FINAL REPORT

1 General information

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2 Summary

Antibiotic resistance is a growing global health threat, driving experimental and theoretical studies to identify factors that prevent or slow its emergence. Studies often compare the efficacy of treatment strategies but rarely consider population bottlenecks—events that drastically reduce population size. In pathogen infections, bottlenecks occur due to pathogen transmission, immune responses, or antibiotic treatment. Despite their known impact on evolution, their role in resistance evolution, especially alongside other infection-related factors, remains largely unexplored. In our study, we used mathematical models informed by data to explore the effect of population bottlenecks on antibiotic resistance evolution.

As a first step, we focused on the interplay between antibiotic pressure and bottleneck size. We built a mathematical model based upon experimental results from Mahrt et al. 2021, exploring trait adaptation and the effect of demographic fluctuations. Our results show that different bottleneck sizes can favour the selection of different resistance traits—for example, small bottlenecks promote the adaptation of the maximum growth rate, while large bottlenecks promote the adaptation of lag time and carrying capacity. These findings provide insight into how different treatment conditions can steer resistance evolution through distinct adaptive pathways, potentially informing the design of more effective antibiotic therapies.

As a second step, we focused on the interplay between migration, bottlenecks, and competition on evolutionary dynamics. This study was motivated by recent experimental work showing that mixing of within-species strains and bacterial interactions can influence resistance evolution in polymicrobial infections (Batra et al., submitted to Nature Ecology and Evolution). Using a mathematical approach, we developed a meta-population model to explore how migration between demes (isolated subpopulations) affects adaptive outcomes. We compared two extreme regimes: full isolation, where demes evolve independently, and full migration, where demes are well-mixed. Our study identifies the key factors that amplify differences between these regimes, highlighting the relevance of spatial structure and stochastic effects in resistance evolution. These findings have broader implications, extending beyond antibiotic resistance to various ecological contexts.

Antibiotikaresistenz stellt eine zunehmende globale Bedrohung für die öffentliche Gesundheit dar und motiviert experimentelle sowie theoretische Studien, um Faktoren zu identifizieren, die ihre Entstehung verhindern oder verlangsamen können. Während Studien häufig die Wirksamkeit verschiedener Therapien vergleichen, werden genetische Flaschenhälse – die

die Populationsgröße drastisch verringern – nur selten berücksichtigt. Solche Flaschenhälse treten bei Infektionen durch Pathogene infolge von Übertragung, Immunantworten oder Antibiotikabehandlungen auf. Trotz ihres bekannten Einflusses auf evolutionäre Prozesse ist ihre Rolle bei der Resistenzentwicklung, insbesondere im Zusammenspiel mit anderen infektionsbezogenen Faktoren, weitgehend unerforscht. In unserer Studie verwendeten wir dateninformierte mathematische Modelle, um die Auswirkungen genetischer Flaschenhälse auf die Evolution von Antibiotikaresistenzen zu untersuchen.

Im ersten Schritt konzentrierten wir uns auf das Zusammenspiel zwischen Antibiotikadruck und der Größe genetischer Flaschenhälse. Basierend auf experimentellen Ergebnissen von Mahrt et al. (2021) entwickelten wir ein mathematisches Modell, um Merkmalsanpassungen und den Einfluss demografischer Fluktuationen zu analysieren. Unsere Ergebnisse zeigen, dass verschiedene Flaschenkalsgrößen unterschiedliche Resistenzmerkmale begünstigen: Kleine Flaschenhälse fördern die Anpassung der maximalen Wachstumsrate, während große Flaschenhälse die Anpassung der Lag-Phase und der maximalen Populationsdichte begünstigen. Diese Erkenntnisse liefern wichtige Hinweise darauf, wie unterschiedliche Behandlungsbedingungen die Resistenzentwicklung über verschiedene adaptive Pfade steuern können – mit potenziellen Implikationen für die Gestaltung effektiverer Antibiotikatherapien.

Im zweiten Schritt konzentrierten wir uns auf das Zusammenspiel von Migration, genetischen Flaschenhälsen und Konkurrenz im Rahmen evolutionärer Dynamiken. Diese Arbeit wurde durch neue experimentelle Studien motiviert, die zeigen, dass das Mischen von Stämmen innerhalb einer Art und bakterielle Interaktionen die Resistenzentwicklung bei polymikrobiellen Infektionen beeinflussen können (Batra et al., eingereicht bei *Nature Ecology and Evolution*). Mit einem mathematischen Ansatz entwickelten wir ein Meta-Populationsmodell, um zu analysieren, wie Migration zwischen Demes (d. h. isolierten Subpopulationen) adaptive Ergebnisse beeinflusst. Wir verglichen zwei Regime: vollständige Isolation, bei der jede Subpopulation unabhängig evolviert, und vollständige Migration, bei der die Subpopulationen vollständig durchmischt sind. Unsere Analyse identifiziert zentrale Faktoren, die die Unterschiede zwischen diesen Regimen verstärken, und unterstreicht die Relevanz räumlicher Struktur und stochastischer Effekte in der Resistenzentwicklung. Die Ergebnisse haben weitreichende Bedeutung über Antibiotikaresistenz hinaus und sind auch für andere ökologische Kontexte relevant.

3 Scientific progress report

3.1 Background and objectives of the project

The emergence and spread of antibiotic resistance in bacterial pathogens are a global health threat (Aslam et al. 2018). The rise of drug resistance has sparked numerous studies aimed at understanding resistance evolution to prevent or delay its spread. Antibiotic drug therapies aim to minimise the emergence of resistance, in some cases relying on multiple antibiotics (Nosten and White 2007; Nichol et al. 2015). However, the evolution of drug resistance is not only affected by the strategy employed *per se*. Changes in the size of the bacterial population, such as demographic noise and population bottlenecks, have also been shown to influence resistance evolution, and often occur *in vivo* (Willi et al. 2006; MacLean et al. 2010).

Population bottlenecks are events that drastically reduce the size of a population. These events can lead to population extinction or reduced genetic variation by removing alleles (Wahl and Gerrish 2001; Wahl and Zhu 2015). During infections, bacterial pathogens may experience multiple bottlenecks due to transmission, immune responses, or antibiotic treatments, making bottlenecks crucial in antibiotic resistance evolution. While bottlenecks and their effects on population dynamics have been extensively studied, their effects on antibiotic resistance evolution remain largely unexplored.

Experimental results reported in Mahrt et al. 2021 have shown how the strength of bottlenecks and the strength of antibiotic-induced selection can affect the evolutionary path to resistance. In these experiments, *Pseudomonas aeruginosa* populations were exposed to varying degrees of bottleneck strengths and antibiotic concentrations under monotherapies of gentamicin and ciprofloxacin. Stronger bottlenecks (i.e., smaller bottleneck size) led to increased diversity in the outcomes, regardless of whether the antibiotic concentrations were low or high. In contrast, weaker bottlenecks (i.e., larger bottleneck size) were associated with higher resistance levels and reduced diversity in the outcomes.

This project focused on assessing how bottlenecks, combined with different inherent features of bacterial populations, affect antibiotic resistance evolution. We used the study from Mahrt et al. 2021 as a starting point to construct our models. The objectives of the project were:

Objective 1: Study the combined effect of bottleneck size and antibiotic-induced selection using the experimental study from Mahrt et al. 2021 as a starting point by developing a

mathematical that reproduces the experimental outcomes. Generalise the model by going beyond the experimental conditions and assess which factors are most relevant to resistance evolution under the different conditions of antibiotic and bottleneck strengths used in the experiments.

Objective 2: Study how periodic bottlenecks and competition affect resistance evolution by assessing how competition strength and stochasticity affect resistance spread. Explore the effect of stochasticity by considering approaches in which population growth and bottleneck sampling are treated either stochastically or deterministically.

Objective 3: Study the effects of bottlenecks applied randomly in time on antibiotic resistance evolution. Focusing on transmission bottlenecks, study how bottlenecking conditions (i.e., timing and strength) may lead resistant mutants to survive transmission between hosts and, therefore, spread through the population.

3.2 Description of the project-specific results and findings

3.2.1 Adaptation of bacterial populations exposed to periodic bottlenecks and antibiotic drug pressure

Based upon the data reported in Mahrt et al. 2021, we developed a mathematical model for bacterial populations. In the experiments, *Pseudomonas aeruginosa* populations (strain PA14) were exposed to two different antibiotic concentrations: 20% or 80% inhibitory drug concentration (labelled as IC_{20} or IC_{80}) for antibiotics gentamicin (GEN) and ciprofloxacin (CIP). The experimental evolution was performed by serial dilution at two bottleneck sizes: 5×10^4 cells and 5×10^6 cells (labelled as k50 and M5), which represent the number of cells transferred in each bottleneck. In total, four different treatments were considered, resulting from the possible combinations of drug concentrations and bottleneck sizes used: IC_{20}k50, IC_{20}M5, IC_{80}k50, and IC_{80}M5. For our analysis, we proceeded in four steps, where we progressively abstracted from the experimental data.

In *Step 1*, we focused on the analysis of the experimental data set to determine which traits - maximum growth rate, carrying capacity, lag phase, transition rate of the lag phase - evolved and contributed to adaptation. We fitted a Baranyi-Roberts growth model (Baranyi and Roberts 1994) with inter-strain competition to experimentally measured growth curves. We found that treatments with a small bottleneck size favour the adaptation of the maximum growth rate, while treatments with a large bottleneck size favour the adaptation of the lag time and the carrying capacity.

In *Step 2*, we parameterised the model by the median of the replicate trait estimates for each treatment and studied the fixation dynamics of new mutants. We found that the emergence time of successful mutants is earlier for large bottleneck sizes, while it shows a considerable variation when the bottleneck size is small. Both bottleneck sizes showed similar establishment probabilities and fixation times for mutants when exposed to the same antibiotic concentration.

In *Step 3*, we explored scenarios where the traits of the resistant strain differed from those observed experimentally. We systematically varied one trait at a time while maintaining a constant selection coefficient for each trait variation. The results indicated that traits requiring less change to reach a given selection level were most often selected under experimental conditions, suggesting a preference for traits that could quickly confer adaptive benefits under antibiotic pressure.

Finally, in *Step 4*, we compared the dynamics of mutants with low versus high fitness, finding that small bottlenecks favour the fixation of low-fitness mutants, regardless of the antibiotic concentration. This suggests that the size of population bottlenecks is critical in determining which mutations are more likely to become fixed in the population.

Overall, our modelling approach provided valuable insights into how antibiotic selection pressure and bottleneck size shape the adaptive landscape of bacterial populations. By extending the experimental findings, our model offers a more comprehensive understanding of the evolutionary dynamics under different treatment conditions. A manuscript of this study is in preparation.

3.2.2 Resistance variation and bacterial interactions of a genetically diverse bacterial population

Building on the insights gained from our initial modeling work, we also turned our attention to a complementary experimental study carried out by Hinrich Schulenburg's lab, which was not originally included in the proposal. This study offered a unique opportunity to explore additional dimensions of the effects of bottlenecks on antibiotic resistance evolution – particularly by incorporating the mixing of subpopulations. This experimental study focused on assessing how mixing of within-species strains and bacterial interactions can influence resistance evolution in polymicrobial infections (Batra et al., submitted to Nature Ecology and Evolution). I contributed to this project by participating in its conceptualisation and discussion of results. The results from this study motivated the development of a meta-population model which we employed to

address Objective 2 (see Section 3.2.3 below).

In this experimental study, a genetically diverse mixture of 12 *Pseudomonas aeruginosa* strains was evolved under six antibiotic treatment regimes: two monotherapies, two switching treatments, one combination therapy, and a no-drug control. The antibiotics used were gentamicin and piperacillin/tazobactam. The evolving populations were subjected to two distinct serial transfer protocols. The first, a 'no-mixing' meta-treatment, maintained a strict well-to-well correspondence between old and new plates at each transfer, thereby simulating spatially separated subpopulations. The second, a 'mixing' meta-treatment, involved pooling bacteria from all replicate wells in equal proportions at the end of each growth cycle, simulating a well-mixed population without spatial structure.

The results showed that strain diversity plays a key role in helping bacterial populations adapt to strong antibiotic pressure. In the mixing meta-treatment, resistance variants were more uniformly selected—likely favouring those with the highest competitive fitness. In contrast, the no-mixing treatment led to greater variation in the resistance outcomes, likely due to random effects caused by population bottlenecks. The findings suggest that the higher survival rates and faster adaptation observed under mixing conditions were driven by the maintenance of strain diversity. This diversity likely promoted adaptation not just by preventing the random loss of resistant strains, but by preserving beneficial interactions between strains that support the development of antimicrobial resistance.

3.2.3 Stochastic meta–population dynamics with periodic bottlenecks and competition: A comparison of full isolation and full migration

Motivated by the experimental work on mixing of subpopulations, we developed a meta-population model to explore how migration and bottlenecks affect adaptive outcomes. We compared two extreme regimes: full isolation, where bottlenecks are applied to each deme independently, and full migration, where all demes are mixed at each transfer step and distributed uniformly across all demes (migration and bottlenecks were applied simultaneously). These regimes served as theoretical analogues of the no-mixing and mixing meta-treatments used in the experimental study. We aimed to identify the key factors driving differences between both migration scenarios. We compared the mutant frequencies across transfers and the establishment probability of new mutants for different models that varied the interactions between wild-types and mutants.

As a starting point, we implemented a null model in which individuals neither compete for

resources nor interact during any phase of the cycle—neither during growth nor during the bottleneck-migration step. As expected, we found that full migration does not confer any selective advantage to mutants compared to full isolation. In other words, when interactions are absent, migration alone has no effect on the probability of mutant establishment or the overall dynamics of adaptation. This finding is consistent with the experimental results presented in Section 3.2.2, where strain interactions appeared to play a critical role in shaping evolutionary outcomes.

In the next step, we considered competition for resources between the wild-type and mutant populations. We found that mutant frequencies are consistently higher in full migration compared to full isolation, indicating that migration facilitates adaptation by allowing successful lineages to spread across demes. This effect is amplified under stronger bottlenecks and smaller initial population sizes, where demographic noise plays a greater role. The divergence from the null model highlights the importance of competitive interactions in shaping evolutionary outcomes. Additionally, we observed that the establishment probability depends on the interplay between migration and bottleneck strength: it is higher in full isolation compared to full migration under strong bottlenecks, higher in full migration compared to full isolation under intermediate bottlenecks, and similar across both scenarios when bottlenecks are weak. These results show that migration, when combined with ecological interactions and demographic stochasticity, can substantially influence evolutionary dynamics in fragmented populations.

As a final step, we considered competition during bottlenecks, i.e., bottlenecks have a fixed size - after each bottleneck event, each deme is restored to a predetermined population size, mimicking the transfer protocol used in Mahrt et al. 2021. Same as the case when competing for resources, we found that mutant frequencies are higher in full migration compared to full isolation. However, more intriguing differences emerged when we examined the establishment probability of new mutants: for shorter time between bottlenecks, the establishment probability is higher in full isolation, while as the time between bottlenecks increases, differences between both migration scenarios becomes negligible, likely because larger mutant populations become less susceptible to stochastic loss from bottlenecks. Additionally, we compared outcomes under deterministic versus stochastic assumptions in population growth and bottleneck sampling. When either of the sources is deterministic, mutant frequencies were overestimated, and substantial differences emerged between both migration scenarios. Our results underscore the importance of accounting for stochasticity in both population dynamics and bottleneck events, as ignoring these effects can lead to remarkable misestimations of

mutant establishment and evolutionary outcomes.

Although motivated by microbial experiments, this study has broader implications for understanding adaptation in meta-populations, which is also of relevance to ecology and conservation. A manuscript detailing this work is currently in preparation.

3.3 Deviations from the original concept

The original concept of my research proposal included three objectives. Objective 1 was explored as originally intended, with extensions of the model that were not initially planned but that enhanced the analysis of our study. These extensions included the estimations of establishment probabilities of new mutants, the inclusion of mutants with low and high fitness, the estimation of selection coefficients, and a much more profound data analysis.

The experimental study presented in Section 3.2.2 was not initially part of the original proposal. However, the experimental design aligned closely with the models we were developing in Objective 1 to assess the effects of population bottlenecks on antibiotic resistance evolution. Recognizing this alignment, we integrated the study into the project and used it as a foundation for developing a model to address Objective 2. The model developed has the addition of incorporating the mixing of subpopulations, which proved to be of great value for investigating the effects of competition and stochasticity on resistance evolution, in line with the original goals of Objective 2. Due to the substantial time and effort dedicated to the newly integrated experimental study, as well as the extensions to the model developed in Objective 1, it was not feasible to fully pursue Objective 3 within the original project timeline.

3.4 Description of the handling of research data generated in the project and the data infrastructures used

This project used both existing experimental data and new data generated through numerical simulations. Experimental datasets were provided by members of the Schulenburg lab (Dr. Niels Mahrt and Dr. Aditi Batra) and included bacterial growth measurements under various antibiotic treatments. These data were used to inform and validate our models. Simulated data, generated using C++, were performed using the computing clusters of CAU Kiel and Max Planck Institute for Evolutionary Biology.

To ensure data quality, all simulation scripts were version-controlled and well-documented. Metadata was included for all outputs, and simulations were tested for consistency and reproducibility. All code and data will be made publicly available upon publication, either as

supplementary material or through an open-access repository such as Zenodo. These resources will include sufficient documentation to allow for reuse by other researchers using standard software such as MATLAB and R.

3.5 Bibliography

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4 Published Project Results

4.1 Publications with scientific quality assurance

4.2 Other publications and published results

Aditi Batra, Leif Tueffers, Kira Haas, Tabea Loeblein, João Botelho, Michael Habig, Daniel Schuetz, Gabija Sakalyte, Florian Buchholz, Ernesto Berríos-Caro, Hildegard Uecker, Daniel Unterweger, Hinrich Schulenburg, 2025. Resistance variation and bacterial interactions shape the adaptation of a genetically diverse bacterial population to antimicrobial treatment. (bioRxiv, doi: https://doi.org/10.1101/2025.04.01.646401).

4.3 Patents (applied for and granted)

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