Bacterial canker, caused by *Pseudomonas syringae* pathovars including pv. *syringae* (*Pss*) and pv. *morsprunorum* race 1 (*Psm*1) and race 2 (*Psm*2), is a major disease of Prunus species such as cherry (*Prunus avium*). Concerns over the environmental impact of, and developing resistance to, copper controls call for alternative approaches to disease management. One method of control could be achieved using naturally occurring bacteriophage (phage) infective to the bacterial pathogens. We have isolated and characterised phages (MR) with host specificity to *Pss*, *Psm*1 and *Psm*2. However, before being used for field applications as a biocontrol agent, it is important to assess their efficacy as well as changes occurring in the bacterial population. The growth of *Pss* populations co-inoculated with MR phage individually or in combination were measured and showed that phages are likely antagonising one another thus reducing the efficacy of phage control of bacterial populations. To understand this interaction further, Pss and phage were coevolved over 10 generations and the genomic and behavioural changes in bacterial populations were measured. The motility, biofilm formation and growth of coevolved Pss was not affected by the coevolution. *Pss* evolved mechanisms of resistance to phages through modifications to lipopolysaccharide (LPS) or mutations in a glycosyltransferase involved in LPS synthesis. However, the latest generations of coevolved phages were more potent at reducing the coevolved bacterial population, even faster and more efficiently than the earliest generations. Therefore, understanding the genetic mechanisms of coevolution in generating more infective phages for precise targeting of the bacterial population is essential.

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The PA7-like strains: A protruding cluster in *Pseudomonas aeruginosa*

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Abstract

PA7-like strains are a group of bacteria that are outliers within the *Pseudomonas aeruginosa* species. Sequences of 20 PA7-like strains in addition to 2,447 other *P. aeruginosa* strains and 30 Pseudomonas spp. closely related to *P. aeruginosa* were curated and used to create alignments based on the nucleotide sequences of 16 conserved ribosomal proteins and the *P. aeruginosa* core genome. FastTree was used to generate phylogenetic trees based on each alignment with clusters identified by FastBaps. Similarities between genome sequences were compared using the 16S rRNA sequences, Digital DNA:DNA hybridisation (dDDH), average nucleotide identify (ANI), and hash sketching of DNA sequences.

The traditionally used 16S rRNA incorporates PA7-like strains within the *P. aeruginosa* species and BLAST analysis shows identity scores of 99-100% between the 16S rRNA sequences of PA7-like strains and the PAO1 *P. aeruginosa* type strain. Despite this, clustering based on the alignment of 16 ribosomal proteins from multiple *Pseudomonas spp*. shows the PA7-like strains are grouped separately from the main cluster of P. aeruginosa strains. These PA7-like strains had dDDH scores <70% and ANI scores <95% when compared with other members of the *P. aeruginosa* species and thus were below the threshold to be considered part of the same species.

Genotypic analysis of *P. aeruginosa* shows that whilst the 16S rRNA sequence of PA7-like strains is indistinguishable from the rest of the species, expanded analysis including the whole genome indicates that the PA7-like strains have diverged away from *P. aeruginosa* into their own cluster.

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Characterising the evolutionary relationship, domain organisation, and structural similarity of *Pseudomonas* RpoN-dependent enhancer-binding proteins

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Abstract

Success across a broad niche range relies on cellular processes interacting with the external environment, such as motility, virulence, biofilm formation, and nitrogen assimilation. *Pseudomonas*, a genus of gram-negative bacteria, has found such success, occupying a range of niches from human pathogen to growth-promoting diazotroph. Gene regulatory networks (GRNs) control gene expression allowing it to be fine-tuned in response to environmental conditions. This control facilitates the adaptation of a genus such as *Pseudomonas* to a wide range of habitats. In particular, sigma factor 54 and its associated network of RpoN-dependent enhancer-binding proteins (RpoN-EBPs) are critical to the transcription of genes involved in these external functions. In the Taylor lab, we have a tractable experimental system in *P. fluorescens*, where we explore the drivers of GRN rewiring and adaptation between RpoN-EBPs. In gene deletion experiments, functional redundancy is observed between RpoN-EBP family members. However, one of the obstacles in extrapolating experimental outcomes of loss-of-function mutations from one species to another is variation in RpoN-EBPs across *Pseudomonas* species. Here, we comprehensively characterise the evolutionary relationship, domain organisation, and structural similarity of RpoN-EBP members in *Pseudomonas*. Our results highlight the shared traits of this protein family and evaluate the extent of variation present. This work provides a foundation for future work on regulation, function, redundancy potential, and variation in GRN architecture between RpoN-EBPs in Pseudomonas species.

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Air pollution affects the gut microbiome of buff-tailed bumblebees (*Bombus terrestris*)

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Abstract

Bees play an essential role in global pollination and food security making the study of bee health and causes of population decline vitally important. Bees house a defined group of beneficial core gut bacteria, the balance of which is key to maintaining bee health. Bees treated with pesticides show differences in their gut microbiome and subsequent health declines. Our previous work was the first to show that a major air pollutant, black carbon (BC), changes the behaviour of human pathogenic bacteria. We hypothesise that exposure to BC influences bacterial behaviour in the bee gut microbiome, causing an imbalance in this microbial community which may adversely effect bee health.

Important native pollinator, the buff-tailed bumblebee (*Bombus terrestris*) was chosen for this work to provide data for this key UK species and to act as a model for bee species unable to be reared in controlled conditions. *B. terrestris* were reared in controlled laboratory conditions and treated with or without BC, their gut microbiome was sampled and 16S amplicon sequenced to determine the effect of BC on gut microbial composition and diversity. *B. terrestris* treated with BC showed changes to their gut microbiome including a significant increase in viable bacteria. This change to the microbial community demonstrates that exposure to BC has direct, measurable effects on the bee gut microbiome and could have knock-on effects to the host. This data supports the hypothesis, air pollution causes an imbalance in the bee gut microbiome and may adversely influence bee health and pollinator populations.

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Stable antibiotic resistance and rapid human adaptation in livestock-associated MRSA

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Abstract

Mobile genetic elements (MGEs) are agents of horizontal gene transfer in bacteria, but can also be vertically inherited by daughter cells. Establishing the dynamics that led to contemporary patterns of MGEs in bacterial genomes is central to predicting the emergence and evolution of novel and resistant pathogens. Methicillin-resistant *Staphylococcus aureus* (MRSA) clonal-complex (CC) 398 is the dominant MRSA in European livestock and a growing cause of human infections. Previous studies have identified three categories of MGEs whose presence or absence distinguishes livestock-associated from the human-associated CC398. Here, we characterise the evolutionary dynamics of these MGEs using a collection of 1180 CC398 genomes, sampled from livestock and humans, over 27 years. We find that the emergence of livestock-associated CC398 coincided with the acquisition of a Tn*916* transposon carrying a tetracycline resistance gene, which has been stably inherited for 57 years. This was followed by the acquisition of a type V SCC*mec* that carries methicillin, tetracycline, and heavy metal resistance genes, which has been maintained for 35 years. In contrast, a class of prophages that carry a human immune evasion gene cluster and that are largely absent from livestock-associated CC398 have been repeatedly gained and lost in human- and livestock-associated CC398. These contrasting dynamics mean that when livestock-associated MRSA is transmitted to humans, adaptation to human host outpaces loss of antibiotic resistance. In addition, the stable inheritance of resistance-associated MGEs suggests that the impact of ongoing reductions in antibiotic and zinc oxide use in farms on MRSA will be slow to be realised.

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In vitro evolution of *Klebsiella grimontii* to TZP resistance reveals identical and unique genomic changes between lineages compared to in-patient based evolution

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Abstract

Background: Experimental evolution of pathogenic bacteria to antimicrobial resistance (AMR) has the potential to rapidly inform antibiotic therapy. However, there is limited understanding of how *in vitro* AMR evolution can replicate evolution of AMR within the human host.

Methods: A trio of *Klebsiella grimontii* isolates cultured from a hospital in-patient with a recurrent bloodstream infection showed development of piperacillin-tazobactam (TZP) resistance over a four-month period due to a single SNP in the promoter of a chromosomal $bla_{OXY\text{-}6\text{-}4}$. To test if the same evolutionary pathways are followed in laboratory evolution compared to in-patient, the susceptible ancestor was exposed to sub-inhibitory concentrations of TZP in LB broth, followed by growth on TZP-supplemented LB agar. Resistant colonies were selected, and fitness, TZP susceptibility and genomes were compared to the ancestor and the *in vivo* evolved resistant isolate.

Results: In one *in vitro* evolved lineage, we observed the same $bla_{OXY\text{-}6\text{-}4}$ promoter SNP as seen in the *in vivo* evolved resistant strain which conferred high-level TZP resistance, however all other adaptive mutations were unique to either the *in vivo* or *in vitro* evolved lineages. The acquisition of TZP resistance did not confer any negative fitness consequences in the laboratory-evolved strains, in contrast to reduced fitness of the in-patient evolved strain.

Conclusion: This study highlights different evolutionary trajectories in laboratory-based AMR evolution when compared to in-patient evolution and emphasises the need for comprehensive experimental design and cautious translation of findings to the clinic, particularly when interpreting fitness data from laboratory experiments.

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Multiple-trait interactions enable public good overproduction to promote both population productivity and the evolution of cooperation.

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Abstract

Microbial community functioning plays a central role in health, disease and biotechnology. Understanding and predicting their evolutionary dynamics is vital to effectively manage and exploit this role. Microbes enrich their environment via secretions, including to catabolise polymeric nutrients such as the secretion of the sucrolytic enzyme, invertase (*SUC2*), by *Saccharomyces cerevisiae*. The products are often considered cooperative because the enriched environment is shared with neighbouring cells. However, exploitative non-producers can drive a decline of producers by avoiding production costs while reaping the benefits, leading to a loss of population productivity. In short-term competition experiments to investigate the evolutionary dynamics of SUC2 secretion, we found non-producers outcompeted producers. However, producers overcame this competitive disadvantage over long-term evolutionary experiments. What adaptations occurred to achieve this? Although equivalent studies in diverse microbial systems found that producers prevent being outcompeted by down-regulating secretion to reduce costs, we found the opposite whereby overexpressing *SUC2* enhanced producer fitness. How can “hyper-cooperation” be beneficial for an individual when they incur higher costs? We deciphered the mechanism behind this fitness gain by engineering a collection of competitor strains with different sucrose metabolism attributes, including manipulating *SUC2* and hexose transporter expression. We systematically tested three putative mechanisms with a range of phenotypic and competition assays to reveal that interactions between multiple metabolic traits can stabilize the costs of secretion to maintain population productivity. Our findings reveal a novel mechanism for supressing the advantage of non-producers and suggest that SUC2 secretion provides both competitive and cooperative benefits.

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Bacterial adaptation in response of environmental stressors in wild natural communities

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Abstract

All species live in diverse communities with many hundreds of other species, which makes it hard to predict how ecosystems will respond to changing environments. We are trying to understand the evolutionary responses of wild bacterial communities to experimental perturbation. Our field system comprises tree-hole communities found in temporary pools formed by the roots of beech trees in the University of Oxford's Wytham Woods field station. Here, replicate tree hole communities were perturbed by the addition of lime at an interval of 7 days up to 90 days. The pH of the replicate tree hole communities was measured, and samples were collected at every time point. We observed that the overall pH of the limed tree holes increased by 0.2 to 0.6 units after 2 months as compared to the control tree holes. Preliminary 16s sequence data suggested that the frequency of the most abundant taxa, Comamonadaceae has consistently declined over time in control as well as in limed tree holes. We saw the minimal effect of pH on the overall composition of the communities and taxa were consistently maintained throughout the experiment. Further, we think that a combination of metagenomics and high-throughput phenotypic assays in the laboratory would help us track the responses of constituent species and the whole community to experimental changes in pH.

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Colistin resistance evolution in *Pseudomonas aeruginosa*

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Abstract

Background – *Pseudomonas aeruginosa* (*Pa*) is commonly associated with chronic biofilm-associated lung infections in cystic fibrosis (CF) patients. Emergence of resistance against colistin is of concern, as this drug is commonly used to treat *Pa*-associated disease exacerbations. Colistin resistance evolution in biofilms remains undetermined. Here, we characterize the rate and extent of colistin resistance evolution in vitro for *Pa* during biofilm and planktonic growth, exposed to different concentrations of colistin.

Methods – We passaged planktonic and surface-adherent biofilm cultures of the *Pa* PAO-derived strain XEN41 for 10 days. The cultures were treated with static (1xMIC, 2xMIC) or stepwise increasing colistin concentrations (1-16xMIC). We collected daily samples and performed population analysis profiling (PAP) to characterize emergence of the distribution of colistin resistance.

Results – *Pa* biofilms treated with colistin showed an increased rate and magnitude of colistin resistance within the first days of treatment when compared with planktonic cultures. However, no significant differences were identified after days 10 of evolution. Increased concentrations of colistin were associated with a shift in the distribution of colistin resistance in both planktonic and biofilm cultures. We surprisingly observed a lack of fixation of observed heteroresistant subpopulations in both planktonic and biofilm lifestyles.

Conclusion – The rate and extent of colistin resistance evolution in biofilms displayed a marginal increase when compared to planktonic. Clonal interference may explain the observed lack of fixation of heteroresistant subpopulations. Future sequencing analysis will allow us to determine & compare the effect of biofilm-lifestyle and treatment specific genotypic changes.

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Multiple dynamic features of microbial populations are associated with rates of spontaneous mutagenesis

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Abstract

Spontaneous mutagenesis can be a key driver of microbial evolution. Rates of spontaneous mutation have been associated with particular ecological features of microbial populations: population density, growth rate and nutrient availability. Such plasticity in mutation rate is challenging to deconstruct because each of these features is dynamic, even during assays used to estimate mutation rates. Here we dissect and test these features using a combination of dynamic simulation of biochemical networks in silico and mutation rate estimates in both batch and continuous cultures of Escherichia coli. We find that all three effects are detectable and separable – increases in per genome mutation rate occur with decreasing population density, decreasing growth rate and decreasing nutrients. The simulation models suggest a central role for the production and degradation of reactive oxygen species (ROS) in these processes: they can enable the observed association of lower population densities with higher per genome mutation rates. We test this idea by manipulating both environmental oxygen and genes that remove ROS. We find that populations either grown without oxygen, or lacking genes encoding ROS-removing enzymes, lose mutation rate plasticity, having similar mutation rates when grown to different final population densities by manipulating nutrients. This work provides a basis, both for exploring the molecular mechanisms by which microbes' ecology modifies mutagenesis and for understanding how mutation rate plasticity affects the dynamics of evolution.

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Piperacillin and Tazobactam affect the evolutionary trajectory of antibiotic resistance in human pathogens in different ways

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Abstract

Piperacillin-Tazobactam (Pip-Tazo) is a beta-lactam/beta-lactamase inhibitor combination and among the most prescribed antimicrobials in hospital medicine, used to treat pneumonia, septicaemia and urinary tract infections. Piperacillin is inactivated by commonly carried resistance enzymes, but Tazobactam inhibits these allowing successful treatment. The effect of Piperacillin on Gram-negative bacteria has been widely studied, but less attention has been paid to the effects of Tazobactam. We used a massive transposon mutagenesis approach named TraDIS-Xpress to determine the genes in Escherichia coli that affect survival when exposed to Piperacillin, Tazobactam and Pip-Tazo. We found significant differences in the selective pressure of the two drugs: a striking finding was that multiple efflux systems were essential for survival in the presence of Tazobactam and Pip-Tazo, but only one was crucial in the presence of Piperacillin. Evolution experiments supported this finding, where bacteria selected after repeated culture with Tazobactam and Pip-Tazo showed increased efflux activity relative to Piperacillin or no drug. Increased efflux activity is a common precursor to development of high-level antimicrobial resistance as efflux synergises with other mechanisms. Therefore demonstrating that Tazobactam can select for multidrug-resistant pathogens is a worrying concern. We also identified multiple pathways affecting susceptibility to both drugs separately and in conjunction, including genes involved in cell envelope biosynthesis, signalling systems, DNA repair, protein translation and export and acid stress responses. These findings could have consequences for antibiotic prescription and should inform the development of future beta-lactamase inhibitors which should consider selective potential of inhibitors as well as the active drug.

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How do interactions between mobile genetic elements enhance microbial community resilience?

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Abstract

Many ecologically-important traits are transmitted between bacteria by horizontal gene transfer (HGT), driven by the activity of mobile genetic elements (MGEs). MGEs routinely interact with one another, e.g., different transposons on a plasmid enables multiple traits to transfer between bacterial lineages simultaneously, and transposon movement between replicons releases traits from plasmid host-ranges. This modularity of MGEs may help microbial communities in the face of a changing environment, increasing resilience to stressors by accelerating the spread of adaptive traits across community members. Using computer modelling and a laboratory microcosm system, we investigated how different plasmid vehicles affected the spread of a chromosomal, transposon-borne resistance gene. We found that resistance gene mobilisation varied across a panel of four plasmids in a manner independent of conjugation rate, suggesting that other plasmid features, such as gene content, may be key factors influencing the probability of chromosomal gene mobilisation. Using an agent-based model, we explored the consequences for population-level resilience, and show that plasmid maintenance cost has a larger impact on plasmid persistence in the community than plasmid transfer rate under varying levels of environmental poison. Incorporation of transposons and multiple bacterial species into the model helps us map trait spread in a more complex system and make predictions about key factors affecting MGE modularity.

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Experimental evolution of insect pathogens- applying and testing our understanding of virulence.

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Abstract

Experimental evolution of pathogens through host passage has been used to manipulate virulence for decades. Historically, this has worked best for viruses potentially because fitness and virulence of many viruses is often correlated with growth rate within hosts. However, virulence of bacterial and fungal pathogens is more complex, often involving social traits affected by multiple levels of selection. Here we manipulated selection pressure at multiple levels, including selection for infectivity within a metapopulation, in order to "improve" the virulence of a fungal biocontrol agent Akanthomyces muscarius, against an aphid pest. Selection at multiple levels could produce modest increases in virulence of this pathogen, although we saw little evidence for life-history trade-offs seen in other pathogens. However, competition between hosts for speed of kill or competition for infectivity between populations could also produce increases in yield of infectious spores, another valuable biocontrol trait. Passage in vitro lead to substantial declines in spore production, a pattern that could be explained by social conflicts in terms of the timing of sporulation. Shared phenotypic changes across multiple life history traits were accompanied by convergent genomic evolution, creating opportunities for mechanistic understanding of valuable biocontrol traits. In addition, designing passage experiments that account for multiple levels of selection has clear benefits for understanding pathogen evolution as well as for improving biocontrol agents.

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Decoding the synergistic interaction between trimethoprim and nitrofurantoin in *Escherichia coli*

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Abstract

Antibiotic combination therapies are commonly used clinically to treat infections caused by antibiotic resistant bacteria, for mixed species infections or hard-to-treat chronic infections (e.g. lung infections in cystic fibrosis patients). However, the clinical effectiveness of these combinations varies, partly depending on whether the used drug combination shows an increased (synergy), equal (additivity) or reduced (antagonism) effect on efficacy compared to that predicted from the individual antibiotic's effect combined.

Our lab previously identified a synergy interaction between trimethoprim and nitrofurantoin in *Escherichia coli* clinical isolates (Fatsis-Kavalopoulos et al., 2020, PLoS Biol). These two antibiotics are often used to treat urinary infections, although typically not in combination. Recently, we expanded the analysis to 250 isolates and demonstrated that this combination shows synergistic in the majority of the isolates.

The analysis of the underlying molecular mechanism causing synergy is being carried out using two different approaches. Firstly, a transcriptomic analysis was performed using one isolate selected as model, in the presence of one, two or none of the antibiotics, in order to compare gene expression changes. Secondly, a genetic selection was performed using different concentrations of trimethoprim and nitrofurantoin in combination where we identified mutants that had lost synergy. Among the 61 mutants obtained, 53 had lost the trimethoprim-nitrofurantoin synergy when grown at subinhibitory antibiotic concentrations. A whole genome sequencing of these mutants has been performed, and is currently under analysis. By combining the transcriptomic and genetic analysis, we hope to determine the mechanistic basis for the synergy.

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Hot or Not: The Evolutionary and Ecological Consequences of Having a Mutational Hotspot or Not in an Evolving Gene Regulatory Network

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Abstract

Having a mutational hotspot can fundamentally change the evolutionary trajectory of a microbial population. Here we look at the consequences of having a mutational hotspot by comparing two *Pseudomonas fluorescens* lines- one with a hotspot (Pf0-2x-sm) and one without (Pf0-2x).

These bacteria are immotile due to disruption of the flagellar master regulator FleQ. They are selected to restore motility through starvation on 0.25% agar plates, which they do after just a few days, but the routes taken to restore this phenotype and the outcome of this differ between them. We screen them for mutations that have restored the swimming phenotype. We also test the swimming speed of the evolved lines by re-inoculating them into 0.25% agar, and measure them after 24 h. Co-culture competitions with the immotile hotspot and non-hotspot lines are performed in 0.25% agar to see which evolves to swim first.

We find the hotspot line has a less diverse mutational spectrum to restore motility than the non-hotspot line, with the hotspot mutation (A289C *ntrB*) occurring 81% of the time. The hotspot line restores motility the fastest alone and almost exclusively wins when in competition with the non-hotspot line- 100% in complex media and 83% in minimal media. However, we see that the hotspot mutation is not the fastest possible swimming phenotype. This suggests that being able to explore more mutational options can be beneficial for discovering better phenotypes, but the ease of access of a mutational hotspot also provides inherent benefits for out-competing others.

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The evolution of manipulative cheating

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Abstract

A social cheat is typically assumed to be an individual that does not perform a cooperative behaviour, or performs less of it, but can still exploit the cooperative behaviour of others. However, empirical data suggests that cheating can be more subtle, involving evolutionary arms races over the ability to both exploit and resist exploitation. These complications have not been captured by evolutionary theory, which lags behind empirical studies in this area. We bridge this gap with a mixture of game-theoretical models and individual-based simulations, examining what conditions favour more elaborate patterns of cheating. We found that as well as adjusting their own behaviour, individuals can be selected to manipulate the behaviour of others, which we term 'manipulative cheating'. Further, we found that manipulative cheating can lead to dynamic oscillations (arms races), between selfishness, manipulation, and suppression of manipulation. Our results can help explain both variation in the level of cheating, and genetic variation in the extent to which individuals can be exploited by cheats.

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Gene loss and compensatory evolution promotes the emergence of morphological novelties in budding yeast

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Abstract

Deleterious mutations are generally considered to be irrelevant for morphological evolution. However, they could be compensated by conditionally beneficial mutations, thereby providing access to new adaptive paths. Here we use high-dimensional phenotyping of laboratory-evolved budding yeast lineages to demonstrate that new cellular morphologies emerge exceptionally rapidly as a by-product of gene loss and subsequent compensatory evolution. Unexpectedly, the capacities for invasive growth, multicellular aggregation and biofilm formation also spontaneously evolve in response to gene loss. These multicellular phenotypes can be achieved by diverse mutational routes and without reactivating the canonical regulatory pathways. These ecologically and clinically relevant traits originate as pleiotropic side effects of compensatory evolution and have no obvious utility in the laboratory environment. The extent of morphological diversity in the evolved lineages is comparable to that of natural yeast isolates with diverse genetic backgrounds and lifestyles. Finally, we show that both the initial gene loss and subsequent compensatory mutations contribute to new morphologies, with their synergistic effects underlying specific morphological changes. We conclude that compensatory evolution is a previously unrecognized source of morphological diversity and phenotypic novelties.

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Tools for teaching evolution: optimising a predictable microbial experimental evolution model system for a lab practical in secondary schools

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Abstract

We aim to develop and optimise a microbial experimental evolution practical kit for secondary schools to offer a hands-on learning approach that fosters deeper experiential learning, to facilitate a better comprehension of evolution. Current teaching of evolution relies on classroom-based presentations that lack tangible, experiment-driven learning leading to disengagement and misconceptions. Practical teaching of evolution that demonstrates natural selection through microbial experimental evolution promotes greater investment and better understanding. We will modify an experimental system commonly used in our lab: the re-evolution to bacterial flagellar motility. This model system lends itself to a classroom practical environment as the desired phenotype evolves very quickly (over the course of a weekend) and the phenotype itself is obvious once evolved (motility). The practical will form part of a three-lesson plan. Students will inoculate motility agar with an engineered non-motile strain of Pseudomonas fluorescens. After 3 to 4 days several bacteria will evolve motility and will visibly cover a larger proportion of the agar plate. These bacteria will then be passaged on to a new agar plate after 24 hours, the fastest evolved line, and variation in motility speed across evolved lines, will be measured. This experiment will be bookended by class discussions to develop the students' understanding of experimental evolution. We hope this approach will inspire young people to get excited about microbiology and create opportunity for discussions on the importance of microbial evolution in wider society.

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How fast do antibiotic resistance genes move? Quantifying generalised transduction in S. aureus

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Abstract

Background: Antibiotic resistance gene diversity in bacteria suggests that there must be frequent gain and loss events. However, the dynamics of generalised transduction, the main mechanism of horizontal gene transfer (HGT) in the important pathogen *S. aureus* are unknown. Understanding how the fundamental interactions of growth, competition and HGT events drive the rise of multidrug resistance requires novel tools such as mathematical modelling.

Methods: We combined mathematical modelling with *in vitro* and *in vivo S. aureus* data. Firstly, we cultured single-resistant bacteria with 80α phage to generate double-resistant progeny. Our time-series data parameterized novel mathematical models representing microbial dynamics. Secondly, a novel model framework was fit to data from *S. aureus* in piglets to determine multiple HGT rates.

Results: A new model, that requires a limit on bacterial-phage interactions and a growth dependent burst size, captured *in vitro* dynamics and estimated that one in every 10^8 new phage generated carried a resistance gene, generating double-resistant bacteria in just 7h. *In vivo* dynamics were more complex and resulted in wider uncertainty in transduction rates.

Conclusions: Our insights into the rates of HGT by transduction will support resistance evolution prediction and need comparison to better-studied conjugation rates. Linking mathematical modelling to experimental results allows us to reveal and quantify fundamental processes behind the complex dynamics of resistance evolution. The success of this relies on interdisciplinary teams and frequent iterative analysis.

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A genetic approach to understand beta-lactam + aminoglycoside interactions in *Escherichia coli*

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Abstract

Antibiotic combination therapies are commonly used in the clinic, but their effect on bacterial growth can vary from synergy (increased efficacy) to antagonism (decreased efficacy). Interactions between antibiotics vary between strains of the same species in a yet unpredictable way and the evolution of antibiotic antagonism is not well studied. Here, we aim to identify the underlying genetic mechanisms that change the interaction between beta-lactam and aminoglycoside combinations towards antagonism.

We obtained spontaneous mutants in *E. coli* clinical isolates and a model organism by exposing these to various inhibitory concentration combinations of beta-lactams and aminoglycosides. We characterised a subset of mutants, excluding those that showed an increased minimal inhibitory concentration to just one of the antibiotics to avoid mutants that simply became resistant instead of antagonistic.

Selected mutants showed an increase in antagonism with no correlation to fitness. Genome sequencing revealed mostly SNPs and indels in a total of 70 different genes with the majority of mutants showing a single mutation. Except for a few known resistance genes, there was little overlap in the mutations found between different parental strains or different selection conditions. However, there was convergence as many of the encoded proteins play a role in proton motive force generation and are known to provide aminoglycoside resistance. Interestingly, several mutants displayed mutations in the lipopolysaccharide synthesis pathway, which we have now verified enhance antagonism.

These results add to our understanding of the evolution of antibiotic antagonism and could help identify differences between strains that could predict antibiotic interactions.

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Understanding the role of genetic background in determining the evolutionary outcome of gene regulatory network rewiring events

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Abstract

Previous work in the Taylor lab, using motility rescue as a model, has shown rapid and repeatable rewiring between the nitrogen and flagellar networks in *Pseudomonas fluorescens*. In the absence of the flagellar master regulator (fleQ), ntrC (response regulator associated with nitrogen uptake) is co-opted to resurrect motility. My research aims to explore the role of global regulatory network architecture, and the wider genome, in determining gene regulatory rewiring pathways. Initial work explores whether flagellar motility rescue can be repeatably achieved in other gram-negative bacteria, and the rewiring routes capable of this. We aim to delete the flagellar master regulator across several different bacteria: *Pseudomonas aeruginosa, Caulobacter crescentus, Vibrio cholerae and Escherichia coli*, which represent a diverse arrangement of flagellar regulatory networks. Replicate independent lines will be placed under strong selection for motility, and motile isolates sampled, and genome sequenced, to identify rewiring routes. Initial results from *P. aeruginosa* show flagellar motility rapidly evolved, similar to *P. fluorescens*. Isolates evolved slow motility after as early as 42 hours, with fast mutants emerging after as early as 66 hours. Evolved motile mutants will be genome sequenced to identify the rewiring routes used to regain motility. This work will contribute to a larger aim, which is to identify how differences in gene regulatory architecture determines the predictability of evolved rewiring pathways across different bacteria using motility rescue as a model system.

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Evolution of bacteriophage T7 on a bacterial lawn: Genotypic and phenotypic characterisation and the search for the mechanism enabling fast plaque growth

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Abstract

Much of our understanding of the evolution of bacteriophages originates from experiments in well-mixed environments. This contrasts with many bacteria living in dense, spatially structured populations. What are the consequences of this host lifestyle on bacteriophage evolution?

To address this question, we evolved bacteriophage T7 on lawns of the bacterium *Escherichia coli* by repeatedly transferring the phages furthest from the inoculation site onto a fresh lawn. Across eight evolving lines, we observed fast and recurrent evolution with a more than twofold increase in plaque front speed over the course of 14 rounds, corresponding to roughly 700 infection cycles.

To understand the evolutionary strategy taken, we sequenced populations after 5 and 14 rounds as well as selected clones. We found mutation predominantly in genes 7.3, 11, 12, and 17 as well as, in selected lines, a large deletion in the early genes. These genotypic changes must be accompanied by changes in life history parameters to explain the evolution towards faster plaque growth. Yet, initial measurements of life history parameters under standard conditions did not reveal a convincing pattern matching the physics of plaque growth. However, repeating measurements in conditions of lower metabolic activity revealed a different set of changes to life history parameters that can potentially explain the mechanism for accelerated plaque growth.

Our findings highlight that for recurrent evolution of a quantitative trait in a given environment, rationalising or even predicting the evolution of said trait is a challenge, which can, however, be addressed using mathematical modelling.

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The influence of genetic background on the evolvability of antimicrobial resistance

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Abstract

Mutations can allow bacteria to acquire resistance to antibiotics and at the same time, they may affect cell fitness and sensitivity toward other antibiotics. The genetic background of a bacteria may also influence the effects of such mutations. For example, the same resistance-causing mutation in two different genetic backgrounds can provide different levels of resistance and fitness effects, due to epistatic interactions. Understanding such interactions could help us predict how resistance evolves and therefore develop methods to slow down or reverse the rapid evolution of antimicrobial resistance in bacterial populations. We have been working to understand how genetic background may influence the evolvability of AMR by comparing the mutational selection windows of strains that vary in genetic background. We find that the width of mutational windows to different antibiotics can vary in different clinical isolates of *Escherichia coli*. We also find that mutations that confer increased resistance to one antibiotic affect the evolvability of resistance to a second antibiotic in *E. coli* K-12. The results from these analyses will provide insight into how genetic interactions may affect resistance, fitness and evolvability. This could be important for designing treatment strategies that minimise the risk of resistance evolution.

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Effect of genetic background and order of antibiotic selection on fitness and collateral susceptibility in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*

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Abstract

Mutations conferring antimicrobial resistance (AMR) often result in a fitness cost to bacteria in the absence of antibiotics. Understanding these fitness effects following selection in the presence of antibiotics may inform strategies to limit the emergence of AMR.

Ten independent lineages of two clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* were selected in the presence of sub-inhibitory concentrations of amoxicillin-clavulanic acid (AMC) or gentamicin (GEN). Five independent lineages of each isolate-antibiotic combination for one *E. coli* and *K. pneumoniae* isolate were subsequently selected for in the alternative antibiotic, creating 25 AMC-GEN and 25 GEN-AMC independent lineages per isolate. Comparative fitness and disc diffusion assays of all independent lineages were assessed.

We found that, while fitness effects of antibiotic selection in individual lineages varied, overall, the fitness effect of single selection in AMC or GEN is dependent on the genetic background. Additionally, relative to both the original and immediate single-selected ancestors, sequential selection in GEN-AMC resulted in a larger, overall fitness cost compared to AMC-GEN, which is replicated in both *E. coli* and *K. pneumoniae*. Collateral effects on susceptibility to other antibiotics can be either dependent or independent on the order of antibiotic selection.

This study indicates that the order of antibiotic selection can impact the overall fitness costs of AMR and collateral effects on other antibiotics. Therefore, if we can determine the antibiotics, or order of antibiotics, which maximise the potential fitness costs of AMR mutations, we may be able to limit the emergence of AMR.

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Resistance evolution against functionally diverse phage combinations

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Abstract

Phage therapy offers a potential treatment against multi-drug resistant infections. But understanding the potential for resistance evolution against phage therapeutics is key to developing effective and durable treatment options. Here, we explore how a key functional phage trait, the receptor which they utilise to adsorb to their bacterial host, influences how and when resistance to phage combinations arises. We test whether increasing the functional diversity of a phage combination (i.e., including phages targeting different bacterial receptors) alters resistance evolution by *Pseudomonas aeruginosa* PAO1 over 12 days of co-evolution with phages. Throughout this time frame we observed multiple cycles of phage killing causing decline in the bacterial population quickly followed by sweeps of resistance allowing bacterial recovery, indicating an arms-race between phage and host. However, the degree and frequency of these cycles was increased when phage combinations were functionally diverse. Further, we characterised the resistance landscape of the bacterial populations through time, to explore how phage functionally diversity affects both the mechanism and variation in resistance which arises.

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