Understanding and predicting microbial evolutionary dynamics – 2025 meeting

Hilton Liverpool City Centre
3 Thomas Steers Way
Liverpool, UK

26 – 27 November 2025

INVITED AND OFFERED TALKS

MICROBIOLOGY SOCIETY

#MicroEvo25

Invited talk

Plasmids and cancer: insights into the evolutionary roles of extrachromosomal DNA

Liam Shaw

University of Bristol, Bristol, United Kingdom

Abstract

Bacterial plasmids are probably the best-studied extrachromosomal elements in biology. But there are others: recently, genome sequencing has revealed that many human cancer cells contain extrachromosomal circular DNA that strikingly resembles plasmids. These elements are associated with oncogenes and the development of drug resistance. The uncontrolled growth of cancer cells in a multicellular organism has been described as an atavistic return to a more 'single-celled' existence, making the presence of such 'plasmid-like' elements particularly intriguing. Evolutionary theory provides a bridge between these different disciplines, helping us to better understand the dynamics of extrachromosomal DNA. I'll use examples from my own work on plasmids and antibiotic resistance together with the work of others, discussing these connections and what they can tell us about genome evolution.

Invited talk

Evolutionary Trajectories Are Largely Independent of Community Context in Microbial Cocultures

Jonathan Friedman, Nittay Meroz, Tal Livny, Yael Sorokin

Hebrew University, Rehovot, Israel

Abstract

Evolutionary dynamics in laboratory microbial populations are often repeatable across replicates, suggesting they may be predictable. However, in natural settings, species evolve within complex communities, and the extent to which such biotic contexts shape evolutionary outcomes remains unclear. To investigate this, we evolved 11 bacterial species alone and in diverse pairwise co-cultures. While some partner-specific effects occured, evolutionary outcomes were largely independent of co-culture identity—even for species already pre-adapted to the abiotic environment. To explain this robustness to community context, we developed a theoretical model capturing the boom-and-bust dynamics imposed by serial passaging in experimental evolution. The model shows that selection acts mainly early in the growth cycle, when population densities are low and interspecific interactions are weak, limiting the influence of co-cultured species on adaptive trajectories. These results suggest that under typical experimental conditions, biotic context may play a surprisingly limited role in shaping evolution. This decoupling may help explain the consistency of microbial evolution and could simplify predictions of evolutionary outcomes in both laboratory and natural microbial communities experiencing intermittent growth.

Invited talk

Vancomycin-resistant enterococci colonise the antibiotic-treated intestine by occupying overlapping but distinct nutrient- and metabolite-defined intestinal niches

Julie McDonald

Imperial College London, London, United Kingdom

Abstract

Treatment with broad-spectrum antibiotics promotes the intestinal colonisation with vancomycin-resistant enterococci (VRE), where this intestinal colonisation often precedes the development of difficult-to-treat invasive VRE infections (e.g. bloodstream infections). Healthy gut microbiomes restrict VRE growth in the intestine, however broad-spectrum antibiotics significantly disrupt the gut microbiome and make the host susceptible to VRE intestinal colonisation. Therefore, understanding how antibiotics disrupt the gut microbiome will allow us to develop new treatments to prevent or treat VRE intestinal colonisation, which will prevent the subsequent development of invasive VRE infections. We showed that antibiotics (that promote VRE intestinal colonisation) killed gut commensals, enriched for a wide range of nutrients, and depleted a wide range of microbial metabolites. We found that a mixture of short chain fatty acids (that were depleted with antibiotics treatment) provided complete or near complete inhibition of VRE growth. We also showed that monosaccharides, disaccharides, and amino acids (that were enriched with antibiotic treatment) acted as carbon or nitrogen sources to promote VRE growth. Finally, we showed that vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* occupied overlapping but distinct nutrient-defined intestinal niches where each pathogen achieved high growth when cultured with each other and when cultured with other multidrug-resistant pathogens (carbapenem-resistant *Enterobacteriaceae*). In summary, we showed that VRE can grow in antibiotic-treated intestines by utilising specific enriched nutrients while in the presence of reduced concentrations of inhibitory microbial metabolites.

Invited talk

Harnessing growth-coupling and evolutionary engineering to establish novel chemistries in bacteria

Pablo Nikel

DTU Biosustain, Kgs Lyngby, Denmark

Abstract

Formate is an attractive one-carbon (C_1) feedstock for biomanufacturing due to its low cost, high solubility, and compatibility with electricity-driven CO_2 reduction. However, natural assimilation of formate is limited to a few bacterial species. I will discuss the engineering of the soil bacterium *Pseudomonas putida* to assimilate formate as its sole carbon and energy source *via* the linear reductive glycine pathway. Through a combination of rational design and adaptive laboratory evolution (ALE), initial strains were optimized for mixotrophic growth, leading to mutations in promoter regions of synthetic genes and the native genome that facilitated metabolic integration. Strict formatotrophy was established by introducing a formate dehydrogenase gene and applying growth-coupled selection. Building on this platform, microbial growth was linked to the biosynthesis of complex metabolites. As an example, a formatotrophic *P. putida* strain was engineered to couple xanthommatin production—a color-shifting animal pigment—with growth by creating a 5,10-methylenetetrahydrofolate auxotrophy dependent on endogenous formate levels. This modular system was further refined through ALE, enabling efficient, gram-scale pigment biosynthesis. These findings establish *P. putida* as the first strictly formatotrophic member of its genus and highlight its potential as a robust host for scalable, growth-coupled production of high-value compounds from C_1 substrates.

Invited talk

Predictable effects of environmental stress on microbial communities

Jana Huisman

Massachusetts Institute of Technology, Cambridge, USA

Abstract

Environmental stress reduces species growth rates, but its impact on the structure and function of microbial communities is less clear. Using salinity stress as an example, we recently demonstrated that increasing salinity stress shifts community composition towards species with higher growth rates. As a result, the mean community growth rate is more robust to increasing stress than the growth of individual species. We showed this by propagating natural aquatic communities at multiple salinities and mapping the observed diversity onto the measured salinity performance curves of >80 bacterial isolates. We further validated these results with pairwise species competitions and in metagenomic data of natural communities sampled from estuarine environments. A minimal ecological model with mortality and stress-dependent growth rates could recapitulate the observed robustness, which suggests that these results extend to other environmental stressors. In this talk I will further highlight what I think the evolutionary implications of these ecological dynamics may be.

Invited talk

Antibiotic use drives AMR… right? Rethinking the importance of selection in the real world

Gwen Knight

AMR Centre at the London School of Hygiene and Tropical Medicine, London, United Kingdom

Abstract

Antimicrobial use (AMU) is often targeted as the primary driver of antimicrobial resistance (AMR), yet population data reveal a far more complex picture. Across Europe, women receive more antibiotics than men but experience fewer drug-resistant bloodstream infections. By age, resistance shows complex and varying patterns across sub-national regions and bacteria–antibiotic combinations, even though antibiotic exposure often increases in older groups. These contrasting trends suggest that selection may not be the main driver and that interventions should consider targeting different sex and age groups differently. Could this also reflect some level of ecological fallacy? To explore this, I will show that individual-level patient data on *Staphylococcus aureus* reveal similarly weak or indirect associations between antibiotic exposure and resistance acquisition. Even without fully resolving these mechanisms, explicitly modelling demographic structure helps refine baseline AMR burden estimates in ageing populations. Ultimately, capturing this complexity requires rethinking the importance of selection in the real world.

Invited talk

Plasmid evolution: a (copy) numbers game

Jerónimo Rodríguez Beltrán

Ramón y Cajal Hospital – IRYCIS, Madrid, Spain

Abstract

Plasmids are autonomously replicating DNA molecules that are widespread across the microbial world, acting as major drivers of evolution through horizontal gene transfer. Plasmids vary broadly in replication strategies, mobility, genetic content, host range, size, and copy number. Among these features, plasmid copy number (PCN) influences key aspects of plasmid biology, including gene dosage, expression levels, and plasmid stability, while also modulating the evolution of plasmid-encoded genes. Therefore, PCN stands out as both a product and a driver of plasmid evolution. However, the evolutionary forces shaping PCN and their consequences for bacterial evolution remain controversial, with studies supporting or opposing the role of plasmids as catalysts of bacterial evolution.

Here, we first investigated the evolutionary rules governing PCN using large-scale sequencing data and quantitative analyses. We found that PCN is highly variable among plasmids but remarkably consistent across different bacterial hosts, genetic cargos, and co-resident plasmids. We uncover a conserved inverse relationship between plasmid size and copy number, independent of host phylogeny, and reveal that plasmids consistently comprise ~2.5% of their host's chromosome size, pointing to a universal scaling law that constrains plasmid biology. Next, we explored how PCN influences evolution. Through a combination of theoretical, computational, experimental, and bioinformatic approaches, we show that plasmid mutation rates scale logarithmically with copy number: increasing PCN enhances plasmid evolvability but with diminishing returns. Altogether, our results resolve a longstanding controversy in plasmid biology while providing a quantitative framework for understanding the evolutionary dynamics of PCN.

Invited talk

Within-patient evolution of ciprofloxacin resistance in Pseudomonas aeruginosa across a large-scale clinical trial

Matthew Shepherd[1], Niamh Harrington[2], Taoran Fu[1], Anastasia Kottara[3], Kendall Cagney[2], James Chalmers[4], Joanne Fothergill[2], Dylan Childs[5], Steve Paterson[2], Michael Brockhurst[1]

[1]University of Manchester, Manchester, United Kingdom. [2]University of Liverpool, Liverpool, United Kingdom. [3]Manchester, Manchester, United Kingdom. [4]University of Dundee, Dundee, United Kingdom. [5]University of Sheffield, Sheffield, United Kingdom

Abstract

The antimicrobial resistance (AMR) crisis threatens to endanger modern medicine within the next 20-30 years. A key part of the problem is the emergence of AMR within patients themselves, during courses of antibiotic therapy. Whilst within-patient AMR emergence is increasingly receiving attention in the literature, investigations commonly involve a small number of patients and study single-patient cases which may poorly represent the more general patient population. Here we report finding on the evolution of ciprofloxacin resistance by *Pseudomonas aeruginosa* across a large-scale clinical trial. This trial utilised inhaled liposomal ciprofloxacin as therapy for patients with the lung condition bronchiectasis, who suffered with *P. aeruginosa* infection. We isolated a total of ~25,000 independent bacterial colonies, prior to treatment and during the year-long the trial, measured the ciprofloxacin MIC and growth rates in KB broth for all isolates, and performed whole genome sequencing of ~4069 isolates. Across the trial, ciprofloxacin MICs increased during periods of treatment and decreased during treatment withdrawal, in a pattern suggestive of resistance fitness trade-offs operating within patients. We also find that distinct phenotypic adaptive trajectories are followed in different patient cases, indicating diverse evolutionary dynamics driving resistance emergence. We also characterise the genetic mechanisms driving the within-patient evolution of resistance and highlight that this occurs through at least 3 distinct mechanisms for *P. aeruginosa*. These findings allow us to start characterising the mechanisms through with AMR can emerge within-patients during treatment, and to ask questions on how best we could predict resistance emergence in the future.

Offered Talks

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From noise to novelty: Selecting *de novo* genes from random sequence in *E. coli*

Arianne Babina[1,2], Michael Knopp[2], Dan Andersson[2]

[1]University of Glasgow, Glasgow, United Kingdom. [2]Uppsala University, Uppsala, Sweden

Abstract

The origination of new genes is a major driver of evolutionary innovation, providing raw material for the emergence of adaptive novelties. While gene birth from pre-existing genes (e.g. horizontal gene transfer or duplication-divergence) is relatively well understood, the *de novo* emergence of genes from nonfunctional sequence remains poorly characterized. Although recent computational analyses have identified many candidate *de novo* genes across diverse organisms, *in vivo* experimental demonstration of this process is limited. To investigate how novel genes might arise *de novo* from nonfunctional sequence, we introduced highly diverse plasmid libraries encoding randomly-generated small open reading frames of varying lengths into *Escherichia coli* and selected for novel functions that enabled bacterial growth under non-permissive conditions. In one screen, we identified small proteins that confer resistance to the last-resort antibiotic colistin via direct protein–protein interactions with a sensor kinase that lead to Lipid A modifications. In another screen, we isolated novel RNA-binding small proteins that rescue an auxotrophic strain by upregulating an alternative enzyme. Additional ongoing work has uncovered novel noncoding RNAs that rewire regulatory networks involved in thiamine metabolism and nitrogen starvation. This study provides experimental evidence that new functions can indeed originate *de novo* from entirely random or nonfunctional DNA. These findings shed light on the molecular constraints governing the *de novo* evolution of novel beneficial functions, with broad implications for understanding and predicting microbial evolution, including how bacteria adapt to antibiotics or new ecological niches, as well as for the design of synthetic genetic circuits.

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Dissecting pOXA-48 fitness effects in clinical Enterobacterales using plasmid-wide CRISPRi screens

Alicia Calvo-Villamañán, Jorge Sastre-Dominguez, Álvaro Barrera-Martín, Coloma Costas, Álvaro San Millán

National Centre for Biotechnology, CNB-CSIC, Madrid, Spain

Abstract

Conjugative plasmids are the main vehicle for the spread of antimicrobial resistance (AMR) genes in clinical bacteria. AMR plasmids allow bacteria to survive antibiotic treatments, but they also produce physiological alterations in their hosts that commonly translate into fitness costs. Despite the key role of plasmid-associated fitness effects in AMR evolution, their origin and molecular bases remain poorly understood. In this study, we introduce plasmid-wide CRISPR interference (CRISPRi) screens as a tool to dissect plasmid-associated fitness effects. We designed and performed CRISPRi screens targeting the globally distributed carbapenem resistance plasmid pOXA-48 in 13 different multidrug resistant clinical Enterobacterales. Our results revealed that pOXA-48 gene-level effects are conserved across clinical strains, and exposed the key role of the carbapenemase-encoding gene, blaOXA-48, as the main responsible for pOXA-48 fitness costs. Moreover, our results highlighted the relevance of postsegregational killing systems in pOXA-48 vertical transmission, and uncovered new genes implicated in pOXA-48 stability. This study sheds new light on the biology and evolution of carbapenem resistant Enterobacterales and endorses CRISPRi screens as a powerful method for studying plasmid-mediated AMR.

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Evolution induced state shifts revealed by a long term microbial community experiment

Mikko Kivikoski[1,2], Johannes Cairns[1,2,3,4], Shane Hogle[3], Sanna Pausio[3], Lutz Becks[5], Ville Mustonen[1,2], Teppo Hiltunen[3]

[1]Department of Computer Science, University of Helsinki, Helsinki, Finland. [2]Organismal and Evolutionary Biology Research Programme, University of Helsinki, Helsinki, Finland. [3]Department of Biology, University of Turku, Turku, Finland. [4]Turku Collegium for Science, Medicine and Technology (TCSMT), University of Turku, Turku, Finland. [5]Aquatic Ecology and Evolution, Limnological Institute, University of Konstanz, Konstanz, Germany

Abstract

Microbial community composition is affected by environmental conditions, ecological interactions among the member species, and the evolution of the member species. Several studies have investigated bacterial community compositions and their state shifts in response to environmental conditions and/or over ecological time scales, but studies on how evolution of the member species affects community assembly are still limited. Here, we present a long-term study of a 23-species synthetic bacterial community. We tracked the community composition and the evolution of the member species in two environments: presence and absence of the antibiotic streptomycin. Six replicate communities in both conditions were propagated for 4 years and the state shifts were inferred from longitudinal 16S rRNA gene amplicon sequencing. The evolution of the member species was analysed from longitudinal whole-genome sequencing data. We show that (i) environment (streptomycin *vs.* no streptomycin) affects the community composition, (ii) that community composition changes over time in a repeatable manner, and (iii) that evolution of streptomycin resistance in certain streptomycin-sensitive species, namely, *Aeromonas caviae* and *Pseudomonas putida*, induces community state shifts. The state shifts were linked to rapid sweeps of a known streptomycin resistance causing point mutation in the *rpsL* gene of the species. In summary, we demonstrate evolution induced community state shifts in a long-term study of a complex bacterial community.

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Using Genome-Scale Metabolic models to predict community coexistence, stability and resource use.

Terence McAvoy, Elze Hesse, Angus Buckling

University of Exeter, Falmouth, United Kingdom

Abstract

Genome-Scale Metabolic models (GSMs) use the annotated genome of an individual to predict gene function and metabolite production in different nutrient contexts.

In community models, GSM's can be used to find metabolites responsible for mediating interactions between community members, for example through predicting cross-feeding or competition for limited resources. Furthermore, coexistence of different species can be tested in different nutritional environments. As predictive models, validation is key, however studies validating community model predictions are lacking.

In this study, a community model was generated for a characterised 5 species stably coexisting bacterial soil community. GSMs were used to predict whether this community would continue to coexist if an additional isolate was added and this was repeated with 25 different soil isolates. Model predictions were experimentally validated, and these were done across different nutrient contexts.

Modelling predictions ranged from the original community coexisting with the new isolate, an original member being knocked out or the introduced isolate not surviving. This enabled the validation of community GSMs across a range of community contexts, addressing the gap in this area of research.

The accuracy of GSMs in predicting community coexistence and stability was high, but individual biomass production predictions varied across nutrient contexts. Additionally, we showed the importance of trace minerals in mediating model predictions, with their presence and fine tuning being critical to match experimental data.

Overall, this study provided novel validation for community GSMs in predicting coexistence and stability with implications for using these methods in applied microbiology, such as microbiome manipulations.

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Long-range sequence context shapes mutational bias in *Escherichia coli*

Matthew Jago[1], Rowan Green[2,1], Mato Lagator[1]

[1]University of Manchester, Manchester, United Kingdom. [2]University of British Columbia, Vancouver, Canada

Abstract

Understanding how the local sequence context influences the likelihood of mutation at any given locus is critical for deciphering evolutionary processes and predicting evolution. While the role of immediately adjacent nucleotides in determining mutation rates is well established, the impact of more distal bases remains poorly characterized. Here, we analyzed the positions of over 100,000 mutations from five mutation accumulation studies that utilized *Escherichia coli* strains with varying DNA proofreading and mismatch repair capabilities. By calculating the frequency of each nucleotide at positions up to 6 bp away from mutation sites, we uncovered complex repair-dependent biases that extend beyond the immediately adjacent nucleotides and are unique for each base pair substitution. Notably, we identified G followed by a run of Cs as a hotspot for G:C → C:G mutations, which varies dramatically between strains with different DNA repair capabilities. Extending our analysis up to 100 bp away from mutation sites revealed significant sequence context effects over unexpectedly long distances. Most prominently, A:T → G:C mutations are more common in A:T rich regions only when mismatch repair is active, indicating an inherent bias in this repair pathway. These results show how extended sequence context and DNA repair activity collectively shape bacterial mutational spectra, providing new insights into the mechanistic origins of mutational signatures and their evolutionary consequences.

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Molecular Drivers of Within-Host AMR Evolution in *Pseudomonas aeruginosa*

Adam Mulkern

University of Oxford, Oxford, United Kingdom

Abstract

Renowned for its adaptive versatility, *Pseudomonas aeruginosa* is a leading opportunistic pathogen that frequently exhibits resistance to multiple antibiotics, complicating treatment in diverse clinical settings. A key driver of this resilience is the genetic heterogeneity found within patient bacterial populations, which can harbor diverse variants poised to adapt under antimicrobial pressure. To investigate how pre-existing genetic diversity and population composition influence antibiotic resistance trajectories, we employ a high-throughput experimental evolution framework using a collection of barcoded clinical *P. aeruginosa* isolates.

By exposing these barcoded bacterial populations to a range of clinically relevant antibiotics, we can track the frequency and spread of individual lineages in real time. This approach enables us to dissect how different initial population compositions—specifically, the level of intra-strain heterogeneity and the presence of subpopulations with partial resistance—affect the speed and direction of adaptive evolution. The barcoded system further reveals the contributions of specific mutations and regulatory changes to resistance by linking evolutionary outcomes to each lineage's genetic background.

Our preliminary findings suggest that even low-frequency variants with modest resistance can swiftly dominate under certain drug regimens, underscoring the critical role of hidden genetic diversity in clinical failures. These insights enhance our understanding of the molecular drivers that underlie within-host resistance evolution. By leveraging this knowledge to tailor antibiotic combinations and dosing strategies to each patient's unique bacterial population, we can make informed treatment decisions that minimise the selective advantage of resistant subpopulations and help curb the emergence of multidrug-resistant *P. aeruginosa*.

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Exploring community and plasmid dynamics in the hospital sink drain microbiome

Gregory McCallum[1], Matthew Kelbrick[1], Danielle McLaughlan[2], Jonathan Folb[2], Ginny Moore[3], James Hall[1]

[1]University of Liverpool, Liverpool, United Kingdom. [2]Liverpool University Hospitals NHS Foundation Trust, Liverpool, United Kingdom. [3]United Kingdom Health Security Agency, Salisbury, United Kingdom

Abstract

Hospital sink drains contain a complex microbial ecosystem termed the hospital sink drain microbiome, which acts as a reservoir for antimicrobial resistance genes (ARGs). Many of these ARGs are carried on mobile genetic elements such as plasmids. The hospital sink drain acts as a prime location for plasmid transmission, which has been shown *in vitro* to be increased by chlorine-based disinfectants, and therefore could be a major contributor to ARG transfer into opportunistic pathogens. However, plasmid dynamics in the sink drain microbiome are often overlooked in favour of horizontal transfer in the context of infection or patient colonisation. To gain insight into microbial ecology and patterns and drivers of gene exchange in this habitat, we characterised microbial community and plasmid dynamics in real sink traps in an experimental mock hospital setting. Traps were inoculated with samples from real-world hospital sinks and allowed to establish before being installed onto 12 sinks in a mock hospital at UKHSA. Taps were flushed four times a day and a small amount of nutrients was added daily to all sinks to simulate real-world use. Chlorine-based disinfectant was added weekly to the half of the sinks. Sinks were sampled weekly over 9 weeks and changes in community composition were analysed using culturing and metagenomics. Hi-C coupled with long-read metagenomic sequencing will be used to assess differences in plasmid transfer in the disinfectant and control sinks. Overall, this work aims to characterise the impact of disinfectant use on plasmid evolution and exchange in this clinically-proximal environmental microbiome.

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The evolution of gut colonisation strategies of widespread high-risk *E. coli* clones

Pramod K Jangir, Leonardo Lemos Rocha, Médéric Diard

Biozentrum, University of Basel, Basel, Switzerland

Abstract

Extended-spectrum β-lactamases (ESBL)-producing *E. coli,* particularly high-risk clones, such as ST131, ST69, and ST73, are one of the leading causes of multidrug-resistant infections globally. Notably, these clones show a remarkable ability to colonise the mammalian gut by overcoming gut microbiota-mediated competition. However, we have a very limited understanding of (i) genetic factors that are specifically required for overcoming gut microbiota competition, and (ii) how the microbiota-driven selective pressures shape the evolution of these clones in the gut.

To address this, we integrated random-barcoded transposon sequencing (RB-TnSeq) data from several prevalent ESBL *E. coli* screened in mice with and without microbiota, together with long-term in vivo evolution in mice with microbiota. Interestingly, gut microbiota-mediated competitions have considerable effects on the fitness landscape of ESBL *E. coli*. In particular, the comparative RB-TnSeq reveals that pathoadaptive genes, such as those involved in biofilm formation and adhesion, are specifically required when the microbiota is present, but largely dispensable in germ-free mice. Moreover, the majority of these microbiota-specific pathoadaptive genes are part of the accessory genome, highlighting the importance of microbiota-mediated selection in pathogen evolution. Our long-term in vivo evolutionary experiment in the presence of microbiota also shows signatures of convergent evolution towards certain pathoadaptive functions. In sum, these findings demonstrate that the selection of pathoadaptive traits in ESBL *E. coli* is driven by gut microbiota-mediated competitions. This study underscores the dynamic influence of the gut microbiota on pathogen evolution and suggests that targeting microbiota-pathogen interactions may offer effective strategies for controlling enteric infections.

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Arbitrium phages can manipulate each other's lysis - lysogeny decisions

Robyn Manley[1], Rebecca Woodhams[2], JOhn Bruce[3], Elvina Smith[2], Ben Temperton[1], Westra Edze[2]

[1]University of Exeter, Exeter, United Kingdom. [2]University of Exeter, Penryn, United Kingdom. [3]Strathclyde university, Glasgow, United Kingdom

Abstract

Many viruses can switch between lytic replication and dormancy (or lysogeny). It was recently discovered that some viruses that infect bacteria (known as bacteriophage, or phage) employ peptide-based ("arbitrium") communication systems to optimise their lysis/lysogeny switch: high peptide concentrations signal a lack of susceptible hosts and trigger lysogeny, while low peptide concentrations signal an abundance of uninfected hosts and prompt lysis. Here we demonstrate that Arbitrium-phages belonging to different species and genera can influence each others' infection dynamics by secreting similar communication peptides, leading to early lysogenisation of the signal-receiving phage, and elevated fitness of the signal-emitting phage. Antagonistic coevolution between signal emitting and signal receiving phages to manipulate each other's infection behaviours may explain the rapid diversification of arbitrium systems and their frequent horizontal exchange to escape the noise of cross talk.

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Donor-dependent *Escherichia coli* invasion dynamics across Human gut microbiotas using an *in vitro* gut simulator

Hugo Barreto, Caroline Steiner, Olivier Tenaillon

Institut Cochin, Paris, France

Abstract

The human gut microbiota provides protection against pathogen colonization, a phenomenon known as colonization resistance. However, the mechanisms by which commensal bacteria bypass colonization resistance and are able to invade and colonize remain understudied. Using MiniBioReactor Arrays, *in vitro* systems that simulate the human gut environment, we investigated the colonization ability of 26 barcoded commensal *Escherichia coli* natural isolates across 16 distinct gut microbiotas from healthy donors. In the absence of any perturbation to the human gut microbiota, we found that *E. coli* was able to invade and colonize for at least 10 days in all donor microbiotas. Colonization levels varied by up to 3 logs between donors, suggesting substantial donor-specific differences in their susceptibility to invasion. We observed that invasion success was not associated with the initial abundance of *Enterobacteriaceae*, the most likely competitors of *E. coli* within the gut microbiota. In contrast, we observed an inverse relationship between microbiota diversity and invasion susceptibility, with low diversity communities being more permissive to *E. coli* invasion. Furthermore, the identity and frequencies of the barcoded *E. coli* strains after 10 days of colonization were also donor-dependent, and ongoing genomic analyses aim to identify genes or mutations linked to colonization success. Our results suggest that the interplay between microbiota diversity and *E. coli* genomic background influences invasion success, highlighting the importance of these factors in determining colonization outcomes.

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Phage-steering of bacterial evolution permits antibody-mediated clearance of Escherichia coli K1 from the gut

Anouk Bertola[1], Louise Larsson[2,1], Nicolas Alexandre Wenner[1], Leonardo Lemos Rocha[1], Despoina Dimakou[2], Niina Ruoho[2], Emma Slack[2,3,4], Médéric Diard[1,4]

[1]Biozentrum, University of Basel, Basel, Switzerland. [2]Institute for Food, Nutrition and Health, ETH Zürich, Zürich, Switzerland. [3]Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom. [4]Basel Research Centre for Child Health, Basel, Switzerland

Abstract

Opportunistic infections emerging from bacteria colonising the gut are becoming more and more problematic with the rise of antibiotic resistance. Gut-resident pathogens such as multidrug resistant *Escherichia coli* K1 can be transferred from mother to child and are a major cause of meningitis and sepsis in new-borns. The K1 capsule mimics host structures and is thus poorly immunogenic. We developed a combinatory approach of phage steering, immunisation and competition, to exclude *E. coli* K1 from the gut. K1-specific bacteriophages were used to drive the intra intestinal evolution of capsule-less mutants, that are exposing the highly immunogenic O-antigen moiety of the LPS. Under these conditions, capsule-less *E. coli* K1 could be excluded from the gut by applying selective pressure with vaccine-induced IgA in combination with a probiotic competitor. This combination allows the clearance of *E. coli* K1 from the gut of colonised mice. When applying this approach to a murine vertical transmission model, the combination of phage, vaccine and competitor delayed transmission of *E. coli* K1 from the mothers to the pups, showing an additive effect of the individual components. Combining phage-steering with vaccination is a promising avenue for the prevention of neonatal bacterial infections.

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Evolutionary tradeoffs in facultative symbiosis; reciprocal effects of phage predation and nodulation on rhizobia

Charly Pain[1], Ville Friman[2], Alex Best[1], Ellie Harrison[3]

[1]University of Sheffield, Sheffield, United Kingdom. [2]University of Helsinki, Helsinki, Finland. [3]Sheffield, Sheffield, United Kingdom

Abstract

Facultative symbionts such as rhizobia live between radically different worlds. Within the symbiosis they engage in specialised interactions with their hosts, while free-living individuals living in complex communities are exposed to dangers such as predation. These different environments impose very different selective pressures, potentially leading to evolutionary tradeoffs. Here we examine the reciprocal effects for rhizobia between phage predation in the environment and the nitrogen-fixing symbiosis within legume hosts. Using experimental evolution, we find that antagonistic coevolution driven by phages can drive loss of symbiotic potential in free-living rhizobia. But rhizobia that enter legume hosts early can escape this arms race and associated tradeoffs. The implications of this are context dependent - symbiotic rhizobia are outcompeted by more resistant rhizobia in the soil when phages are present. Ultimately however, this cost is outweighed in competition for hosts; despite higher rates of predation outside the host, rhizobia isolated from plant hosts were better able to colonise new hosts than those isolated from the soil. These results therefore suggest that continuity of symbiotic interactions can be important for maintaining symbiotic efficiency over evolutionary time. Selection outside the host however may drive specialisation in free-living environments. Taken together this illustrates how tradeoffs driven by conflicting selection can drive diversity in symbiont populations.

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Increased plasmid copy number reduces establishment probability of beneficial mutations

Mario Santer, Tal Dagan

Kiel University, Kiel, Germany

Abstract

Plasmids are key drivers of microbial evolution and major contributors to the spread of antibiotic resistance. While they can be maintained through both vertical inheritance and horizontal gene transfer, plasmids are also subject to loss due to fitness costs and segregational instability. We developed mathematical models to investigate the population dynamics and genetics of plasmid-encoded alleles, incorporating plasmid copy number. We analysed the establishment and fixation dynamics of resistance-conferring mutations under selective conditions. In the absence of horizontal transfer, we found that high plasmid copy numbers reduce the establishment probability of beneficial alleles. This effect is driven by **segregational drift**—the random partitioning of plasmid copies during cell division—which impedes the establishment and fixation of initially rare alleles. Evolution experiments using multicopy plasmids in *Acinetobacter baylyi* confirmed these predictions: both the initial survival probability of beneficial alleles was reduced, and their fixation time increased, compared to mutations on the bacterial chromosome. Our comparison with experimental data demonstrates the predictive power of mechanistic models for plasmid allele dynamics. We further extended the framework to include plasmid loss and horizontal plasmid transmission. Our results highlight how plasmid-specific inheritance processes—particularly segregational drift—critically shape the evolutionary fate of plasmid-borne traits. This work underscores the importance of integrating theoretical and experimental approaches to understand the evolutionary dynamics of mobile genetic elements.

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Inflammation-like environments limit the loss of quorum sensing in Pseudomonas aeruginosa: Evolutionary insights from host-mimicking experiments

TAORAN FU, Rosanna Wright, Danna Gifford, Christopher Knight, Michael Brockhurst

University of Manchester, Manchester, United Kingdom

Abstract

Understanding the molecular evolution of pathogens under multidimensional selective pressures remains challenging. Here, we used real-time evolution in host-mimicking media to show how an opportunistic pathogen, *Pseudomonas aeruginosa*, disentangles its regulation of oxidative stress responses from quorum sensing (QS). Clinical isolates frequently lose QS via mutations in the master regulator *lasR*, but this increases susceptibility to hydrogen peroxide. Through 42-day daily passaging, we found that *lasR* mutations consistently emerged only after the evolution of oxidative stress tolerance via *katA* promoter or *oxyR* mutations. These early mutations decoupled oxidative stress responses from QS, enabling survival under inflammation-like conditions before nutrient competition drove QS loss. These mutations decouple oxidative stress responses from QS, allowing pathogens to survive lethal stress before responding to nutritional selective pressures favoring QS loss. Evidence of natural selection for this trajectory is shown by analyzing the synonymous and nonsynonymous mutations observed in parallel-evolved clonal populations. Our findings highlight the interplay between environmental stressors and the stepwise molecular evolution of pathogens during host colonisation. By using experimental evolution to observe real-time pathogen adaptation, we provide novel insights into how inflammatory responses shape the molecular evolution of virulence and survival strategies in chronic infections and how pathogens follow multi-step adaptation in highly complex and multidimensional environments.

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The role of anti-phage defence systems in shaping the genomes of temperate phages

Aleksei Agapov, Anna Olina, Rama Bhatia, Edze Westra, Stineke van Houte

University of Exeter, Penryn, United Kingdom

Abstract

Bacteriophages play an important role in controlling bacterial population sizes through cell lysis. In response to phage predation, bacteria evolved a multitude of anti-phage defence systems (DSs). Remarkably, DSs are carried by mobile genetic elements, such as plasmids, integrons or temperate phages. In contrast to lytic phages, temperate phages can insert themselves in the host genome and be inherited by the cell progeny in the form of a prophage. We hypothesise that carrying DSs provides additional benefits for the host and helps the temperate phage to compete with other phages.

In this work we sequenced a hundred temperate phages isolated from *Pseudomonas aeruginosa* strains. We clustered these phage genomes together with 2500 publicly available *P. aeruginosa* temperate phage sequences based on their predicted proteomes and looked at the distribution of the DSs in these genomes. genomic hotspots encoding defence genes. Our analysis reveals that DSs often occur in specific position in the genome, “defence hotspots”. We identified six distinct types of hotspots and suggests candidates for novel DSs in *P. aeruginosa* temperate phages. We are testing the identified hotspots and individual candidate DSs in a lab strain of *P. aeruginosa*. Our results demonstrate strong protection from a wide panel of phages.

Given the compact nature of phage genomes, genomic locations of DSs are expected to have a strong impact on phage performance. We hypothesise that the regulation of DSs depends on their position in the phage genome.

This works identifies novel DSs and contributes to understanding of phage genome organisation.

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Microbiology Society 14–16 Meredith Street, London EC1R 0AB, UK +44 (0)20 3034 4870 microbiologysociety.org