# Pathogen evolution and spread in the face of host genetic diversity




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*A thesis submitted for the degree of*

**Doctor of Philosophy**

Hilary 2020

# Declaration

I declare that I wrote this thesis and that the work is my own except where explicitly stated in the text. This work has not been submitted for any degree or professional qualification except as specified.

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Hilary 2020

# Acknowledgements

I whole heartedly thank my amazing supervisor, Kayla King, for her unwavering support, encouragement, and guidance throughout this PhD experience. In addition to providing endless chocolate supplies during lengthy experiment procedures. I thank Mike Bonsall for the many interesting conversations about evolutionary theory. I would also like thank all the past and present members of the King Group: Suzie Ford, Charlotte Rafaluk-Mohr, Alex Betts, Jordan Sealey, Georgia Drew, Kieran Bates, Emily Stevens, Maria Ordovas-Montanes, Dylan Dahan, Anke Kloock, Signe Lagercrantz, Vicky Pike, and Andrea Gomez Chamorro. I do not know how I would've managed without 'crafternoons' or 'zoo support'. Also, a big shout-out for all the friends made at Hertford college who adopted me early on in my time at Oxford. Especially the pals: Liisa Parts, Georgia Hole, Emily Baker, Antonis Dimopolous, and Brooke Johnson. You guys introduced me to many fancy-pants oxford dinners, summer boat races, pub quizzes, wine bar times, endless coffee breaks, and fun chill-out times in the beautiful parks of Oxford.

A special thanks to the Falmouth-made buddies: Olivia Lee, Abi Colclough, and Aimee Murray. Thank you for always having wine and/or tea, for being great listeners, for always being around, and for the many laughs.

Finally, I would like to thank my family, especially my parents Jill and Lennart who have been incredibly supportive throughout. I am truly fortunate to have such funny, strong, and intelligent people in my life. I dedicate this thesis to you both.

# Publications and Contributions

The following published (and in prep) papers have arisen from this thesis, and are presented in Chapters 2,3, and 4:

**Chapter 2**

**Ekroth, A.K.E.**, Rafaluk-Mohr, C. & King, K.C. (2019) Host genetic diversity limits parasite success beyond agricultural systems: a meta-analysis. *Proceedings of the Royal Society B: Biological Sciences*, 286 (1911), 20191811.

A.K.E.E. and K.C.K. conceived and designed the study. A.K.E.E. gathered the data and performed the statistical analysis with C.R-M. A.K.E.E. and K.C.K. wrote the paper.

**Chapter 3 and 4**

**Ekroth, A.K.E.**, Gerth, M., Stevens, E.J., Ford, S.A. & King, K.C. (2020) Host relatedness shapes the evolution of a novel bacterial infection. (*Submitted*)

A.K.E.E. and K.C.K. conceived and designed the study. A.K.E.E. performed the experiments and analyzed the data. A.K.E.E., S.A.F., E.J.S., and K.C.K. interpreted

the data. M.G. and A.K.E.E. conducted the bioinformatic analysis. A.K.E.E. and K.C.K. wrote the paper with input from all authors.

I contributed to one other project, currently in prep for journal submission:

Rafaluk-Mohr, C., Gerth, M., **Ekroth, A.K.E.**, Sealey, J., Kloock, A., Aboobaker, A.A. & King, K.C. Host adaptation to microbe-mediated protection limits the Red Queen. (*In prep*)

# Abstract

Despite the strong forces of genetic drift and directional selection, virtually all natural populations are genetically diverse. Declining within-host population diversity is nevertheless a growing problem, with homogenous populations (known as 'monocultures') predicted to suffer the most from pathogen (and/or parasite, herein used interchangeably where the same principles apply to both) infection given the increasing likelihood of uniform susceptibility. The literature has shown conclusively that genetically diverse populations stand a better chance of limiting infection by natural pathogens (otherwise known as the monoculture effect) or pathogens for which genetic variation for resistance exists in the host population. However, the impacts of within- and between-host population genetic relatedness in the face of novel pathogens has not been tested. In this thesis, I combined meta-analytic and experimental evolution approaches to explore the impacts of host population genetic diversity on pathogen spread and evolution. Firstly, I gathered data from a wide range of host-pathogen interactions to test the effects of two levels of host within-population diversity (low and high) on parasite success in a formal meta-analysis. Among other findings, this study revealed broad-spectrum support for the observation that low diversity increases the chance of parasite success across systems. Secondly, I used experimental evolution and follow-on genomics analysis to track the evolutionary dynamics of *Staphylococcus aureus* as a novel infection in an array of natural, susceptible *Caenorhabditis elegans* isolates. I found that the pathogen evolved similar virulence in closely-related hosts, with infection load evolving along an independent trajectory. I also found that *S. aureus* performs better (i.e., more virulent and higher infection loads) in a diverse population of distantly-related hosts

compared to a population of closely-related hosts. Thirdly, I investigated whether *S. aureus* maintained its generalist infection ability throughout evolution in the novel *C. elegans* populations. I discovered that despite evolving in monocultures of particular host isolates, evolved *S. aureus* was able to infect sympatric and novel hosts equally. Moreover, pathogen performance between these host types was not negatively related to the genetic distance between them. As a whole, this body of work confirms that the host genetic diversity shapes the ecology and evolution of natural and novel pathogens across the tree of life. It additionally extends on knowledge of the monoculture effect to the wider concern of novel infections, and thereby improves our understanding of how host population genetic composition might be used to control the spread and evolution of new emerging infectious diseases.

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# 1

# Introduction

## 1.1 The ‘monoculture effect’

Host diversity is predicted to keep disease spread at bay. By increasing the variety of individuals or species we are reducing what is known as a ‘monoculture effect’. The term ‘monoculture effect’ was first described in agriculture where pathogen spread resulted in heavy yield losses of homogenous crops[1–5]. As a result, multiline cultivars and cultivar mixtures were introduced to increase host genetic diversity[1,2,6]. By increasing host genetic diversity, this increases the likelihood of pathogens coming into contact with resistant hosts, thus reducing the probability of widespread infection[7,8].

Theory has also predicted that, relative to genetically diverse host populations, homogenous host populations are more likely to suffer from pathogen infection[9–14]. When one host genotype can only be infected by one pathogen genotype, Lively (2010) showed that the

number of genotypes within a host population was inversely proportional to the pathogen's ability to establish. By modelling this highly specific interaction, Lively (2010) revealed that through a 'matching alleles' infection genetics framework[15], host diversity could inhibit pathogen spread through a population. Even without a strict matching allele scenario, whereby host genotypes merely vary in levels of susceptibility, genetically diverse host populations reduce the severity of epidemics[11]. Together, these theories show the effectiveness of genetic variation in resistance in a host population to counter the transmission and impacts of infectious disease.

There has been a surge in experimental work both in the lab[7,8,16,17] and field[18–21] showing that host population genetic diversity can limit disease spread. However, it is unclear whether additional factors may also be influencing parasite infection success within a host population, such as host density, host-parasite specificity, and multiple parasite species infection. These factors, among others, have separately been shown to determine parasite transmission. Contact rates, for example, may increase in dense host populations[22,23]. The size of the parasite (microparasite or macroparasite) may influence parasite genotype specialism[24–26]. Multiple parasite infection can sometimes cause higher parasite success in homogenous host populations in comparison to single parasite species infections[21,27]. Moreover, parasite success is recorded in a variety of different ways (e.g., parasite load, parasite prevalence, or host mortality in the honeybee (*Apis mellifera*)[17]), which may affect whether the monoculture effect is detected in a study. There is therefore a need to determine whether, among all these factors in host-parasite systems, host genetic diversity has a main effect in parasite success. Furthermore, the monoculture effect is often measured in naturally occurring, likely evolving host-pathogen interactions, where underlying host resistance is likely to be found. It is

therefore unclear whether diversity might offer the same protection in host populations that are completely susceptible to novel pathogen infection.

## 1.2 Novel host-pathogen interactions

Rapid worldwide ecological change, largely due to human activity, is escalating the occurrence of emerging infectious diseases[28–30]. These emerging diseases are often different from coevolving and longstanding interactions as host populations are unlikely to share any evolutionary history with the novel pathogen. Thus, understanding when and how these pathogens might spread and evolve within a novel host is important to managing their spread.

There are a range of opportunities under which a novel pathogen can emerge in a new host population[30,31]. Novel host-pathogen interactions occur as a result of host spill-overs[32,33], host species jumps[34], the evolution of a new pathogen trait[35,36], or pathogen invasion of a new environment[37,38]. These scenarios will differ depending on the nature of host-pathogen interaction. For example, the emergence and transmission of rabies into African wild dogs (*Lycaon pictus*) was the result of a spill-over from domesticated dogs as they increasingly shared a geographic area and frequently came into contact[32,39]. The introduction of the myxoma virus is a classic example of a species jump via a mosquito vector from the South American tapeti (*Sylvilagus brasiliensis*), in which the virus causes limited harm, into the European rabbit (*Oryctolagus cuniculus*), a host which suffers much more from viral infection[34,40]. Pathogens may also suddenly become hypervirulent. The emergence of novel traits in the H5 and H7 avian influenza viruses are shown to have frequent increases in virulence due to high mutation rates[35,41]. Finally, Chikungunya virus is an example where a pathogen invades a new environment through the movement of infected humans from one continent to another[37]. Novel infections can occur in host populations that vary in

resistance[8,42] or have no prior resistance to a pathogen[43]. In this thesis, I focus on novel infections whereby a host species has no prior genetic variation for resistance towards the pathogen (unlike in Longdon *et al.* 2011).

It is unclear how the genetic composition of the host population will affect the evolutionary dynamics and outcomes of emerging infectious diseases. Pathogens are sometimes predicted to evolve into generalists when faced with genetically diverse hosts, and evolve into specialists in genetically homogenous hosts[44,45]. Indeed, some *Salmonella* species have evolved wide host ranges which permit them to spill-over and infect a multitude of animals, including domesticated species[46]. By having static host populations, i.e. host populations that are not allowed to evolve, we can observe the changes in pathogen virulence over time[47], before resistance emerges.

## 1.3 Experimental evolution of pathogens

Experimental evolution is a widely used approach to studying the processes of pathogen evolution in real time. By manipulating abiotic and/or biotic factors, experimenters can track the frequency of pathogen adaptations at the genotypic and phenotypic level[48–50]. For example, the use of antibiotics in human and animal medicine can shape pathogen evolution by inhibiting bacterial growth, before resistant genotypes emerge and spread through the host population. These resistant genes, found on the chromosome or in mobile genetic elements, allows for gene presence to be measured and the spread within a bacterial population to be quantified[48]. Experimental evolution has also revealed that pathogen evolution can be driven by the presence of a protective microbiota within the host. Antimicrobial superoxides produced by the protective microbes can suppress within-host growth, leading to reduced pathogen fitness[49]. The protective microbe also limits pathogen within-host growth through

the uptake of siderophores produced by the pathogen. As siderophores are known to regulate the production of virulence factors in the pathogen, the selection pressure produced by competition with the protective microbe favours the evolution of reduced virulence[51]. By manipulating the ability of hosts to also evolve in the passaging procedures, it has been shown that host coevolution is a major selective force against pathogens. The evolution of detection and removal mechanisms in hosts, and the evolution of avoidance or suppression mechanisms in the pathogen can occur[50]. For example, in the bacteria-phage system, the CRISPR-Cas adaptive immune system will recognise and clear phage infection by inserting a piece of phage DNA into its own genome (known as a 'spacer')[52]. The phage will then evolve a mutation in the target sequence to regain infectivity (known as a "protospacer")[53].

As host genetic diversity gives rise to more variation in resistance encountered by pathogens, there is stronger selection on pathogens to evolve in response[8,54]. Experimental evolution of a virus in a mouse host model has shown that pathogen fitness and virulence levels are affected by the variability in host genetic backgrounds[54]. As pathogens pay limited costs associated with causing host mortality in serial passage experiments (transmission is not reduced), we may expect an increase in pathogen virulence over time[55,56]. All work to date has been conducted on natural host-pathogen interactions for which there is likely an evolutionary association. We thus have less understanding of initial pathogen evolution in a novel host population with no evolutionary history of infection.

## 1.4 *Caenorhabditis elegans* as a model animal host

The free-living nematode, *C. elegans*, is a useful system for exploring host-pathogen interactions by applying an experimental evolution approach. These animals feed off a plethora of bacterial species found in their natural soil and compost environments[57]. With

well over 150 different wild strain isotypes (and even higher numbers of different isolates) archived by the Caenorhabditis Genetics Center (https://cgc.umn.edu/strains?vt=wild), these animals are adapted to climates globally. Isolate variation has been found at the phenotypic level[58], whereby animals exert different avoidance strategies to reduce infection. However, even though these animals are ubiquitous, extensive genomic investigation has shown limited diversity at the genomic level[59,60]. This is likely to be due to hermaphroditic selfing in *C. elegans*, resulting in reduced within-species diversity, and subsequently leading to haplotype similarity across the globe[61]. Alternatively, Andersen *et al* (2012) argue that their comprehensive study of 200 wild isolates has shown that the sharing of large haplotypes is likely due to a global sweep of beneficial alleles driven by positive selection[60]. However, as there are hundreds of fitness related *C. elegans* genes, the identification of beneficial alleles is unclear.

There are many advantages in using *C. elegans* as a model organism for studying host-pathogen interactions[62–65] and animal innate immune defence[66]. With a completely sequenced and highly annotated genome (100 Mb), analysis into innate immune defence along with genomic evolution can also be determined. Upon pathogen infection, *C. elegans* are able to activate defence-related molecules, such as the MAPK cascade signalling pathways, similar to those found across plants, invertebrates, and mammals[66–68] making it possible to draw parallels between innate immune functions across species. The innate immune system of *C. elegans* is also highly specific, where it is capable of recognising and responding to specific pathogens[66]. With the lack of an adaptive immune system, exploring the mechanisms of the innate immune system is easier in this non-vertebrate host.

There are numerous practical benefits to using *C. elegans* as a host animal. It is relatively easy and cheap to maintain large population sizes: they are fed *E. coli* strain OP50 bacteria which grows quickly in basic Luria Broth, they are kept on Nematode Growth Media which has a well-established recipe (wormbook.org), and can be archived at -80°C for rapid resurrection at a later date. The nematodes are simultaneous hermaphrodites, capable of self-fertilization, and so homogenous lines of hermaphrodites can be easily maintained, as males arise at population frequencies of <0.2%[69]. Morphological differences between males and hermaphrodites, where the tails of males are hooked shaped, are easy to spot between individuals under a microscope. These animals also have distinguishable life stages and short life cycles of three days (Figure 1), attributes that make these animals amenable for long-term evolution experiments[66].

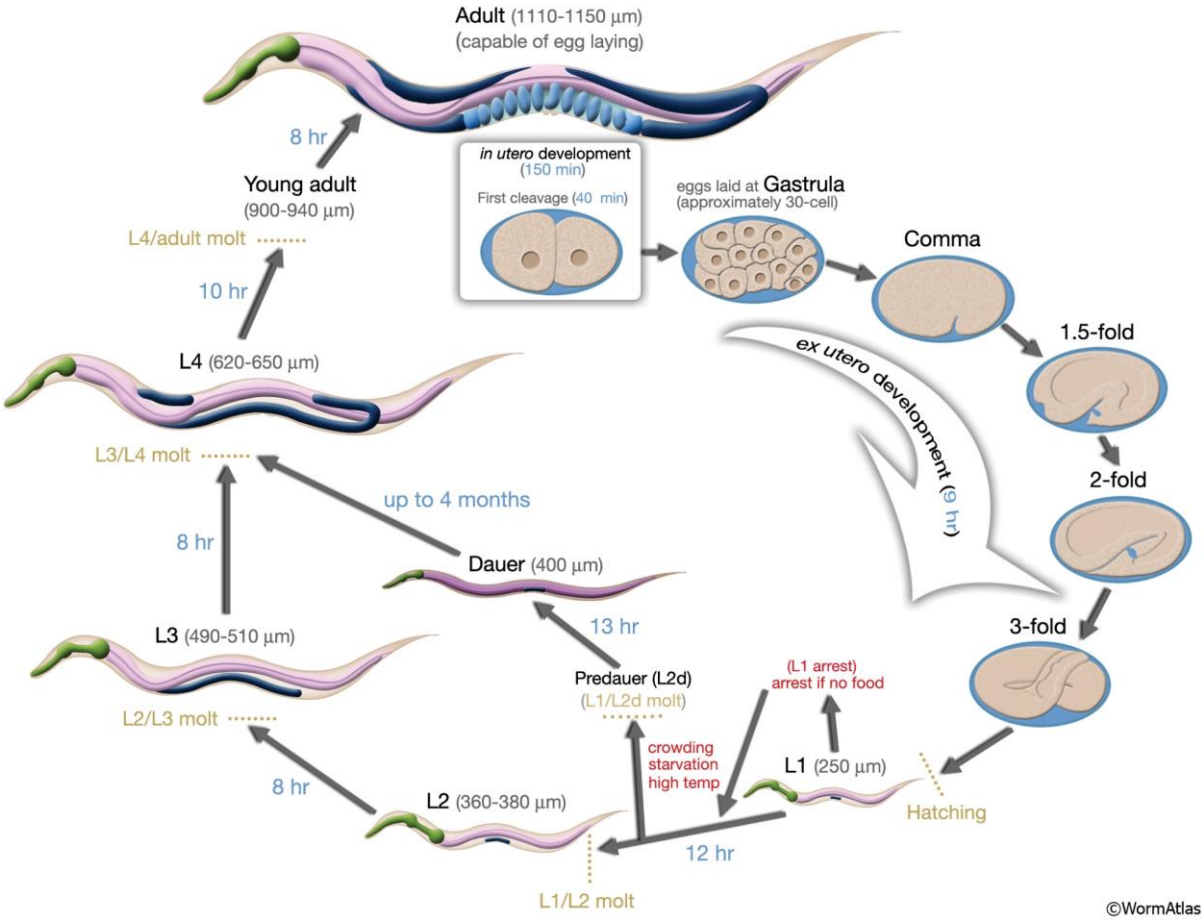



**Figure 1:** *C. elegans* life cycle. *C. elegans* hermaphrodite nematodes develop from egg to adults producing their own fertilized eggs in approximately 3.5 days at 20°C (wormbook.org). Hatched larvae, known as Larval stage 1 or L1s, will feed on NGM (Nematode Growth Medium) plates seeded with *Escherichia coli* strain OP50. If no food is available L1 larvae will arrest their development. Larvae can survive several months of starvation by entering into the stress-resistant larval stage known as the dauer stage[70]. Each larval stage lasts between 8-12h and ends with a period of inactivity, known as lethargus[71], where a new cuticle is formed with the moulting of the old cuticle. After approximately 10h at L4 stage, nematodes will use their self-produced sperm to produce fertile eggs[72]. This period can last between 2-3 days, whereupon the animals will live for several more weeks before dying of senescence (wormbook.org). Image taken from wormatlas.org.

*C. elegans* can be infected by a diverse set of pathogens, natural and novel, which are mostly culturable and easily maintained in a laboratory environment, thereby presenting an ideal system to study pathogen infection dynamics[57]. Naturally coexisting bacterial pathogens will either infect by cuticle attachment (e.g. *Leucobacter* spp.[73]), or ingestion and colonisation of the gut lumen (e.g. *Pseudomonas aeruginosa*[64], *Bacillus thuringiensis*[74], and *Serratia marcescens*[75]). Cuticle attachment will occur next to the rectum, causing swelling and adherence to other animals (known as "worm stars"), and affect locomotion[57,73]. Intestinal damage occurs from bacterial gut accumulation, where released virulence factors[64] or toxin producing spores[57,76] will eventually kill the host. Moreover, fungal pathogens, such as *Drechmeria* and *Harposporium*, are found naturally with *C. elegans*, wherein infection occurs from ingested spores or hyphae adherence to the animal cuticle[77]. Microsporidia (e.g. *Nematocida parisii*[78]) and viruses (e.g. Orsay virus[79]) are also found alongside *C. elegans* and cause infection through the intestinal cells by DNA or RNA injection resulting in the loss of the internal gut structure of the host. Indeed, as all these naturally occurring pathogens and parasites infect the gut and/or cuticle of the *C. elegans* host, much is known about this host-pathogen system.

Opportunistic pathogens, such as *Staphylococcus aureus*[80], *Enterococcus faecalis*[51], and *Streptococcus pneumoniae*[81] have also been extensively used to study host-pathogen dynamics in evolution[51] and coevolution[80] experiments with *C. elegans*. These interactions have allowed for the measurement of trade-offs between resistance and host growth rates[82] and the assessment of patterns of pathogen local adaptation[74,83]. Pathogen load measurements can be directly taken as these pathogens colonise the nematode gut. As bacterial pathogens can cause mortality in *C. elegans*, virulence factors relating to degree of host harm can be identified in this animal model[84]. In fact, the exploration into virulence factors that target host

immune responses or cellular processes, similar to those shared by other animals, have provided useful insights into immune system functions, which are otherwise difficult to measure in a more complex animal[68,85].

## 1.5 *Staphylococcus aureus*

*Staphylococcus aureus* is a Gram-positive bacterium, capable of being a commensal and an opportunistic pathogen in humans as well as animals[86]. When pathogenic, *S. aureus* is capable of causing a wide range of conditions, some of which include; skin infections, endocarditis, abscesses, and bacteraemia[87]. Due to the fact that more than 80% of *S. aureus* strains produce penicillinases, the likelihood of acquiring resistance towards β-lactam antibiotics such as methicillin is high[88]. Indeed, MRSA (Methicillim-Resistant *Staphylococcus aureus*) is a problem found across animals; humans[89], cattle[90], horses[91], poultry[92], sheep[93], pigs[94], dogs[95], and cats[96] as antibiotics are frequently used in attempts to minimise spread. As such, other methods to reduce the spread of *S. aureus*-related infections are highly important for the improvement of both human and animal medicine.

As a result of the close proximity of humans and domesticated animals, host shifts between species have been observed on multiple occasions[97–100]. For example, human to livestock host jumps has resulted in mastitis in cows[98] and ‘bumble foot’ in chickens[99]. In one instance, the change in a single nucleotide polymorphism resulted in an infection causing skin abscesses in a novel rabbit host[100]. Host shifts back into humans from cows has on occasion resulted in a pandemic[101]. Whereas in other cases, adaptation to cows has resulted in the inability to re-infect humans[102]. This ability to host switch between species shows the high adaptability of this pathogen and the large host range it has acquired as a result.

Mechanisms underlying pathogenic response to host innate immune defences can also be explored in *S. aureus*. *S. aureus* produces a host of disease-causing virulence factors, many of which are the result of metal limitation in the host environment[103]. As a response to infection, the host innate immune system will restrict essential nutrients required for bacterial growth, such as iron, manganese, and zinc[103,104]. To circumvent this limitation, bacteria can produce iron-binding compounds known as siderophores, utilise host heme through cell surface receptors to obtain host iron, or express ferrous iron transporters or lactoferrin receptors to steal iron from host proteins[103]. In the absence of other metals, *S. aureus* will express Mn transporters MntH (a protein) and MntABC (an ATP-binding permease)[105] for manganese uptake, and Zn importers AdcABC permease and CntABCDF[106] to compete with the host for Zinc uptake. Therefore, when studying host immunity, upregulation or downregulation of genes associated with metal production can inform on the dynamics of *S. aureus* infection.

## 1.6 Thesis outline

In this thesis I will develop on the above themes. Specifically:

In **Chapter 2**, I review and combine published data on the effects of within-population genetic diversity on parasite success in a meta-analysis. I examine whether there is broad support for the role of host genetic diversity on parasite success and account for the contribution of other factors beyond diversity – e.g., biological traits of the host-parasite system, study settings, and measures of parasite success. I found that (i) host diversity defends against parasite success across systems, (ii) host diversity offers greater protection towards microparasites whereas it has little to no effect on macroparasites, specifically ones that transmit in a density-dependent fashion, and (iii) the effect of host diversity is stronger in

field studies compared to lab studies. I discuss the importance of preserving within-species genetic diversity for vulnerable populations.

In **Chapter 3**, I investigate the effects of host relatedness on pathogen evolutionary dynamics and outcomes. I show that pathogen traits evolve along different trajectories in monoculture host populations, with virulence linked to host genetic relatedness and pathogen load linked to initial colonisation capabilities of host isolate. I also show that pathogens evolving in diverse host backgrounds have higher infection loads and cause higher host mortality in comparison to selection in host monocultures. The results highlight that host diversity may not reduce pathogen prevalence if all hosts within the population show variation in levels of susceptibility with no underlying resistance present.

In **Chapter 4**, I extend the work of Chapter 3, to consider the role of host relatedness in shaping parasite specialism or generalism in a novel infection. I show that, despite evolving in either monoculture or polyculture host populations, *S. aureus* remains a generalist pathogen. In fact, I find that *S. aureus* is capable of infecting sympatric and novel hosts equally, even as the genetic relatedness between host isolates decreases. This work highlights the reduced potential for coevolution between host and novel pathogens as the latter have the ability to jump between hosts.

Finally, in **Chapter 5**, I summarise the main findings from Chapters 2-4 and discuss the wider implications of emerging infectious diseases in the current climate. I conclude with suggesting future directions in experimental and theoretical research.

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# 2

# Host genetic diversity limits parasite success beyond agricultural systems: a meta-analysis

This Chapter has been published in *Proceedings of the Royal Society B: Biological Sciences*.

## 2.1 Abstract

There is evidence that human activities are reducing the population genetic diversity of species worldwide. Given the prediction that parasites better exploit genetically homogeneous host populations, many species could be vulnerable to disease outbreaks. Whilst agricultural studies have shown the devastating effects of infectious disease in crop monocultures, the widespread nature of this diversity-disease relationship remains unclear in natural systems. Here, we provide broad support that high population genetic diversity can protect against infectious disease by conducting a meta-analysis of 23 studies, with a total of 67 effect sizes. We found that parasite functional group (micro- or macroparasite) affects the presence of the effect and study setting (field or lab-based environment) influences the magnitude. Our study also suggests that host genetic diversity is overall a robust defence against infection regardless of host reproduction, parasite's host range, parasite diversity, virulence, and the method by which parasite success was recorded.

Combined, these results highlight the importance of monitoring declines of host population genetic diversity as shifts in parasite distributions could have devastating effects on at-risk populations in nature.

## 2.2 Introduction

Most natural populations are genetically diverse[1]. Given there is often specificity between hosts and parasites[2], host population genetic diversity is thought to increase the chance that one or more individuals is resistant to infection. The likelihood of a parasite encountering a susceptible host is thus reduced[3]. Genetically homogenous host populations are conversely predicted to be more vulnerable to infection given the uniformity of host susceptibility. This negative relationship between host genetic diversity and parasite success is often referred to as the 'monoculture effect'[4].

The study of the monoculture effect in agricultural settings is extensive[5–7]. A recent meta-analysis showed that with increased diversity in intraspecific cultivar mixtures, disease presence is reduced and crop yields increased[7]. However, crop plants are under artificial selection for high yield, and may therefore exhibit less genetic polymorphism than hosts in the wild. We consequently know little of the extent to which low genetic diversity influences parasite success across species and environments beyond agricultural contexts.

Threats to within-species genetic diversity are on the rise. There is evidence that habitat alterations, pollution, and global temperature changes, as well as the restriction of species geographical ranges may lead to increased genetic drift and reduced population genetic diversity[8,9]. Impacts of humans on local species biodiversity, however, remain controversial[10,11]. Populations with reduced genetic diversity might suffer diminished evolutionary potential[12] and increased inbreeding depression[13,14]. Knowing whether there is an additional threat of outbreaks in these populations is crucial for disease management and species conservation approaches.

Theory has illuminated the dynamics of parasite spread[4,15–18] in diverse host populations as well as examined the level of diversity required to stop transmission[19,20]. However, whether population genetic diversity can impact parasite success in nature more broadly remains unclear for several reasons. Firstly, given that parasite transmission can be determined by host density[3], the relative effects of density versus host genetic diversity need to be elucidated[20]. Shrinking habitats, for example, can result in higher population densities (and lower resource availability) where parasites can transmit better due to more contact between hosts[21,22]. Secondly, even when focusing on host genetic diversity alone, there is great variation across systems in the conditions under which infection and diversity are measured. For example, there is a clear difference between microsporidian *Nosema bombi* infections of diverse versus genetically homogenous bumble bee (*Bombus terrestris L.*) populations in comparison to trypanosomelid *Crithidia bombi* infections under the same host conditions[23]. There is also a clear difference when parasite diversity is taken into account. For example, we see an increase in parasite success on homogenous host populations when multiple parasite species infect[23–26] but not always between one host-parasite species pair[27,28]. Thirdly, because parasite success is measured differently across studies, and even within systems, there is the potential that the relevant measure of parasite success is not used. For example, in honeybee (*Apis mellifera)* host populations, genetic diversity has a negative impact on parasite success when infection prevalence or parasite load is measured, but not always when host survival is calculated[29]. Host survival might be less informative because of the interplay of virulence, force of infection (i.e. the rate of infection and within population pathogen colonisation), and the timing of infection (i.e. seasonality) might determine the overall spread of pathogens in host populations[30]. It is therefore unclear whether the effect of low host genetic diversity on parasite success is relevant to host-parasite interactions in non-agricultural systems across the tree of life.

We tested the effect of host population genetic diversity on parasite success with a formal meta-analysis across a range of host-parasite systems. We searched the published literature for all publicly available data sources and compared the effects of low and high host genetic diversity on parasite success using Hedges's effect size *g* (with positive values indicating an effect of low host genetic diversity on parasite success) with a nested random mixed effects meta-analysis model. We also tested whether biological traits, associated with the species in the interaction, as well as study settings and measures could explain variation in the effects of genetic diversity on parasite success.

## 2.3 Materials and methods

### 2.3.1 Literature search

In July 2019, the literature was searched using keyword searches on Web of Knowledge, Google Scholar and PubMed, with a subset of the terms 'host genetic diversity', 'low versus/and high host genetic diversity', 'heterogeneous versus/and homogenous host populations', 'monoculture effect', 'disease spread', and 'parasite prevalence' to investigate the effect of low versus high host population diversity on parasite disease impact (see Supp. Fig. 1 for PRISMA flowchart[31] summarising study collection process). We gathered data of parasite success in host populations of varying genetic diversity. We define 'parasite success' as any measure of a parasite's ability to proliferate within a host population reported in a given study. As parasite presence within a host population is measured differently across studies, the following terms were included as measurements of parasite success; parasite load, parasite virulence, parasite abundance, host mortality rate, viral concentrations, viral

load, infection rate, and infection intensity. We also checked reference lists for other potential papers. Studies were also searched for and extracted from review papers.

Papers were included in this study if they met the following inclusion criteria:

i. The study was published in a peer reviewed academic journal.

ii. The study collected parasite success data from two distinct comparable host population groups with any measured difference in diversity, such as low versus high genetic diversity, inbred versus outbred, and monoculture versus polyculture.

iii. In the study, both host population groups contained the same species.

iv. The study measured genetic diversity at the host population level and not community diversity or individual-level genetic heterozygosity.

v. The study was not conducted in an agricultural system.

vi. The study did not interfere with parasite or host lifecycle, as in passaging manipulations.

We excluded agricultural studies as a recent meta-analysis had already demonstrated the benefits of intraspecific diversity to crop yields (and thus host fitness) in the presence of infectious disease[7].

### 2.3.2 Statistical analysis

We calculated Hedges's *g*, from studies using the method described in Hedges 1981[32] . This is a standard and widely used method of calculating effect sizes in meta-analyses which takes into account small sample sizes[33,34]. To calculate effect size *g*, mean parasite measurements and their standard deviation for each treatment were extracted in the order of low host population diversity and high host population diversity. We extracted data from either paper figures, reported statistics in the text, or raw data received from authors. Where means and standard deviations in each group were not available (2 out of 23 studies), t-values and degrees of freedom were extracted.

We calculated the standard mean differences using the *escalc* function in the package *metafor* in R version 3.6.0 (R core development team) before performing a nested random mixed effects meta-analysis model using the *rma.mv* function. We chose this model to account for the fact that we collected several effect sizes per study, where some studies shared the same host species, which has the potential for pseudo-replication and phylogenetic non-independence. Estimates of effect size *g* were extracted from the model. We first tested for an overall relationship between host population genetic diversity and parasite success using the entire dataset. We then tested whether the magnitude of the relationship was dependent on the following moderator variables: study setting, parasite success measure, host reproduction, parasite functional group, parasite's host range, parasite diversity, and ability of parasite to cause host death (See Supp. Table 1 for variable definitions). The measure of heterogeneity of moderator variables was reported as Cochran's Q test, where Q is the weighted sum of squares about the fixed effect estimate between subgroups[35].

We tested for an effect of both study setting (field or lab-based environments) and parasite success measure on the relationship between host genetic diversity and parasite success. For the latter, we separated measures into three groups based on those used in studies included in the meta-analysis: parasite prevalence, parasite load, and host mortality (Supp. Table 1). Studies looking at overall parasite presence in a host population were placed under the category 'parasite prevalence'. Where measures of parasite propagules per host were taken, studies were placed under 'parasite load'. Measures of mortality within a population were placed under 'host mortality'. In order to incorporate studies publishing survival data, measures of host mortality were taken as the inverse of published survival measures.

We then focused on the impact of host and parasite biological traits on variation in the magnitude and direction of effect sizes. We firstly considered host reproductive mode, given sexual and asexual strategies can generate disparate levels of population genetic diversity. However, one study was placed under a separate reproduction group as the host (*Daphnia magna*) had undergone both sexual and asexual reproduction in the study. Secondly, we looked at infection by parasite functional group ( micro- or macroparasites) as the former tends to be associated with higher mortality[36], and thirdly the parasite's host range (1 host species or >1 host species) as this factor has been shown to have an impact in crop studies[37,38] due to the reduced genetic specificity between hosts and multi-host parasites. Fourthly, we separated studies into three categories – one genotype of one parasite species (1 Genotype), multiple parasite genotypes of one parasite species (>1 Genotypes), and multiple parasite species (>1 Species) – to determine whether the diversity-disease relationship was dependent on parasite diversity. Higher levels of parasite diversity might increase the pool of susceptible hosts in a diverse population. Lastly, we tested whether effect sizes were dependent on the

parasite's ability to cause host death. Compared to less harmful parasites, virulent parasites could select for greater levels and variation of resistance in the host population.

### 2.3.3 Assessing for potential publication bias

Studies that report larger effects are more likely to get published in comparison to studies reporting smaller effects[39]. To check for publication bias, we visualised the spread of our effect sizes by creating a funnel plot (Supp. Fig. 2). We then performed a Fail-Safe N analysis to calculate the number of additional studies needed to reduce the significance level of the weighted average effect size[40].

## 2.4 Results

We found 32 unique host-parasite interactions in 23 papers containing data that followed the inclusion criteria. Papers often included results from multiple experiments or exposures to multiple parasite species. A total of 67 effect sizes were retrieved from this data set, covering a diverse range of host and parasite species (Table 1).

After the construction of a funnel plot, we find no indication of a publication bias in this meta-analysis data set, with the majority of points falling symmetrically within the plot (Supp. Fig. 1). The unusual shape of the plot can be explained by the fact that small sample sizes were predominantly found in laboratory studies, whereas large sample sizes were predominantly found in field studies. Consequently, studies with large sample sizes had higher errors than those with small sample sizes explaining the shape of the plot (we highlight this by colourising the plot by study setting). Rosenberg's Fail-safe N analysis

showed that an additional 604 studies would need to be added to reduce the significance level of this meta-analysis.

Our results are consistent with the hypothesis that low host genetic diversity results in higher parasite success ($g = 0.3527$, $p < 0.0001$, Fig. 1A). We found that the effect size is influenced by study setting ($Q = 9.2111$, d.f. = 1, $p = 0.0024$, Fig. 1B), where the magnitude of the effect size is significantly greater for field studies ($g = 0.7003$) in comparison to lab studies ($g = -0.5249$). Parasite success measures used in the studies do not significantly influence the effect size ($Q = 2.6526$, d.f. = 2, $p = 0.2655$, Fig. 1C).

We found no evidence of an effect of host reproduction on the direction or magnitude of the effect size ($Q = 4.0711$, d.f. = 2, $p = 0.1306$, Fig. 2A), even when we excluded the Daphnia study by Altermatt & Ebert (2008) ($Q = 0.9147$, d.f. = 1, $p = 0.3389$). Conversely, we found that the effect size was dependent on parasite functional group ($Q = 8.3621$, d.f. = 1, $p = 0.0038$, Fig. 2B). The success of microparasites ($g = 0.6277$), and not macroparasites ($g = -0.1725$) was limited by high host population genetic diversity. Neither the direction nor magnitude of the effect size was influenced by host range ($Q = 0.2864$, d.f. = 1, $p = 0.5925$, Fig. 2C), parasite diversity ($Q = 3.1047$, d.f. = 2, $p = 0.2118$, Fig. 2D), or whether parasites caused host mortality ($Q = 3.5504$, d.f. = 1, $p = 0.0595$, Fig. 2E).

Table 1: Summary of literature on the effect of host population genetic diversity on measures of parasite success across host-parasite systems.

| Source paper | Paper number | Host | Measure of host diversity | Parasite | Host range (ref) | Parasite type | Infection measure | Data source | Data extracted | n Effect sizes |
|---|---|---|---|---|---|---|---|---|---|---|
| **Altermatt and Ebert (2008)** | 1 | *Daphnia magna* | 1 vs 10 genotypes | *Octosporea bayeri* | 1 Host species (41) | Fungus | Parasite load | Figure 2, Raw data | Mean ± SD | 2 |
| **Baer and Schmid-Hempel (1999)** | 2 | Bumblebee (*Bombus terrestris*) | Queens inseminated with sperm from 1 vs 2 or 4 males | *Crithidia bombi*, *Nosema bombi* | >1 Host species (60) | Protozoa, Fungus | Parasite load | Figure 1, Raw data | Mean ± SE | 4 |
| **Baer and Schmid-Hempel (2001)** | 3 | Bumblebee (*Bombus terrestris*) | Queens inseminated with sperm from 1 vs 2 or 4 males | *Crithidia bombi* | >1 Host species (60) | Protozoa | Parasite load, Parasite prevalence | Figure 1, Raw data | Mean ± SD | 4 |
| **Baer and Schmid-Hempel (2003)** | 4 | Bumblebee (*Bombus terrestris*) | Queens inseminated with sperm from 1 vs 2 or 4 males | *Crithidia bombi* | >1 Host species (60) | Protozoa | Parasite load, Parasite prevalence | Raw data | Mean ± SD | 4 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Calleri *et al.* (2006)** | 5 | Termite (*Zootermopsis angusticollis*) | Inbred vs outbred | *Metarhizium anisopliae* | >1 Host species (61) | Fungus | Parasite load | In text | Mean ± SD | 1 |
| **Desai and Currie (2015)** | 6 | Honeybee (*Apis mellifera L.*) | Queens inseminated with sperm from 1 vs 12 drones | *Varroa destructor*, Deformed Wing Virus, Black Queen Cell Virus, Israeli Acute Paralysis Virus | >1 Host species (62-64) | Mite, Virus, Virus, Virus | Parasite load, Host mortality, Parasite prevalence | Figure 1, 2, 4, 5, 7, 8 | Mean ± SE | 11 |
| **Ganz & Ebert (2010)** | 7 | *Daphnia magna* | 1 vs 10 genotypes | *Glugoides intestinalis*, *Ordospora colligate*, *Microsporidium sp. (undescribed species)* | 1 Host species (65) and >1 Host species (65) | Fungus, Fungus, Fungus | Parasite prevalence | Figure 2 | Mean ± SE | 3 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Hale & Briskie (2007)** | 8 | New Zealand Robin *(Petroica australis)* | Bottleneck vs source population | Hippoboscid flies (*Ornithomya spp*. and O*rnithoica spp*.), Feather mite | >1 Host species (66) | Fly, Mite | Parasite load | Figure 1 | Mean ± SD | 2 |
| **Hughes & Boomsma (2004)** | 9 | Ant *(Acromyrmex echinatior)* | 1 patriline vs 3 patrilines | *Metarhizium anisopliae* (strain KVL 02-73) | >1 Host species (61) | Fungus | Host mortality | Figure 4 | Mean ± SE | 2 |
| **Liersch and Schmid-Hempel (1998)** | 10 | Bumblebee (*Bombus terrestris*) | Full sister workers vs mixed workers | *Crithidia bombi*, *Nosema bombi*, *Apicystis (Mattesia) bombi* | >1 Host species (60, 67) | Protozoa, Fungus, Protozoa | Parasite prevalence, Parasite load | Figure 1 | Mean + CI | 2 |
| **Manlik *et al.* (2017)** | 11 | Bumblebee (*Bombus terrestris*) | Island vs land population | *Nosema bombi* | >1 Host species (60) | Fungus | Parasite prevalence | In text | Mean ± SE | 1 |
| **Pearman & Garner (2005)** | 12 | Italian agile frog (*Rana latastei*) | Low vs high population genetic variability | *Ranavirus* (frog virus 3) | >1 Host species (44) | Virus | Host mortality | Figure 2, Raw data | Mean ± SD | 3 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Reber *et al.* (2008)** | 13 | Ant (*Formica selysi*) | Monogynous vs polygynous colonies | *Metarhizium anisopliae* | >1 Host species (61) | Fungus | Host mortality | Figure 1, 2 | Mean ± SE | 3 |
| **Schmidt *et al.* (2011)** | 14 | Ant (*Monomorium pharaonis*) | Inbred vs mixed colonies | *Beauveria bassiana* | >1 Host species (61) | Fungus | Host mortality | Figure 3 | Mean + CI | 3 |
| **Seeley and Tarpy (2007)** | 15 | Honeybee (*Apis mellifera L.*) | Queens inseminated with sperm from 1 vs 10 drones | American foulbrood (*Paenibacillus larvae*) | >1 Host species (71) | Bacteria | Parasite prevalence | Figure 2, Raw data | Mean ± SD | 2 |
| **Shykoff and Schmid-Hempel (1991)** | 16 | Bumblebee (*Bombus terrestris*) | 1 genotype vs 3 genotypes | *Crithidia bombi* | >1 Host species (60) | Protozoa | Parasite prevalence | Figure 2 | t - value | 2 |
| **Smallbone *et al.* (2016)** | 17 | Guppy (*Poecilia reticulata*) | Inbred vs outbred | *Gyrodactylus turnbulli* (strain Gt3) | >1 Host species (68) | Worm | Parasite load | Figure 2 | Mean ± SE | 1 |
| **Strauss *et al.* (2017)** | 18 | *Daphnia dentifera* | 1 genotype vs 3 genotypes | *Metschnikowia bicuspidata* | >1 Host species (70) | Fungus | Parasite prevalence | Figure S1B | Mean ± SE | 1 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Tarpy (2003)** | 19 | Honeybee (*Apis mellifera L.*) | Queens inseminated with sperm from 1 vs multiple drones | Chalkbrood disease (*Acosphaera apis*) | >1 Host species (74) | Fungus | Parasite prevalence | Figure 2 | Mean ± SD | 1 |
| **Tarpy and Seeley (2006)** | 20 | Honeybee (*Apis mellifera L.*) | Queens inseminated with sperm from 1 vs 10 drones | Sacbrood (Iflavirus genus), Chalkbrood disease (*Acosphaera apis*), European foulbrood (*Melissococcus plutonius*), American foulbrood (*Paenibacillus larvae*) | >1 Host species (69,71,74,75) | Virus, Fungus, Bacteria, Bacteria | Parasite prevalence | In text | t - value | 4 |
| **van Houte *et al.* (2016)** | 21 | *Pseudomonas aeruginosa*, *Streptococcus thermophilus* | 1 genotype vs 6, 8, 12, 24, and 48 genotypes and 1 genotype vs 44 genotypes | Bacteriophage (DMS3), Bacteriophage (2972) | 1 Host species (72,73) | Virus, Virus | Parasite prevalence | Figure 2, Raw data | Mean ± SD | 5 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Wargo *et al.* (2012)** | 22 | Rainbow trout (*Oncorhynchus mykiss*) | Inbred vs outbred | Infectious hematopoietic necrosis virus (IHNV) isolates: 220:90 (HV), WRAC 039-82 (LV), FF020-91 (B), FF030-91(C) | 1 Host species (42) | Virus | Parasite prevalence | Figure 2, Raw data | Mean ± SE | 4 |
| **Whiteman *et al.* (2006)** | 23 | Galapagos Hawk (*Buteo galapagoensis*) | Inbred vs outbred | *Colpocephalum turbinatum*, *Degeerialla regalis* | >1 Host species (56) | Louse, Louse | Parasite load | Figure 2, Raw data | Mean ± SD | 2 |

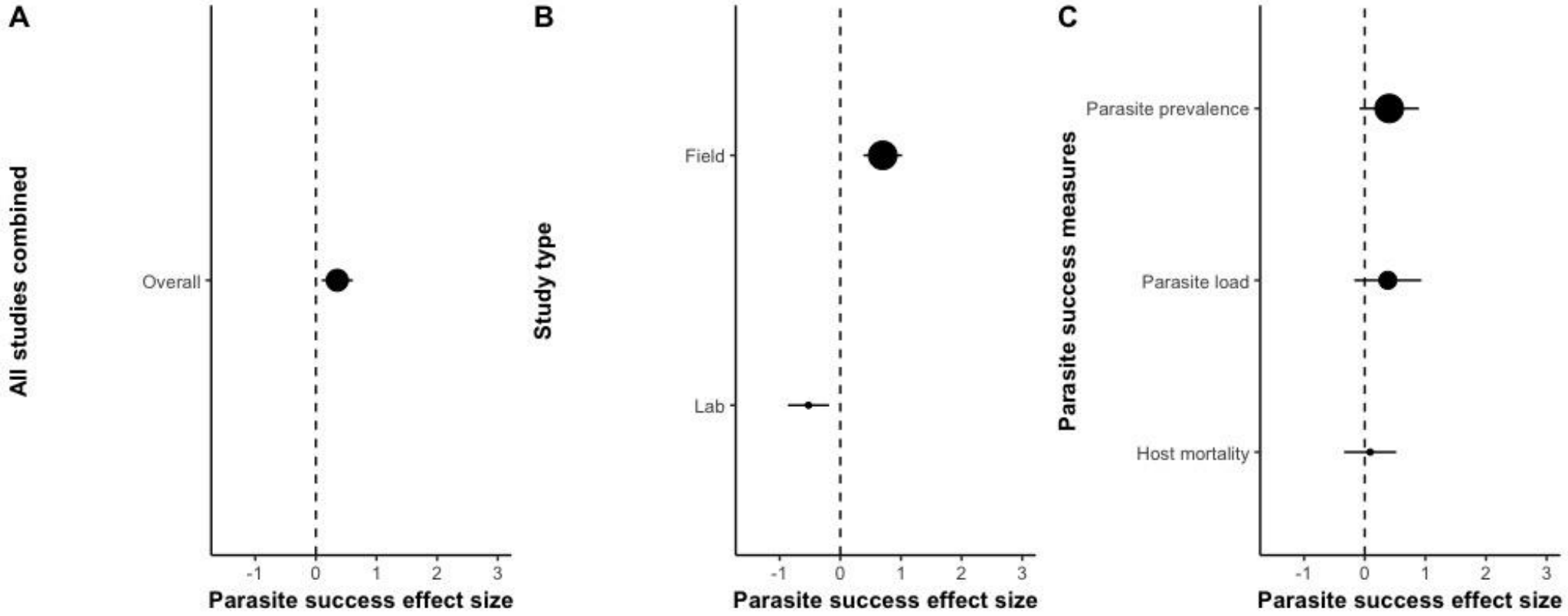



**Figure 1:** Impact of study setting on the effect of host genetic diversity on parasite success. Positive values indicate that low host genetic diversity has an impact on parasite success (i.e., a negative association between genetic diversity and parasite success). Negative values represent the opposite relationship. At an effect size of zero (dashed line), there is no relationship between host genetic diversity and parasite success. (A) Overall effect size (n = 67). (B) Moderator analysis of study type between field (n = 36) and lab (n = 31) studies. (C) Moderator analysis of parasite success measures between parasite load (n = 19), parasite prevalence (n = 35), and host mortality (n = 13). The size of the dot corresponds to the sample size. Effect sizes are shown with 95% confidence intervals.

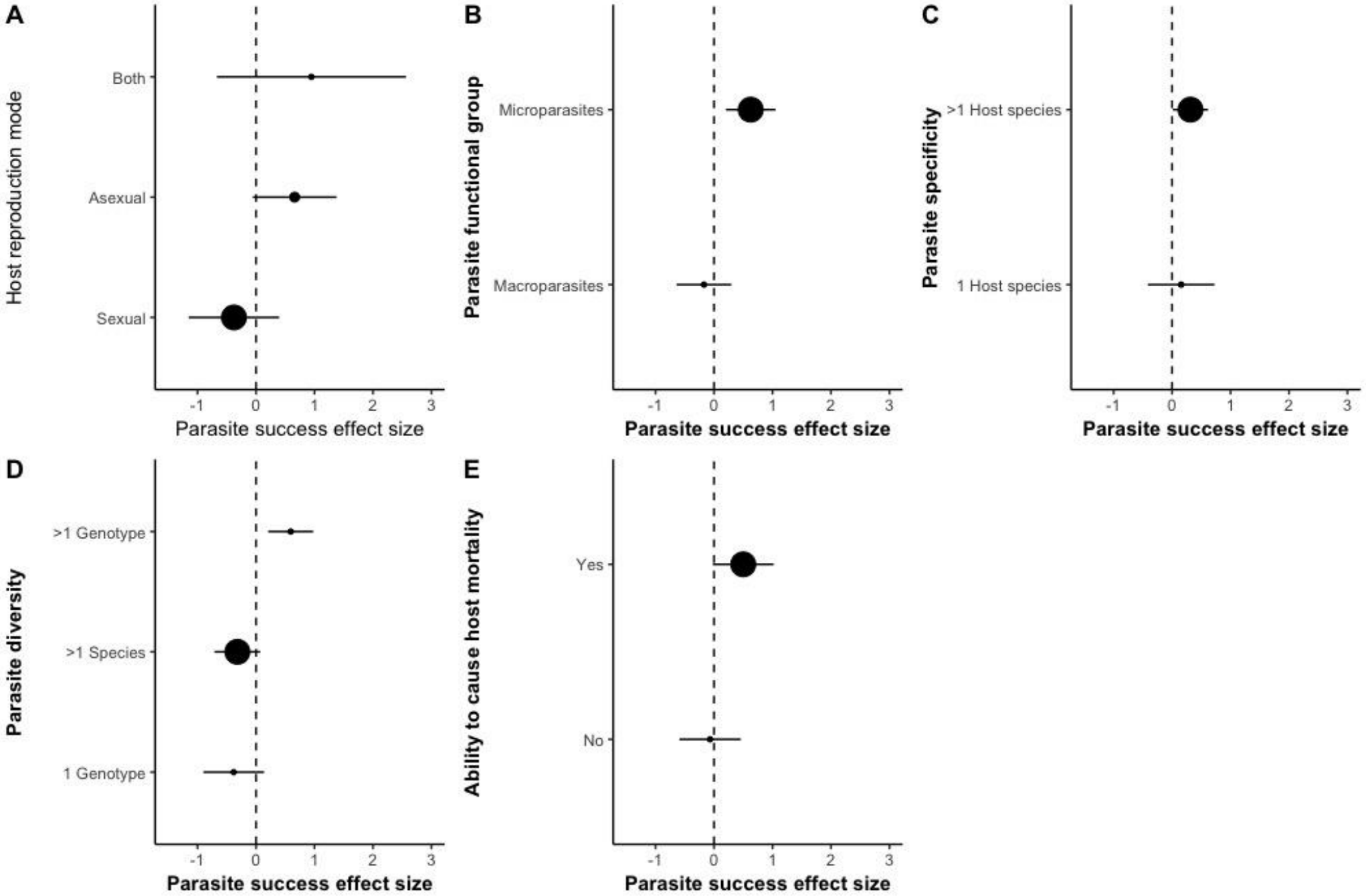

A
Host reproduction mode
Both
Asexual
Sexual
-1
0
1
2
3
Parasite success effect size
B
Parasite functional group
Microparasites
Macroparasites
-1
0
1
2
3
Parasite success effect size
C
Parasite specificity
>1 Host species
1 Host species
-1
0
1
2
3
Parasite success effect size
D
Parasite diversity
>1 Genotype
>1 Species
1 Genotype
-1
0
1
2
3
Parasite success effect size
E
Ability to cause host mortality
Yes
No
-1
0
1
2
3
Parasite success effect size

**Figure 2**: Impact of host and parasite characteristics on the effect of host genetic diversity on parasite success. Positive values indicate that low host genetic diversity has an impact on parasite success (i.e., a negative association between genetic diversity and parasite success). Negative values represent the opposite relationship. The dashed line (effect size of zero) represents no relationship between host genetic diversity and disease spread. Moderator analysis of (A) host reproduction mode: asexual (n = 5), both (n = 2), and sexual (n = 60) effect sizes, (B) of parasite functional group between microparasite (n = 57) and macroparasite (n = 10) effect sizes, (C) host range between 1 host (n = 13) and >1 host (n = 54) parasite effect sizes, (D) parasite diversity between >1 genotype (n = 15), 1 genotype(n = 15), and >1 species (n = 37) effect sizes, and (E) of the ability of a parasite to cause host death, displayed as yes (n = 57) and no (n = 10) effect sizes. The size of the dot corresponds to the sample size. Effect sizes are shown with 95% confidence intervals.

## 2.5 Discussion

Our meta-analysis shows that host population genetic diversity reduces parasite success across multiple natural systems. In particular, we find that host population genetic diversity is effective at limiting microparasite infection success, with little to no effect on the macroparasites tested, and the protection is stronger when measured in the field. Our findings additionally highlight the potential damage that emerging infectious diseases may have on genetically homogenous host populations.

The parasites included in our meta-analysis were highly variable in terms of their host range. However, we found no evidence that a parasite's host range affected its success in host populations of low or high genetic diversity. Indeed, we see evidence of resistance in more diverse populations involving highly specialised interactions[41–43], in broad spectrum interactions at the genotypic level[44], and in those that cross host-species boundaries[25,26,45]. That host range is not a factor here is in contrast to those results found in crop studies. For example, in rusts and powdery mildews, disease severity is driven by a pathogen's host specificity[6]. The mirroring of parasite virulence genes to host resistance genes means that crop mixtures need to contain both susceptible and resistant cultivars to avoid a monoculture effect. When there is a lack of host specificity, mixed cultivar populations are just as susceptible as monocultures. For example, mixed cultivar populations have been observed to be slightly more susceptible to infection[37] or completely susceptible[38] in comparison to monocultures to the fungal pathogen *Mycosphaerella graminicola*. These findings suggest that the threat to crops from generalist parasites is greater than specialist parasites.

Given that host range did not influence whether parasite success was reduced by host genetic diversity, it is possible that novel parasites, just as well-adapted parasites, could have high success in host populations with low genetic diversity. Essentially, homogenous populations could be vulnerable to outbreaks with spill-over or emerging infectious diseases which are less likely to be host specific[46], but for which there is clearly genetic variation for resistance. The resistance to emerging parasites in these cases could be due to historical contact or similar mechanisms of infection applied by parasites with an evolutionary history to the host[8]. Nevertheless, this result is concerning from a conservation perspective as global climate change has the potential to reduce within-species genetic diversity[47] and alter host population ranges[9,48]. Natural movement of individuals between populations has always served to bolster host diversity[9], and introducing new genotypes is an approach applied by conservation biologists to improve population viability[14]. Whilst adding individuals to a population could increase diversity and reduce inbreeding[49], a risk may be that new individuals, new species, and changes in ecological opportunities bring in new parasites to the population[50,51]. There is potential here for an increased overlap between host populations with low genetic diversity and novel infections. Given that we found a stronger effect in field studies, these consequences are of real concern.

The difference in parasite success between diverse and homogeneous host populations was more pronounced in field studies, compared to lab studies, despite the additional environmental noise data collection in nature might involve. One reason could be that less diverse populations in the wild are more susceptible to infection than they are in the lab for reasons unrelated to genetic diversity. Hosts on islands as well as social insects, such as bees[52], ants[53], and termites[54], live in tight proximities to each other making parasite

transmission easier in homogenous populations. The stronger effect in field studies highlights the importance of the maintenance of diversity in natural populations.

In our meta-analysis, the success of macroparasites was not impeded by genetic heterogeneity in host populations. The macroparasites in the studies included herein were all ectoparasites, and their biology may explain our result. Ectoparasite transmission is often dependent on host-to-host contact[55,56] and thus host density is likely a critical factor in parasite success[55]. Host density may play a more important role than host genetic diversity here such that similarly aggregated populations varying in diversity might be equally susceptible to infection. It has been shown that clustering of captive animal populations restricted by movement or wild animal populations restricted by ranges are highly vulnerable to ectoparasites[50,57]. Moreover, host social behaviours, such as grooming[29] or preening[26] can reduce ectoparasite success. In fact, in populations where social grooming is correlated with relatedness, ectoparasite load is dramatically reduced in highly related individuals[58]. Taken together, host diversity on its own does not always explain a reduction in parasite success, particularly in the case of ectoparasites.

Understanding the impact of host population genetic diversity on parasite infection outside of agricultural systems is crucial because of anthropogenic threats to the diversity of wild populations. This meta-analysis reveals that the susceptibility conferred by low host genetic diversity is a widespread phenomenon in nature, with microparasites most likely to encounter resistance in diverse host populations. Indeed, these broad patterns show that genetic diversity is a robust weapon against infection, similar to the effects of species biodiversity[59]. Our findings suggest that further erosion of within-species genetic diversity could drive outbreaks of both coevolving and emerging infectious diseases. Conservation efforts should

focus on preserving population genetic diversity in vulnerable populations to improve their ability to fight off infections.

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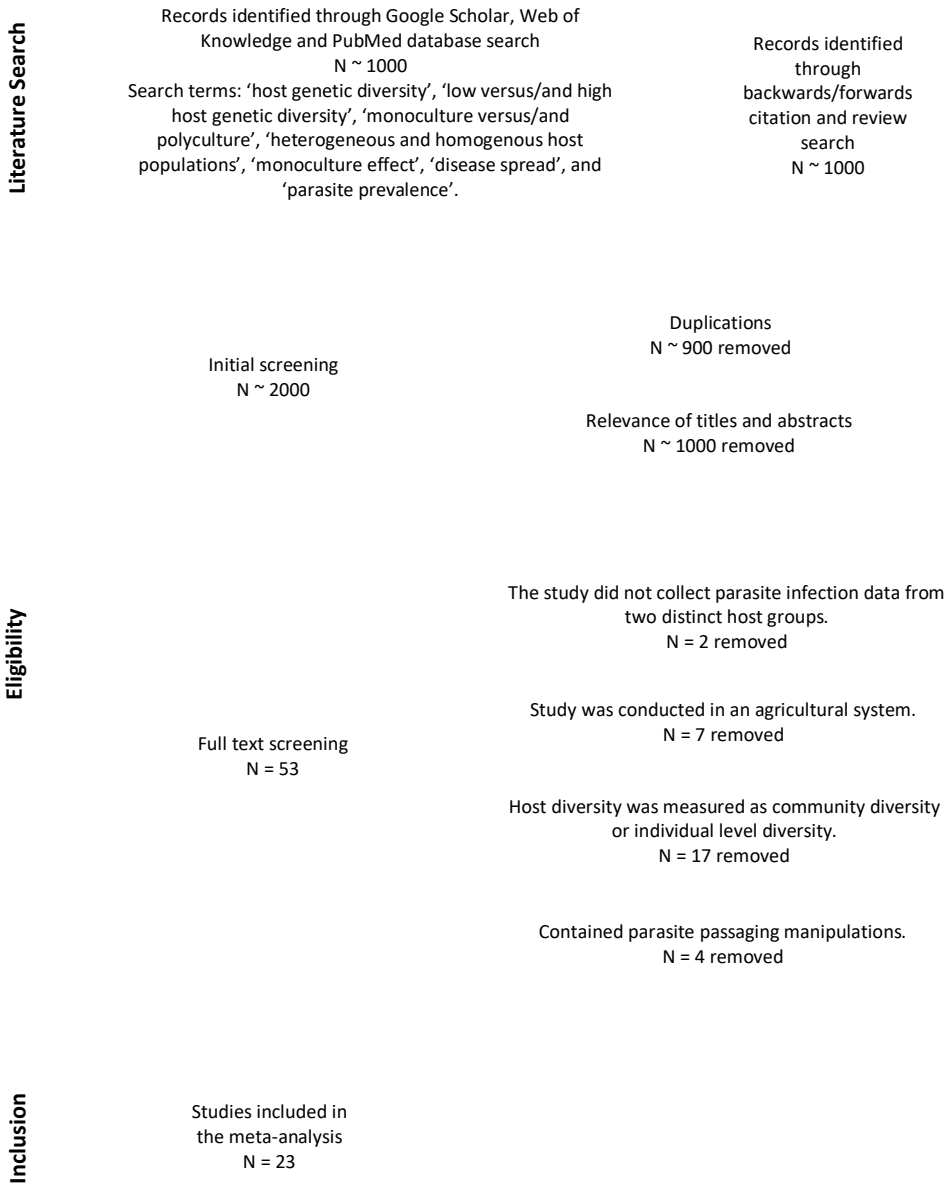
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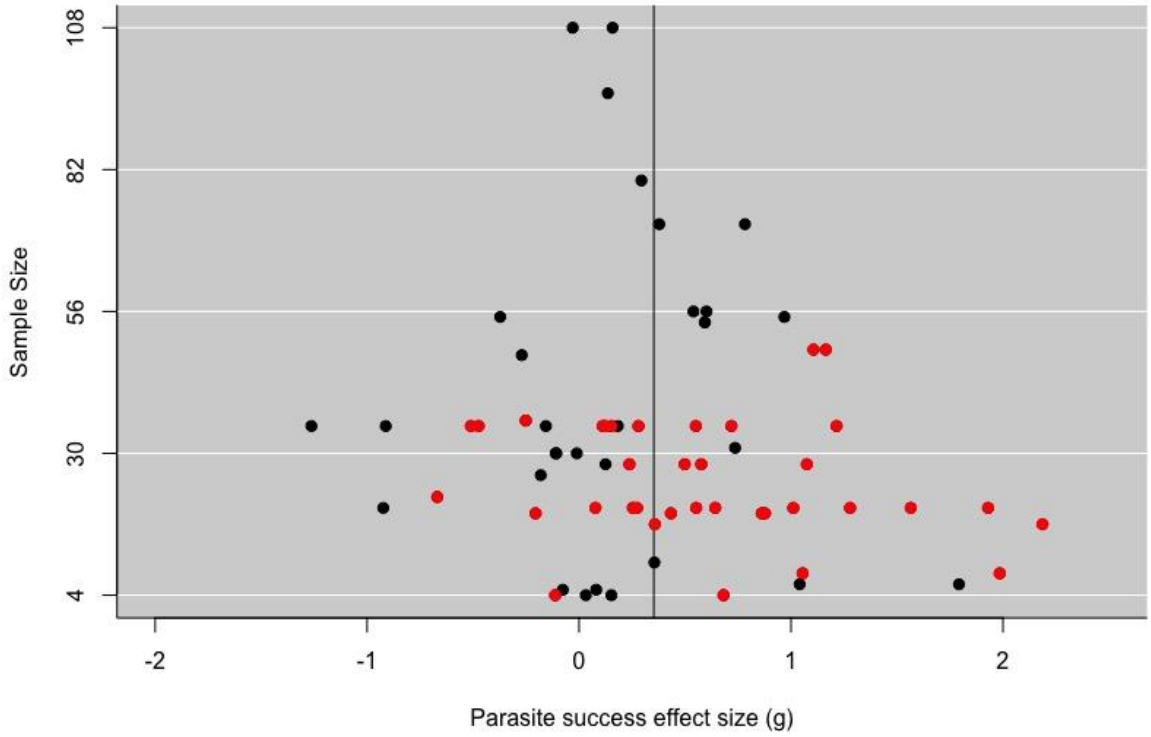
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# Chapter 2:
# Supplementary material




**Supplementary Figure 1:** PRISMA flow chart of literature search and study selection process. Studies excluded from the analysis at the full text screening stage are listed in Supplementary Table 2.




**Supplementary Figure 2:** Funnel plot of the meta-analysis data set. Points on the graph represent the relationship between effect size and experiment sample size for host genetic diversity and parasite success for each study. Points in red represent lab studies and points in black represent field studies. The vertical line shows the effect size predicted by the meta-analysis model.

**Supplementary Table 1:** Definition of variable terms used in the meta-analysis

| Moderator variable | Definition |
|---|---|
| **Study setting** | Lab – An experimental study that occurred in a controlled lab environment<br>Field – An experimental or observational study that occurred in the field |
| **Parasite infection measure** | Parasite prevalence –Measures of proportion of individuals infected within a given population (e.g., parasite prevalence, virus concentration, % infection of queens, and brood disease infection intensity)<br>Parasite load – Measures of parasite propagules per host (e.g., parasite load, infection intensity, viral load, and parasite abundance)<br>Host mortality – Measures of host mortality and transformed measures of host survival (e.g., mortality, mortality rate, proportion dead, and proportion survived) |
| **Host reproduction** | Sexual – A host species within a study that only undergoes sexual reproduction<br>Asexual – A host species within a study only undergoes asexual reproduction<br>Both – A host population within a study undergoing both sexual and asexual reproduction during an experiment |
| **Parasite functional group** | Microparasite – A parasite that has short generation times and high rates of reproduction within its host (e.g., fungi, protozoa, viruses, and bacteria)<br>Macroparasite – A parasite that has long generation times and does not necessarily reproduce within its host (e.g., lice, worms, mites, and flies) |
| **Host range** | 1 Host species – A parasite found to only infect one host species<br>>1 Host species – A parasite found to infect multiple host species |
| **Parasite diversity** | 1 Genotype – Host populations were infected by 1 parasite genotype<br>>1 Genotype – Host populations were infected by >1 parasite genotype<br>>1 Species – Host populations were infected by >1 parasite species |
| **Ability to cause host death** | Yes – A parasite that can cause host mortality<br>No – A parasite that does not cause host mortality |

**Supplementary Table 2:** Studies excluded from meta-analysis at full text screen stage

| Study | Reason for exclusion |
|---|---|
| **Abdala-Roberts**, L., Gonzalez-Moreno, A., Mooney, K.A., Moreira, X., Gonzalez-Hernandez, A. & Parra-Tabla, V. (2016) Effects of tree species diversity and genotypic diversity on leafminers and parasitoids in a tropical forest plantation. Agricultural and Forest Entomology, 18, 43-51. | Host diversity was measured as community diversity. |
| **Acevedo-Whitehouse**, K., Gulland, F., Greig, D. & Amos, W. (2003) Disease susceptibility in California sea lions. Nature, 422, 35. | Host diversity was measured as individual level diversity. |
| **Agha**, R., Gross, A., Rohrlack, T. & Wolinska, J. (2018) Adaptation of a Chytrid Parasite to Its Cyanobacterial Host Is Hampered by Host Intraspecific Diversity. Frontiers in Microbiology, 9:921. | Contained parasite passaging manipulations. |
| **Alexander**, H.M., Roelfs, A.P. & Cobbs, G. (1986) Effects of disease and plant competition on yield in monocultures and mixtures of two wheat cultivars. Plant Pathology, 35, 457-465. | Study was conducted in an agricultural system. |
| **Andras**, J.P. (2017) Genetic variation of the Caribbean sea fan coral, *Gorgonia ventalina*, correlates with survival of a fungal epizootic. Marine Biology, 164:130. | Host diversity was measured as individual level diversity. |
| **Browning**, J.A. & Frey, K.J. (1969) Multiline cultivars as a means of disease control. Annual Review of Phytopathology, 7, 355-382. | Study was conducted in an agricultural system. |
| **Campbell**, G., Noble, L.R., Rollinson, D., Southgate, V.R., Webster, J.P. & Jones, C.S. (2010) Low genetic diversity in a snail intermediate host (*Biomphalaria pfeifferi* Krass, 1848) and schistosomiasis transmission in the Senegal River Basin. Molecular Ecology, 19, 241-256. | Host diversity was measured as individual level diversity. |
| **Ellison**, A., Cable, J. & Consuegra, S. (2011) Best of both worlds? Association | Host diversity was measured as individual level diversity. |

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| between outcrossing and parasite loads in a selfing fish. Evolution, 65, 3021-3026. | |
| **Hendrick**, P.W., Kim, T.J & Parker, K.M. (2001) Parasite resistance and genetic variation in the endangered *Gila topminnow*. Animal Conservation Forum, 4, 103-109. | Host diversity was measured as individual level diversity. |
| **Hughes**, W.O.H. & Boomsma, J.J. (2006) Does genetic diversity hinder parasite evolution in social insect colonies? Journal of Evolution Biology, 19, 132-143. | Contained parasite passaging manipulations. |
| **Jinks**, J.L. & Grindle, M. (1963) Changes induced by training in *Phytophthora infestans*. Heredity, 18, 245-264. | Contained parasite passaging manipulations. |
| **Kerstes**, N.A.G. & Wegner, K.M. (2011) The effect of inbreeding and outcrossing of Tribolium castaneum on resistance to the parasite *Nosema whitei*. Evolutionary Ecology Research, 13, 681-696. | Host diversity was measured as individual level diversity. |
| **Knott**, E.A. & Mundt, C.C. (1990) Mixing ability analysis of wheat cultivar mixtures under diseased and nondiseased conditions. Theoretical and Applied Genetics, 80, 313-320. | Study was conducted in an agricultural system. |
| **Kubinak**, J.L., Cornwall, D.H., Hasenkrug, K.J., Adler, F.R. & Potts, W.K. (2014) Serial infection of diverse host (Mus) genotypes rapidly impedes pathogen fitness and virulence. Proceedings of the Royal Society B: Biological Sciences, 282: 20141568. | Contained parasite passaging manipulations. |
| **Lai**, R., You, M., Zhu, C., Gu, G., Lin, Z., Liao, L., Lin, L. & Zhong, X. (2017) Myzus persicae and aphid-transmitted viral disease control via variety intercropping in flue-cured tobacco. Crop protection, 100, 157-162. | Study was conducted in an agricultural system. |
| **Liao**, H., Luo, W., Pal, R., Peng, S. & Callaway, R.M. (2016) Context-dependency and the effects of species diversity on ecosystem function. Biological Invasions, 18, 3063-3079. | Host diversity was measured as community diversity. |

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| **Lopez-Uribe**, M.M., Appler, R.H., Youngsteadt, E., Dunn, R.R., Frank, S.D. & Tarpy, D.R. (2017) Higher immunocompetence is associated with higher genetic diversity in feral honey bee colonies (*Apis mellifera*). Conservation Genetics, 18, 659-666. | Host diversity was measured as individual level diversity. |
| **Meagher**, S. (1999) Genetic diversity and *Capillaria hepatica* (Nematoda) prevalence in Michigan deer mouse populations. Evolution, 53, 1318-1324. | Host diversity was measured as individual level diversity. |
| **Meyer-Lucht**, Y., Otten, C., Puttker, T., Pardini, R., Metzger, J.P.& Sommer, S. (2010) Variety matters: adaptive genetic diversity and parasite load in two mouse opossums from the Brazilian Atlantic forest. Conservation Genetics, 11, 2001-2013. | Host diversity was measured as individual level diversity. |
| **Mitchell**, C.E., Tilman, D. & Groth, J.V. (2002) Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. Ecological Society of America, 83, 1713-1726. | Host diversity was measured as community diversity. |
| **O'Brien**, S.J., Roelke, M.E., Marker, L., Newman, A., Winkler, C.A., Meltzer, D., Colly, L., Evermann, J.F., Bush, M. & Wildt, D.E. (1985) Genetic basis for species vulnerability in the cheetah. Science, 227, 1428-1434. | Host diversity was measured as individual level diversity. |
| **Palmer**, K.A. & Oldroyd, B.P. (2003) Evidence for intra-colonial genetic variance in resistance to American foulbrood of honey bees (*Apis mellifera*): further support for the parasite/pathogen hypothesis for the evolution of polyandry. Naturwissenschaften, 90, 265-268. | Host diversity was measured as individual level diversity. |
| **Pilet**, F., Chacon, G., Forbes, G.A., Andrivon, D. (2006) Protection of susceptible potato cultivars against late blight in mixtures increases with decreasing disease pressure. Phytopathology, 96, 777-783. | Study was conducted in an agricultural system. |

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| **Rottstock**, T., Joshi, J., Kummer, V. & Fischer, M. (2014) Higher plant diversity promotes higher diversity of fungal pathogens, while it decreases pathogen infection per plant. Ecological Society of America, 95, 1907-1917. | Host diversity was measured as community diversity. |
| **Schmid-Hempel**, P. & Crozier, R.H. (1999) Polygyny versus polyandry versus parasites. Philosophical Transactions of the Royal Society Biological Sciences, 354, 507-515. | Host diversity was measured as individual level diversity. |
| **Spielman**, D., Brook, B.W., Briscoe, D.A. & Frankham, R. (2004) Does inbreeding and loss of genetic diversity decrease disease resistance? Conservation Genetics, 5, 439-448. | Host diversity was measured as individual level diversity. |
| **Velo-Anton**, G., Rodriguez, D., Savage, A.E., Parra-Olea, G., Lips, K.R. & Zamudio, K.R. (2012) Amphibian-killing fungus loses genetic diversity as it spread across the New World. Biological Conservation, 146, 213-218. | The study did not collect parasite infection data from two distinct host groups. |
| **Whitehorn**, P.R., Tinsley, M.C., Brown, M.J.F., Darvill, B. & Goulson, D. (2011) Genetic diversity, parasite prevalence and immunity in wild bumblebees. Proceedings of the Royal Society B: Biological Sciences, 278, 1195-1202. | The study did not collect parasite infection data from two distinct host groups. |
| **Yang**, B., Ge, F., Ouyang, F. & Parajulee, M. (2012) Intra-species Mixture Alters Pest and Disease Severity in Cotton. Environmental Entomology, 41, 1029-1036. | Study was conducted in an agricultural system. |
| **Zhu,** Y., Chen, H., Fan, J., Wang, Y., Li, Y., *et al*. (2000) Genetic diversity and disease control in rice. Nature, 406, 718-722. | Study was conducted in an agricultural system. |

# 3

# Host relatedness shapes the evolution of a novel bacterial infection

Data presented in this chapter form a manuscript together with data from Chapter 4.

## 3.1 Abstract

Pathogens continue to emerge from increased contact with novel host species. Whilst phylogenetically distant host species can represent distinct environments for pathogens, the impact of within-species relatedness for pathogen evolution are unclear. In a novel interaction, we experimentally evolved a generalist pathogen (*Staphylococcus aureus*) in naïve nematode *Caenorhabditis elegans*) populations of closely and distantly related wild isolates. We show that the pathogens emerged as more genetically similar with similar levels of virulence, if evolution took place within closely-related host populations. Infection load and virulence increased over time, more so in diverse host populations, but they evolved along independent trajectories. Together, these findings reveal the rapid changes in pathogen traits and genomes can be shaped by the host population genetic background upon emergence. Our study provides unique empirical insight into the evolutionary dynamics that could occur in other novel pathogens of wildlife and humans.

## 3.2 Introduction

Emerging infectious diseases have led to serious declines in wildlife populations. Heavy population losses have been documented in rabbits following the introduction of Myxoma virus[1], amphibians from Chytrid fungus[2,3], Tasmanian devils from Devil Facial Tumour Disease[4], and brown bats from *Pseudogymnoascus destructans* fungus[5]. Emergence events can result from reservoir host spill-overs[6], a jump from another host species[1], the evolution of a new pathogen trait that allows for exploitation of a new host[7], or by invading a new environment[8,9]. These novel interactions can be very harmful[10] or entirely avirulent[11], but nevertheless, virulence and pathogen replication rates can evolve over time[12].

Whether an emerging pathogen can successfully infect a new host can depend on host phylogenetic relatedness. When susceptibility to infection in the natural host species is similar in the novel host species[13–15], pathogens can shift successfully. With increasing genetic distance between two host species, the chance of successful pathogen transmission is reduced[16,17]. Indeed, the importance of host phylogeny in host shifts has been shown in primate lentivirus[18], rabies virus[19], and Drosophila RNA viruses[14], where host phylogenetic relatedness enhances pathogen success upon a host switch, also referred to as a "phylogenetic distance effect"[13]. On the other hand, a "phylogenetic clade effect" shows that closely related species may display similar levels of susceptibility despite the distance from the pathogens original host due to the loss or gain of immune functions associated with a given pathogen[13,20].

Less is known about the degree to which within-host species genetic relatedness can impact the evolution of novel infections. Host genotypes can differ in their susceptibility to

infection[21,22] and in other aspects that might impact upon infection success, such as pathogen avoidance behaviours[23,24] and starvation responses[25]. Given genetic variation for resistance, high host diversity (low within-population relatedness) can increase the odds of pathogens encountering resistant genotypes. This outcome would limit disease spread compared to homogenous populations (high relatedness) of uniform susceptibility[26–32]. Diversity can further protect susceptible individuals by diluting with resistant hosts, which reduces the contact rate between pathogen and susceptible host[33,34]. It is commonly assumed that diverse host populations contain resistant individuals[29](also in theoretical models predicting pathogen emergence[32]). However, there could be no genetic variation for host resistance to a novel, emerging infectious disease[1,4,35]. In fact, host resistance may not spread until well into the outbreak when some pathogen evolution will have already taken place[12]. It is nevertheless unclear whether pathogens will evolve similarly in all naïve populations.

Homogeneous populations might be targets for more novel infections, given increasing numbers of those managed for conservation and agriculture[36,37]. Changes to habitats as well as to geographic ranges because of human activity[30] increase opportunities for contact between novel pathogens and naïve hosts. Moreover, there is concern over the vulnerability of isolated and island host species and populations which might also be lacking diversity[4,28]. As new pathogens continue to emerge, the need to understand their evolution in naïve host populations is central to predicting and managing the consequences.

Here, we tracked, from an ancestral state, the evolution of an opportunistic pathogen, *Staphylococcus aureus*, by serial passage in populations of a novel host species, the nematode *Caenorhabditis elegans*, varying in genetic relatedness (24 different one-isolate nematode monocultures and 6 replicate twenty-four isolate nematode polycultures). The nematode hosts

used were randomly selected across a wild isolate *C. elegans* phylogeny[38]. As *S. aureus* is not commonly found in the soil habitat of *C. elegans*, this is an ideal system to study the evolution of an emerging pathogen after introduction into non-resistant, naive host populations. Pathogenic *S. aureus* is also known to jump regularly between animal host species, including a range of domestic animals, wild hosts, such as rodents, non-human primates and bats[39–41]. Our host-pathogen system also allows for the comparison of different infection measures as *S. aureus* is known to colonise the gut[42,43] and cause mortality, where siderophore production has often been the underlying mechanism for virulence in *C. elegans* hosts[42,44]. Our approach permits a direct test of i) whether host genotypes can predict pathogen adaptation and determine successful spread to a new host genotype, and ii) whether pathogen performance differs between nematode monoculture populations (high within-host relatedness) and highly diverse host polycultures (low within-host relatedness). Thus, we hypothesised that host relatedness determines evolved pathogen fitness. If this is true, closely-related host genotypes should show similar levels of evolved pathogen fitness. We also hypothesised that host genetic diversity limits pathogen evolution, where we predict that pathogens evolving in host polycultures will acquire greater genetic variation given the increased variation in host genetic backgrounds encountered.

## 3.3 Materials and methods

### 3.3.1 Nematode hosts and maintenance

Host populations comprised of 24 different genotypes has previously been shown to drive parasite extinction in a bacteria-phage system [26]. Thus, we chose to replicate this level of host diversity to maximise the selection imposed on parasite populations in an animal host. Twenty-three *Caenorhabditis elegans* wild isolates, which are genetically distinct, were

randomly selected across the phylogenetic tree presented in Andersen *et al* (2012) (Supp. Table 1). The lab-adapted isolate N2 was added to the collection. Genetically homogenous lines for each isolate were generated by passaging a single hermaphrodite from offspring for five generations. Ancestral stock populations were then frozen down in a 1:1 solution of 40% DMSO at -80°C. Nematode populations were kept evolutionarily static throughout experiments. Stock populations of all twenty-four nematode isolates were maintained at 20°C on nematode growth medium (NGM) plates seeded with 600 µL *Escherichia coli* OP50 in Luria-Bertani Broth. As the nematode pharynx grinds *E. coli* bacterial cells[45], these cells never reach the gut intact and will not colonise the intestinal lumen at the age we used in our experiments. Thus, *E. coli* would not compete or interfere with the evolution of a pathogen within the nematode gut.

To synchronise the life stages of all 24 nematode isolates, populations were treated with a 1:1 solution of bleach (NaClO) and 5M sodium hydroxide. This solution kills bacterial cells and nematodes, leaving only unhatched eggs. Eggs were subsequently incubated at 20°C on a shaking platform overnight and then plated out onto *E. coli* seeded NGM plates. Once synchronised, young adult nematodes (L4 larval stage) were washed off *E. coli* seeded NGM plates using 2 mL M9 buffer supplemented with 10% Triton®X-100 (Molecular Biology Grade), and added on the top of 1000 µL filter-tips placed within Eppendorf tubes. Nematodes were then washed three times using 500 µL M9 buffer and centrifuged for 1 min at 755 x *g* to remove any bacterial cells lining the nematode cuticle.

### 3.3.2 Bacterial stain and stock preparation

Nematode populations were exposed to and infected with *Staphylococcus aureus* MSSA476 (GenBank: BX571857.1). This strain is methicillin susceptible sourced from the University

of Liverpool[46]. Bacterial infection occurs upon ingestion, where this pathogen lyses the cells lining the nematode gut wall [24] and ultimately causes host mortality [47]. A randomly selected *S. aureus* colony was inoculated into 20 mL Tryptone Soy broth (TSB) and grown for 24h with shaking at 220 rpm at 30°C. This single ancestral *S. aureus* stock population was used for all experiments and frozen in a 1:1 ratio of sample to 60% glycerol at -80°C.

### 3.3.3 Evolution experiment

To investigate pathogen evolution under two levels of host relatedness, and compare the two treatments to the ancestral pathogen, we passaged pathogens across 10 host generations through (i) 24 biological replicates of monoculture nematode host populations (high within-population relatedness), and (ii) 6 polyculture replicate host populations of all 24 isolates combined (low within-population relatedness). We also had 6 no-host control replicates of pathogen passaging to account for adaptation to lab procedures. The passage experiment was performed in 24-well plates containing 500 μL viscous media and 100 μL of concentrated *S. aureus* inoculum per well. To limit pathogen avoidance [48], as well as digging [49] and aggregating [50,51] nematode behaviours, and thus to ensure equal pathogen exposure, nematodes were infected in 1.1% viscous media[52]. The viscous media solution consisted of TSB media supplemented with 2.3g HPMC cellulose ((Hydroxypropyl) methyl cellulose, Sigma-Aldrich). Nematode populations of approximately 500 L4 individuals were added to each exposure well and incubated at 25°C for 24h. Nematode populations (containing both alive and dead animals) were then removed and placed onto 1000 μL filters within 1.5 mL Eppendorf tubes and centrifuged at 2656 x *g* rpm for 2 min to allow the viscous media to pass through the filters. The supernatant was subsequently removed, and nematodes were re-suspended in 500 μL M9 buffer and further centrifuged at 755 x *g* rpm for 2 min. This washing process was repeated three times. To harvest pathogens to passage, 0.7 mm Zirconia

beads (BioSpec products) were added to a resuspension of 10% nematode population sample in a new Eppendorf tube with 100 μL M9 buffer, and placed in a mini beadbeater (BeadBug™, Microtube homogenizer) at 320 rpm for 45 sec. The crushed nematode solution was then plated onto Mannitol Salt Agar (MSA) which selectively grows *S. aureus*, and incubated for 24h at 37°C. Single colonies were then picked and grown in 5 mL TSB overnight shaking at 220 rpm at 30°C. New host populations were then exposed using these cultures (See Fig. 1).

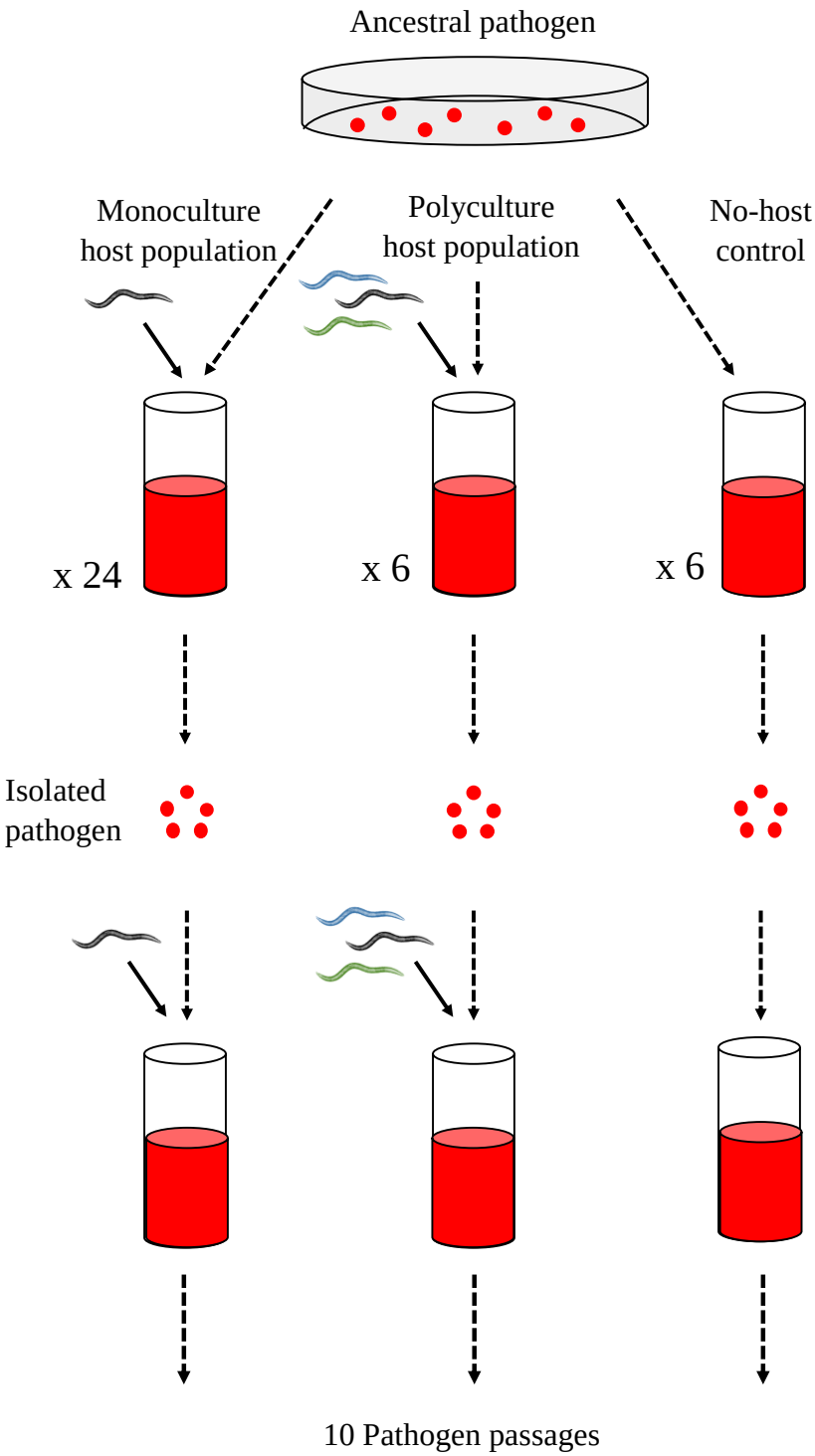

Ancestral pathogen
Monoculture host population
Polyculture host population
No-host control
x 24
x 6
x 6
Isolated pathogen
10 Pathogen passages

**Figure 1:** Experimental evolution of pathogens under two levels of host relatedness. High within-population relatedness was represented in host monocultures (24 biological replicates of nematode isolates). Host polycultures (6 replicate populations comprised of a pool of twenty-four nematode isolates) had comparatively low levels of host relatedness. The pathogen was also passaged in 6 replicate no-host controls to control for lab adaptation. All pathogen populations were passaged across ten host generations from a single ancestral clone. Nematode hosts remained evolutionarily static where pathogens were presented to new hosts populations from stock culture at each passage. Nematodes were exposed to pathogens for 24h, after which 10% of all exposed hosts in the replicate were isolated, crushed, and pathogens was used to re-infect new nematodes.

### 3.3.4 Host mortality and within-host pathogen load

We examined the effects of host relatedness on pathogen evolution by comparing pathogen performance in related (monocultures) to unrelated populations (polycultures). This was achieved by exposing ancestral and evolved (passage 10) pathogen to host monoculture or polyculture populations in a similar manner as one generation of our evolution experiment. Thus, approximately 200 nematodes per replicate population were incubated at 25°C for 24h in 250 μL viscous media supplemented with 50 μL ancestral or evolved pathogen. We then calculated host mortality and infection load differences associated with the relatedness of individuals in host populations. Host mortality was measured by counting the proportion of dead to total nematodes per replicate. Nematodes were considered dead when they did not respond to the touch of a platinum wire[53]. To measure infection load we randomly picked, washed in three 10 μL droplets of M9 buffer, and crushed a random selection of ten alive and dead nematodes per replicate. Pathogen infection load was then estimated by counting colony forming units (cfu) of 100-fold diluted *S. aureus* grown overnight at 37°C on MSA plates. These assays were repeated five times.

### 3.3.5 *In vitro* bacterial growth rate

Bacterial growth rates of monoculture and polycultures of evolved *S. aureus* populations were measured in 96-well plates. A volume of 2 μL overnight grown bacteria was inoculated into 198 μL TSB where growth rates were monitored in a plate reader for 22h at 30°C. The plate reader was programmed to shake intermittently and take OD660 readings every 10min. Bacterial growth in the exponential phase was measured by estimating the slope of each bacterial population from two points by visual inspection. Final OD660 readings were used to calculate differences between treatments in carrying capacity.

### 3.3.6 Siderophore production

Ancestral and evolved populations of *S. aureus* were grown overnight in 5 mL TSB at 220rpm at 30°C. Populations were then standardised by OD630, and subsequently filtered through 22 μm filters to remove bacterial cells. Instructions supplied by the SideroTech (EmergenBio) kit were followed by taking 100 μL of each sample to prepared 100 μL reaction mixture and left at r.t.p for 10 min before OD630 measures were taken. Siderophore concentrations are estimated by measuring the changes in OD630 as the result of ferric iron transfer from the reagent (composed of a dye, iron, and a detergent) to siderophore present. Standards supplied by the kit were used to create a reference curve from which the relationship between desferoxamine siderophore and OD630 could be measured and siderophores present in the samples could be determined.

### 3.3.7 Bacterial genome extraction

Forty clones per replicate population from the ancestor and passages 5 and 10 were grown separately overnight in 200 μL TSB at 30°C at 150 rpm. Bacterial clones were pooled together into 1.2 mL cultures, and DNA was extracted following the DNeasy Blood & Tissue Kit (Qiagen) protocol. The cultures were centrifuged at 8000 rpm for 3 min, where the pellet was re-suspended in 180 μL enzymatic lysis buffer (Qiagen) containing 20 μL Lysostaphin (0.1 mg $mL^{-1}$) (Sigma-Aldrich) and 4 μL RNAse A (20 mg $mL^{-1}$)(Sigma-Aldrich). After a 2h incubation period at 37°C, 25 μL protinase K (Qiagen) and 200 μL Buffer AL (without ethanol) was added to samples. The solution was further incubated at 56°C for 30 min, before adding 200 μL ethanol and samples vortexed. DNA purification then followed the DNeasy Blood and Tissue spin-column protocol (Qiagen). Purity and quality of extracted DNA was assessed using the Nanodrop ND-100 and Qubit® Fluorometer. DNA was normalized to a concentration of around 50 ng/μL in 10mM Tris-Cl and sent to the Oxford Genomics Centre

at the Wellcome Centre for Human Genetics where paired-end libraries were prepared and sequenced as 150bp run on an illumina HiSeq4000.

### 3.3.8 Bacterial genome evolution analysis

Fastq read files were checked for quality and trimmed using fastp 0.19.6 using default options. Reads were then aligned to the *S. aureus* MSSA476 reference genome sequence (NCBI accession number, GCF_000011525.1 using the bwa 0.7.17 "mem" algorithm[54] and the option "-M" to ensure single best alignment. Samtools version 1.9 was then used to filter out PCR duplicates and to remove singletons. The Genome Analysis Toolkit v.4.0.11.0 (GATK) Haplotype caller module was used to detect variants with the options "--sample-ploidy 40 --heterozygosity 0.0001 --indel-heterozygosity 0.00001" as previously described[42]. Variants were then hard-filtered using standard practices as described before[42]. All variants present in the evolved *S. aureus* lines that were also present in the sequenced ancestral strain and/or the no-host control were removed before further analyses. Evolutionary distances between *S. aureus* population and time points were determined by calculating the Euclidian distances between variant frequencies within the R statistical programming environment version 3.6.1 (R Core Team 2019). Phylogenetic trees were calculated by hierarchical clustering of distances in R. To account for the impact of host genetic distance on pathogen evolution in our experiments, we determined the genetic diversity of the monocultures employed in our experiments. We first downloaded the hard-filtered variants of *C. elegans* from the "Caenorhabditis elegans Natural Diversity Resource" (https://www.elegansvariation.org/) and extracted all fixed variants (AF=1) associated with any of the 24 monocultures. Euclidian distances were then determined as described for *S. aureus*, and correlation of distances between host and pathogen was assessed using a Mantel test in R.

Frequency of SNPs was plotted over time for monoculture and polyculture treatment to determine if any mutations were fixed, rare, or fluctuated. To further analyze the importance of SNP changes observed at the end of the evolution experiment, we confirmed corresponding gene product and gene function through a literature search (Supp. Table 3). Gene products were placed under the following twelve function categories; adherence, adherence and metabolism, virulence and adherence, metabolism, metabolism and stress, stress, virulence and stress, virulence, virulence and metabolism, siderophore and metabolism, siderophore, and unknown. Within-population SNP frequencies were then calculated by adding up frequencies and dividing by the number of occurrences of a particular gene product. An average of total gene products of a particular function was then compared between host population treatments. We also combined all virulence related functions to evaluate differences between host population treatments.

### 3.3.9 GO term enrichment analysis

To determine whether worm relatedness has an effect on pathogen virulence between strains, we conducted gene pairwise comparisons across all our worm isolate genomes. To this end, we considered any fixed variant within a protein coding gene and created a list of such loci per nematode isolate. Subsequently, for each pairwise comparison, we discarded the loci shared between the nematode isolate pair, thus creating a list of unique variant loci for each worm isolate pair. We hypothesized that these unique loci would potentially be responsible for the observed infection differences between nematode isolates. We then performed a gene ontology (GO) enrichment analysis using g:Profiler (https://biit.cs.ut.ee/gprofiler/gost). We selected comparisons on the basis of evolved pathogen virulence from Fig.2, and categorized them as follows; different virulence levels and distantly related hosts, different virulence

levels and closely related hosts, similar virulence levels and distantly related hosts, and similar virulence and closely related hosts. By looking at worm isolate comparisons we can get an idea on which similarities and differences in host gene functions could relate to pathogen success. Specifically, could the differences in virulence between two closely related hosts be important in ways that will drive the pathogen to be more virulent? Also, does the similarities in virulence between two distantly related hosts show limited differences in ways that are important for infection?

### 3.3.10 Statistical analysis

All data analysis was carried out in R v 3.6.0. Parametric tests were used in cases where the data met the required assumptions, otherwise equivalent non-parametric tests were used. Assumptions of normality of data was confirmed with Shapiro tests, and equality of variances with F-tests (or Levene's test in cases of comparisons among >2 groups). Data point outliers were confirmed using the Dixon test and removed where appropriate. Multiple comparisons of within-host infection load and host mortality data were corrected for using the FDR method for generating p-values.

Binomial GLMs were used to examine differences in pathogen-induced host mortality across monoculture populations. Most data did not meet the assumption of normality, thus Wilcoxon rank sum tests were performed. Welch two sample t-test was used on host Euclidean genetic distances and *in vitro* bacterial growth data due to unequal variances. As polyculture infection load data met the assumption of equal variances, a two-sample *t*-test was performed. ANOVAs were used for assays comparing ancestral and evolved infection load across host monocultures as well as comparing levels of siderophore production across treatments. Split plot ANOVAs were performed when comparing monoculture to polyculture treatments to

account for within-treatment replicate variation and timepoint (ancestral vs evolved pathogen).

Mantel tests were used to examine the relationships between two distance matrices to avoid pseudo-replication of distance calculations. This test was used to examine relationships among pairwise host and pathogen genetic distances, infection load, and host mortality data. Pearson's product moment correlation tests were used to examine relationships between host mortality data and levels of siderophore production in polycultures. Spearman's rank correlation data were performed on infection load and mortality, and mortality and siderophore production monoculture data that did not meet the assumption for normality. Kendall's rank correlation was used to account for tied pairs in the data when making multiple correlations between non-parametric infection load and host mortality between ancestral and evolved pathogens across host monocultures.

## 3.4 Results

### 3.4.1 Pathogen evolution

To test whether host relatedness predicts pathogen evolution in a novel host-pathogen interaction, we transferred *S. aureus* in *C. elegans* monoculture (high within-host relatedness) and polyculture (low within-host relatedness) populations for 10 passages (Fig. 1). The monoculture treatment consisted of 24 populations of different wild nematode isolates, and the polyculture treatment of 6 replicate populations of twenty-four pooled *C. elegans* isolates. All bacterial cultures were started from one bacterial colony, where after 24h of pathogen exposure, the bacteria were harvested from 10% of the total host population and passaged

into a new host (Fig. 1). We quantified virulence in terms of pathogen-induced host mortality, and infection load as the number of colony-forming units (CFU, or live bacteria cells) per nematode host (see materials and methods for full details). In this study, the more a pathogen genotype can replicate within a host, the more it will be represented in the passaged population. These measures of infection were taken after 24h pathogen exposure as this was the window of selection permitted to operate in the evolution experiment (*methods*).

### 3.4.2 Host genetic variation

Previous studies have found that wild *C. elegans* isolates differed in host immunity to infections of *Bacillus thruringiensis*[22] and *Serratia marcescens*[55,56] implying a shared coevolutionary history or significant standing genetic variation for resistance[35]. *C. elegans* and human-acquired *S. aureus* do not naturally co-occur in the same environment which could explain why pathogen traits (pathogen infection load and virulence) were not markedly variable across host isolates at the beginning of the experiment. We found that the ancestral pathogen colony did not yield significant differences among 24 monoculture host populations for host mortality (GLM, $X^2_{1,23} = 28.738$, $p = 0.1892$, Fig. 2) or infection load (One-way ANOVA, d.f. = 23, $F = 0.377$, $p = 0.995$, Fig.3). This lack of significant variation held even when accounting for the genetic distance between host isolates (pairwise genetic distance and infection load: Mantel test, $r = 0.0049$, $p = 0.45$; pairwise genetic distance and host mortality: Mantel test, $r = -0.086$, $p = 0.704$). There was also no correlation between these two measures of infection across host isolates (Kendall's rank correlation, $tau = 0.0023$, $z = 0.0356$, $p = 0.97$) indicating that infection load is not associated with pathogen virulence.

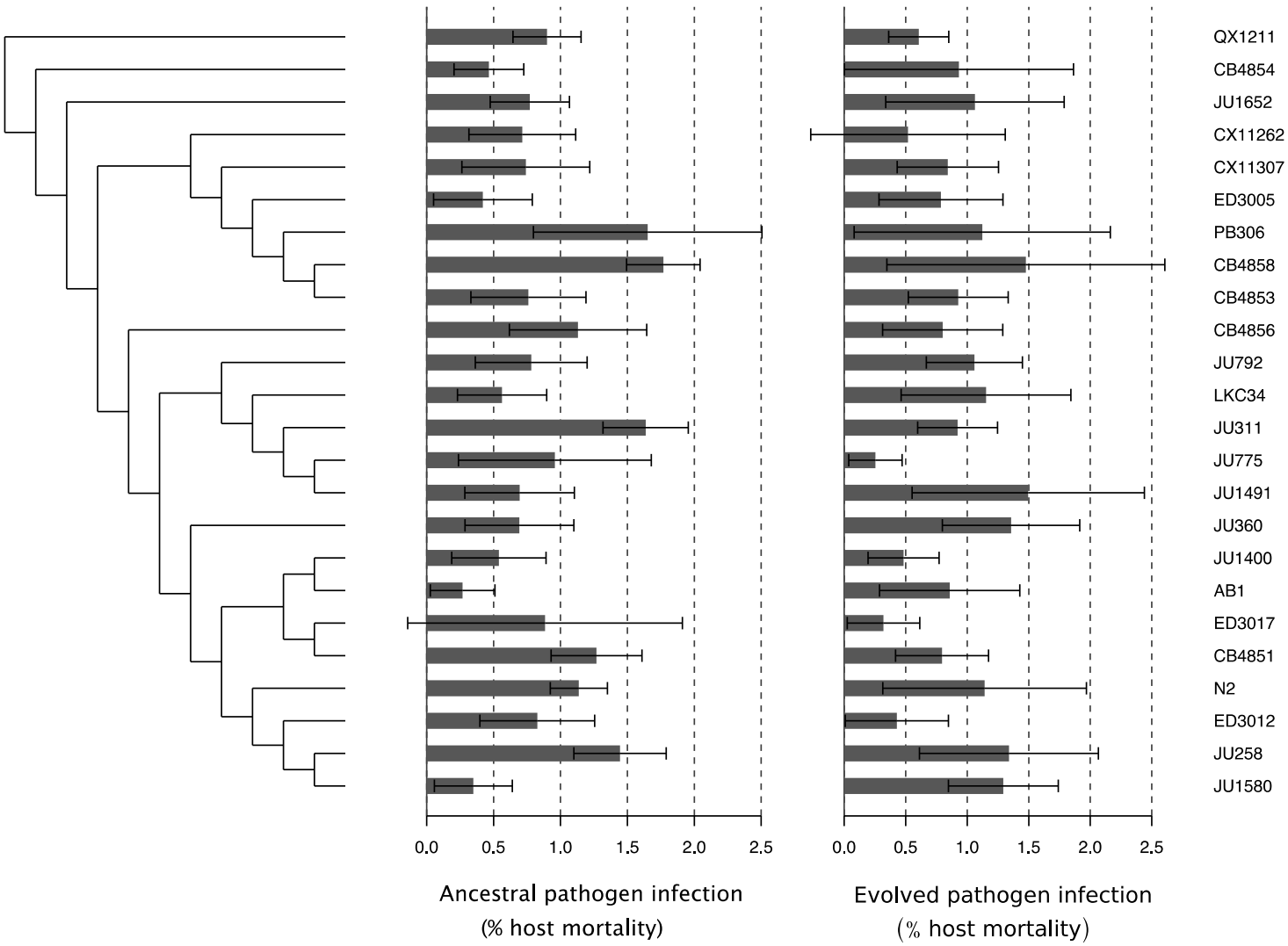

QX1211
CB4854
JU1652
CX11262
CX11307
ED3005
PB306
CB4858
CB4853
CB4856
JU792
LKC34
JU311
JU775
JU1491
JU360
JU1400
AB1
ED3017
CB4851
N2
ED3012
JU258
JU1580
0.0
0.5
1.0
1.5
2.0
2.5
Ancestral pathogen infection
(% host mortality)
Evolved pathogen infection
(% host mortality)

**Figure 2:** Phylogeny of the 24 *C. elegans* natural isolates used in the experiment mapped against their susceptibility to evolved and ancestral pathogens in host monocultures (as measured by mean percent host mortality caused by infection ± SE). Host mortality for each host isolate was measured in five replicates. The tree is rooted by the most genetically disparate host isolate (QX1121), see Anderson et al. 2012 for full description.

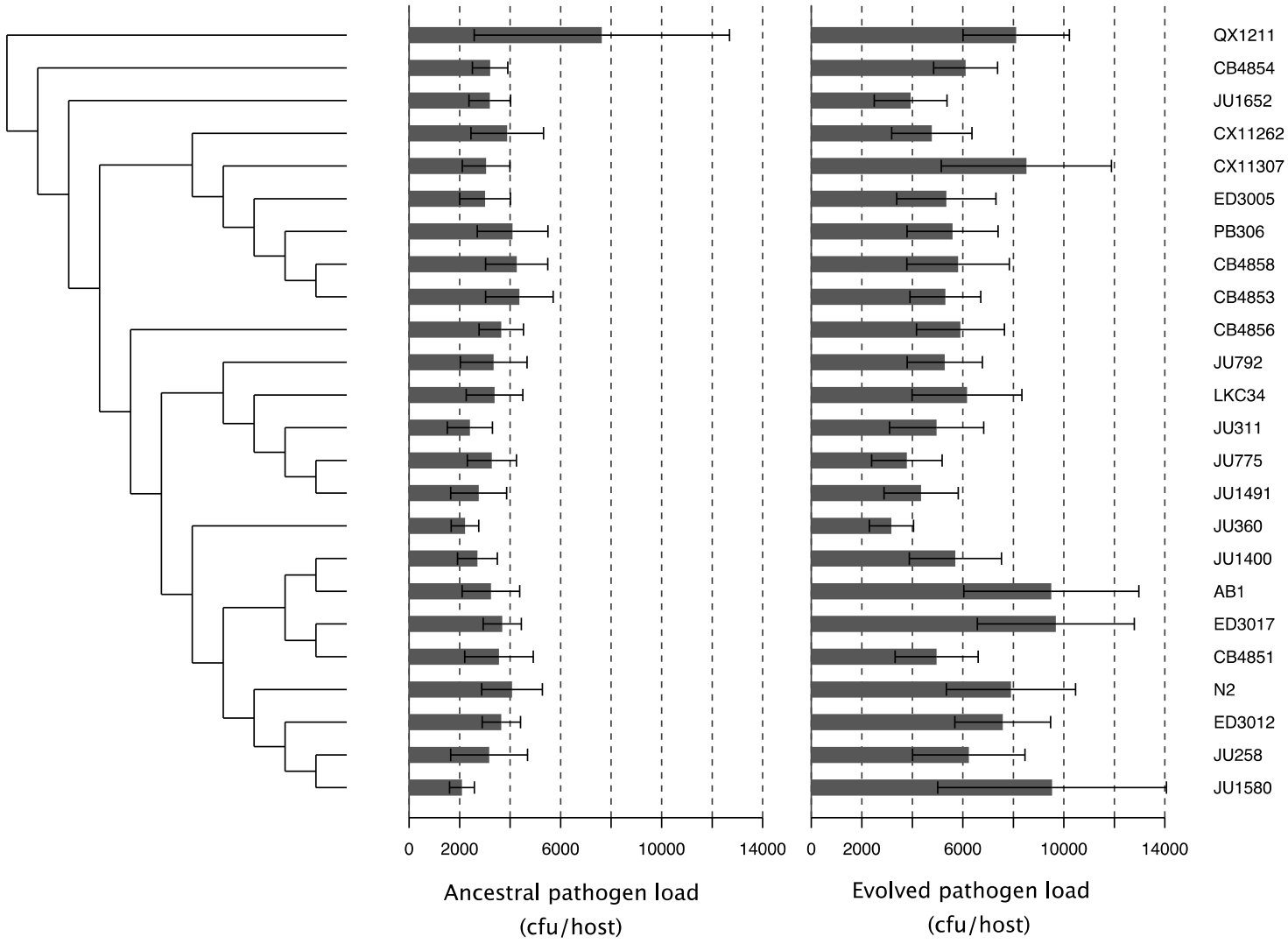

QX1211
CB4854
JU1652
CX11262
CX11307
ED3005
PB306
CB4858
CB4853
CB4856
JU792
LKC34
JU311
JU775
JU1491
JU360
JU1400
AB1
ED3017
CB4851
N2
ED3012
JU258
JU1580
0
2000
6000
10000
14000
Ancestral pathogen load
(cfu/host)
Evolved pathogen load
(cfu/host)

**Figure 3:** Phylogeny of *C. elegans* strains mapped against pathogen infection load. Monocultures of twenty-four *C. elegans* strains were infected with ancestral or evolved *S. aureus* for 24h, whereby pathogen infection load (mean cfu/host ± se) was measured. Each *C. elegans* strain consisted of five replicates. The tree is rooted by the most diverse host strain (QX1121), see Anderson et al. 2012 for full description.

### 3.4.3 Host genotype shapes pathogen evolution

Over evolutionary time, we found that pathogen virulence and infection load evolved differently across host populations. Virulence became significantly variable across different host monocultures (GLM, $X^2_{1,23} = 39.875$, $p = 0.01587$, Fig. 2). This pattern was not found to significantly depend on genetic distance between host isolates in which the pathogens evolved (Mantel test, $r = 0.1287$, $p = 0.166$). These results show that virulence does evolve differently across host genotypes, however as it stands, cannot be predicted by host relatedness.

To understand if host traits could predict pathogen evolution, we conducted a GO-term analysis comparing the putative functions of genes that differ in sequence between host isolates. We first compared isolates that favoured evolution of different levels of virulence evolution. As a control, we then compared host isolates that drove the evolution of similar levels of virulence. We found that in three out of five comparisons made (comparisons 1,2, and 5, Supp. Table 2), host isolates that favoured divergent levels of virulence differed in metal ion binding functions. Conversely, host isolates that favoured similar levels of virulence did not differ in metal ion binding (comparisons 1, 2, and 3, Supp. Table 2), suggesting that differences in host metal binding could predict how pathogen virulence is to evolve. This finding is particularly interesting, given the known link between metal ion binding and pathogen virulence[57]. Metal ion binding is essential to staphylococci as a component of numerous biochemical processes, including energy production, DNA synthesis, and defence against oxidative stress. It is also important in the regulation of virulence factors, and staphylococcal cells that successfully sequester metal ions from a host will exhibit greater pathogenicity[57].

In contrast to virulence, infection load did not evolve to vary among host isolates (One-way ANOVA, d.f. = 23, F = 0.564, p = 0.941, Fig. 3), even when accounting for host genetic distance (Mantel test, r = 0.0299, p = 0.3816). In line with this, we did not find a positive relationship between infection load and host mortality caused by evolved pathogens (Spearman's rank correlation, S = 269590, rho = 0.01544312, p = 0.8682).

### 3.4.4 Initial infection success predicts evolved pathogen load within monocultures

Next, we examined whether the ease with which the novel pathogen initially exploited the monoculture determined the magnitude of change in pathogen traits upon evolution. We found that the ancestral and evolved pathogen trait values were positively associated for infection load (Kendall's rank correlation, z = 8.4909, tau = 0.5255915, p < 0.001, Fig. 4A). These results suggest that the evolutionary outcome of a pathogen for infection load can be determined by the trait's initial value in the host. The better a pathogen can colonise upon introduction, the more the pathogen can evolve to exploit that host genotype over time. We did not see this pattern between the levels of host mortality caused by ancestral and evolved pathogens (Kendall's rank correlation, z = 1.5575, tau = 0.1027, p = 0.1194), potentially because pathogens evolved to become less virulent in some host genotypes and more virulent in others (as described above and in Fig. 2).

### 3.4.5 Closely related hosts represent similar selection environments

Relatedness between two host species can be a predictor of susceptibility to a novel pathogen[14,15]. Thus, we asked whether the patterns of pathogen molecular evolution reflected the genetic relatedness of the host monocultures. We sequenced the evolved *S. aureus* populations mid-way and at the end of the evolution experiment, and calculated pairwise

genetic distances between them using differences in single-nucleotide polymorphisms (SNPs) (*methods*). We then mapped the pathogen distances against those of the corresponding nematode isolates. We found that host and pathogen genetic distances were correlated after passage in ten host generations (Mantel test, $r = 0.4078$, $p = 0.0346$, Fig. 4B) but not after five host generations (Mantel test, $r = 0.197$, $p = 0.1528$).

Together, these results provide support for the hypothesis that closely related hosts represent similar selection environments. These results arose despite a lack of variation in host resistance across isolates prior to bacterial evolution.

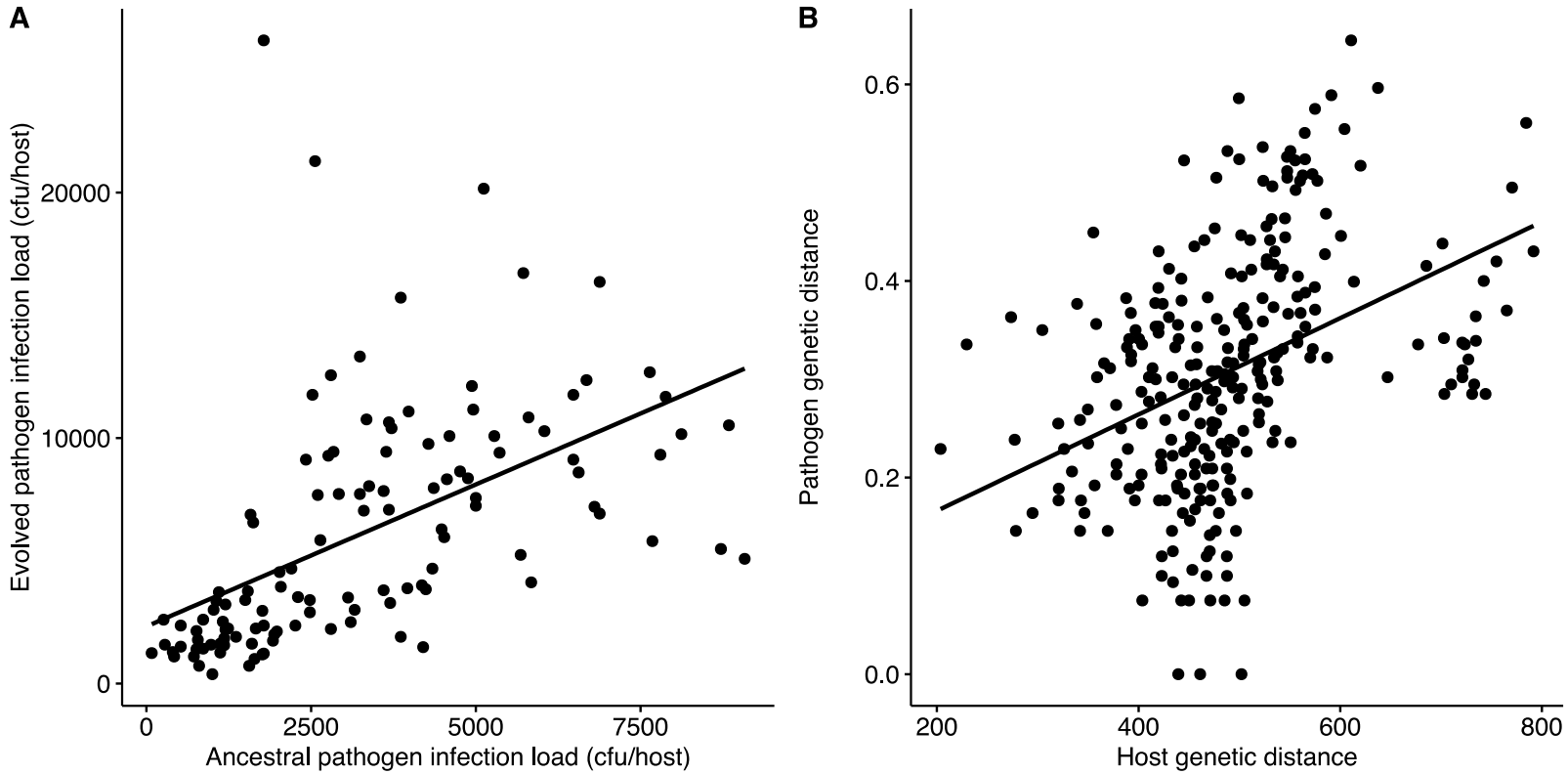



**Figure 4:** Pathogen evolution in host monocultures. (A) Correlation between within-host infection load of ancestral and evolved *S. aureus* across 24 different host monocultures. (B) Correlation between pairwise genetic distance of host isolates and pathogens evolved in monocultures of those isolates. Genetic distance was measured by calculating the Euclidean distances between variant frequencies of each isolate.

### 3.4.6 Pathogen evolution is affected by host population genetic diversity

We examined the impact of host genetic diversity on the novel pathogen's performance. Firstly, we compared within-host infection loads in monoculture (high within-host relatedness) and polyculture (low within-host relatedness) host populations. We found that infection loads of ancestral (Split plot ANOVA, $F_{1,120} = 26.826$, $p < 0.001$, Fig. 5A) and evolved (Split plot ANOVA, $F_{1,120} = 17.599$, $p < 0.001$, Fig. 5A) pathogens were both higher in host polycultures compared to in host monocultures. Infection load continued to increase over evolutionary time in host monocultures (Wilcoxon rank sum test, W = 4662, fdr-corrected $p < 0.001$, Fig. 5A). However, infection load was maintained at the ancestral level in polycultures (Two Sample t test, d.f. = 58, t = -1.9061, fdr-corrected p = 0.0616, Fig. 5A) and ancestral infection loads in host polyculture populations did not correlate with evolved infection loads (Pearson's product-moment correlation, t = 1.8311, cor = 0.33236, p = 0.07814). This finding suggests that, unlike in monocultures, the novel pathogen was limited in its ability to increase colonisation of highly diverse host populations.

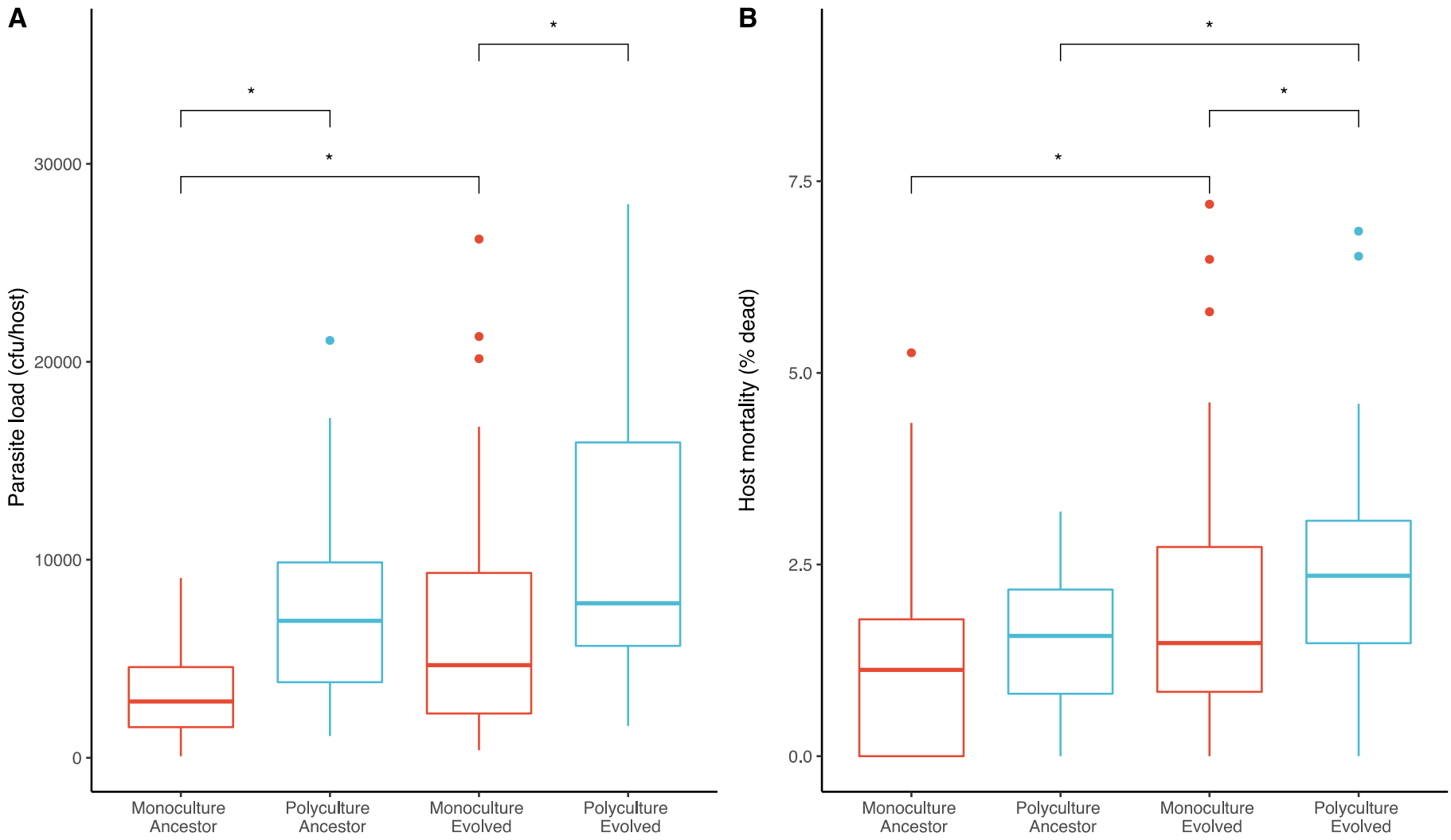

A
B
Parasite load (cfu/host)
Host mortality (% dead)
30000
20000
10000
0
7.5
5.0
2.5
0.0
*
*
*
*
Monoculture Ancestor
Polyculture Ancestor
Monoculture Evolved
Polyculture Evolved

**Figure 5:** Evolution of (A) infection load (cfu/host) and (B) host mortality in host monocultures (high relatedness) and polycultures (low relatedness). Pathogen infection in monocultures is shown in red and for polycultures in blue. Monocultures consisted of 24 biological replicates and polycultures of 6 replicates, with a total of five technical replicates for each. Asterisks show significant comparisons ($p<0.05$).

Secondly, we compared the levels of pathogen virulence in host monoculture and polyculture populations over time. We found that ancestral levels of host mortality did not differ between monocultures and polycultures (Split plot ANOVA, $F_{1,120} = 0.674$, $p = 0.413$, Fig. 5B), and mortality increased over time in both population types (monocultures: Wilcoxon rank sum test, $W = 5508$, fdr-corrected $p = 0.006$, Fig. 5B; polycultures: Wilcoxon rank sum test, $W = 277.5$, fdr-corrected $p = 0.022$, Fig. 5B). The evolutionary change in pathogen virulence was greater in polyculture populations where *S. aureus* caused more host mortality (Split plot ANOVA, $F_{1,269} = 21.995$, $p < 0.001$, Fig. 5B). A common assumption is that more virulent pathogens replicate faster[58,59]. We measured and compared the *in vitro* growth rate of *S. aureus* evolved under the monoculture and polyculture host treatment regimes. Polyculture pathogens did not grow faster (Welch two sample t-test, $t = -0.547$, d.f. = 6.1, $p = 0.604$, Fig. 6), but reached a significantly higher density (Welch two sample t-test, $t = -2.72$, d.f. = 27.84, $p = 0.011$, Fig. 6). This result could help to explain why we find higher loads of evolved pathogens in host polycultures compared to in host monocultures. In the former, pathogens might evolve to be more efficient at assimilating resources per cell in comparison to those evolved in monocultures.

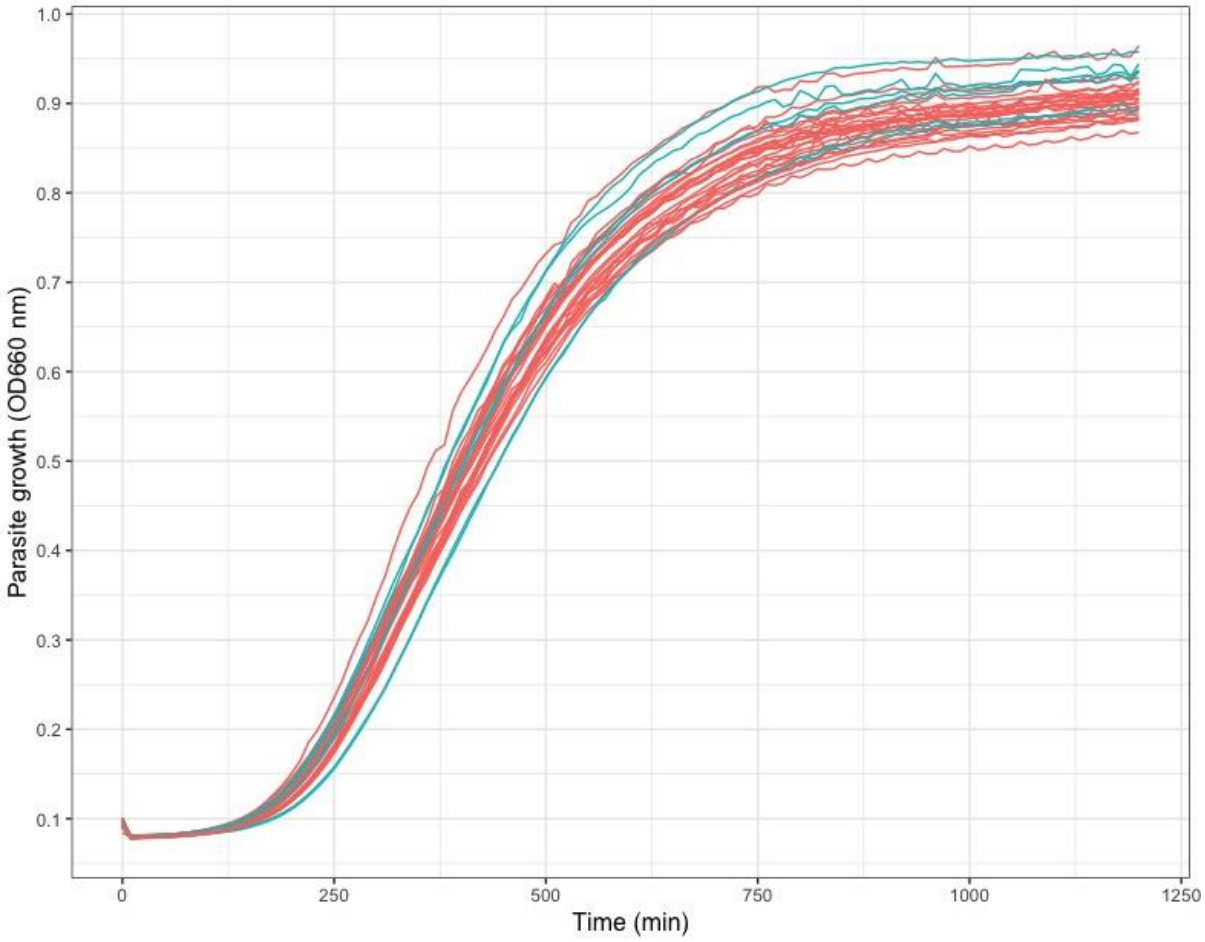



**Figure 6**: Pathogen growth rate *in vitro*. Bacterial growth rates (OD660 nm) of monoculture (red) and polyculture (blue) populations measured over 22h at 30°C. Each line represents the mean value of three replicates of an individual population.

Thirdly, we evaluated whether there were different patterns of molecular evolution underlying the changes in virulence and infection load between host treatments. After measuring genetic distance from the ancestor based on SNPs, we found that on average, genetic divergence was similar between host monocultures and polycultures after five (Welch two sample t-test, d.f. = 7.4052, t = 1.2593, p = 0.2462, Fig. 7) and ten passages (Welch two sample t-test, d.f. = 7.5635, t = 0.35035, p = 0.7356, Fig. 7). In no cases had SNPs reached fixation. Moreover, tracking SNP changes in frequency at passage 5 and 10 (Fig. 8) revealed fluctuations in the frequencies of many alleles over time. This pattern is suggestive of fluctuating selection, or more likely, clonal interference occurring in the pathogen populations of both monocultures and polycultures.

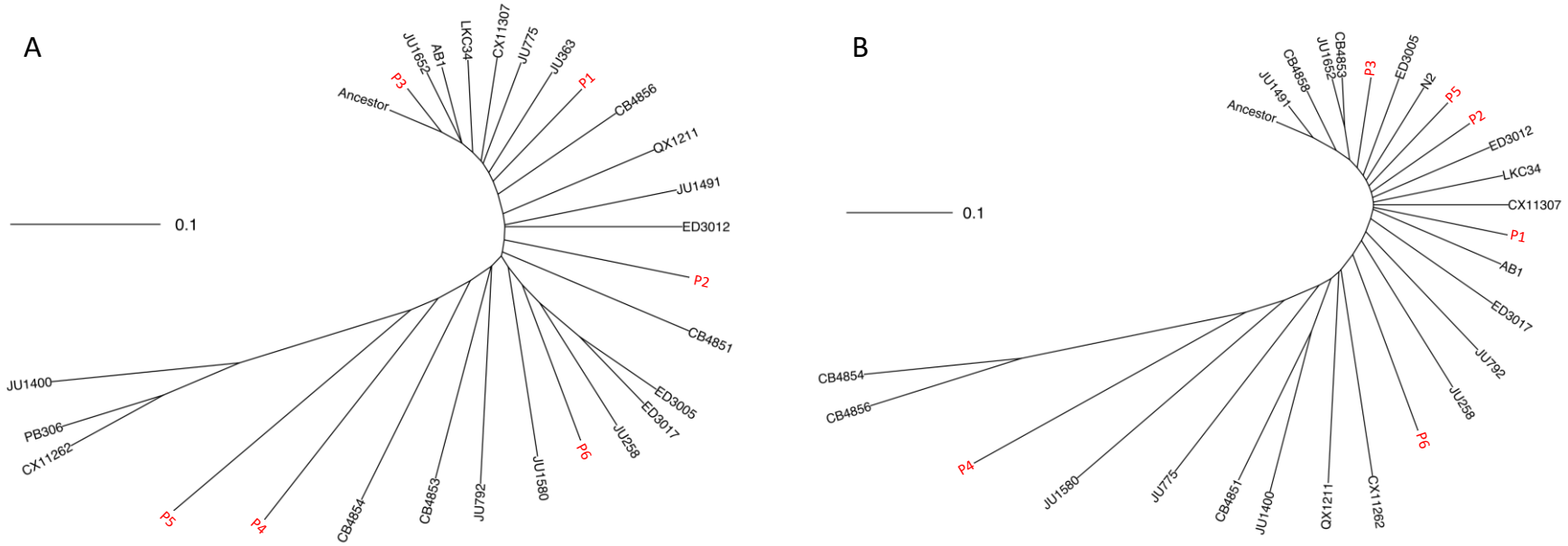



**Figure 7:** Euclidean distance of evolved pathogen populations. (A-B) Phylogenetic tree measuring Euclidean distance of evolved *S. aureus* populations from monoculture (names of wild isolates) and polyculture (in red P1-P6) treatments, along with the ancestor at timepoint 5 (A) and 10 (B). Euclidean distances are measured from pairwise comparisons of strains using SNP frequencies of evolved *S. aureus* populations. In (A) JU311, N2, CB4858 were identical to ancestor. In (B) JU311, JU363, PB306 were identical to ancestor. Euclidean distances were calculated from the frequency of mutations in each population.

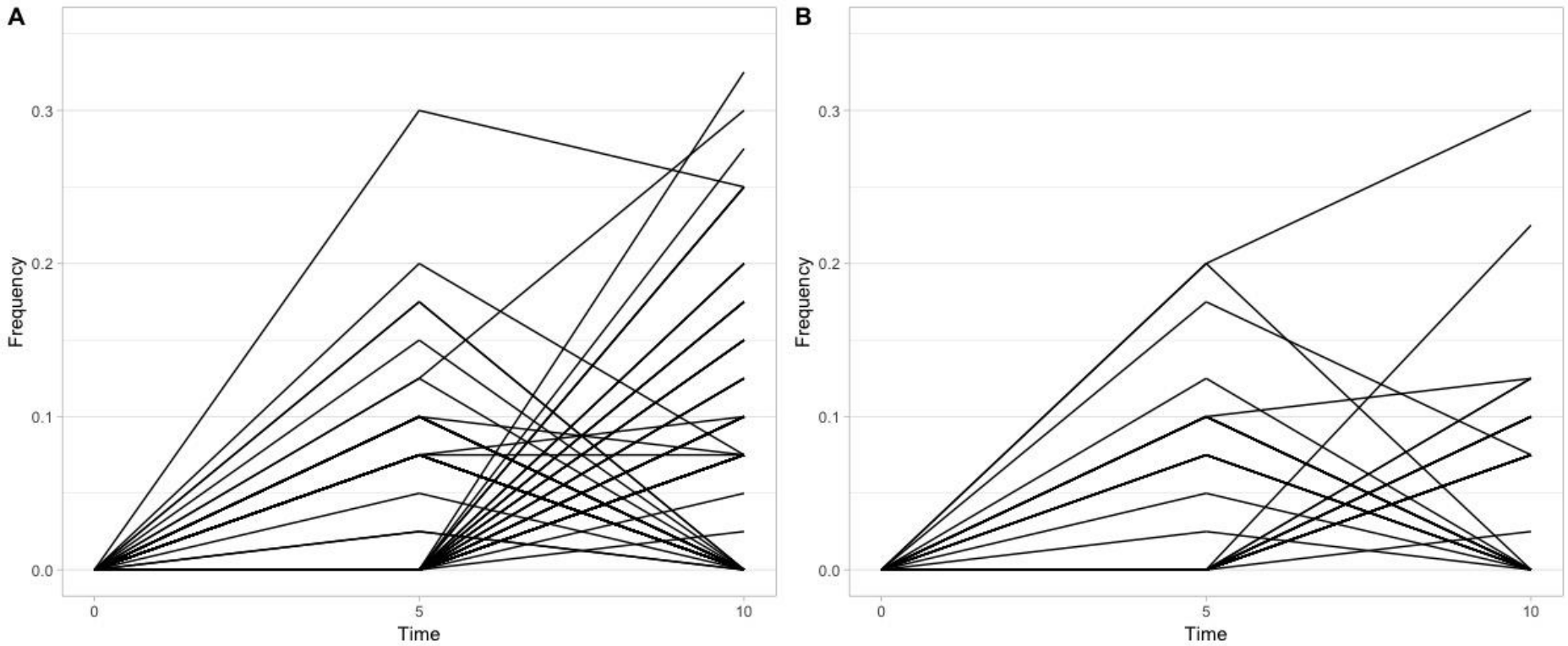



**Figure 8:** SNP frequencies in *Staphylococcus aureus* populations in the evolution experiment over time. The frequency of mutations of 40 sampled clones per population of A) monocultures and B) polycultures. Each line represents a mutation at passage 5 and 10.

Although we observed a burst of pathogen evolution across host populations, selection rarely favoured the same changes in pathogens across host genetic backgrounds after introduction into a novel host species. After identifying SNPs, we grouped the putative functions of the genes in relation to virulence, metabolism, and adherence (Supp. Table 3). We examined the degree to which these functions were under selection by comparing within-population SNP frequencies between evolving pathogens in monocultures and polycultures at passage 10 (Fig. 9). We mostly found no differences in SNP frequencies in pathogen populations between monocultures and polycultures. We found that SNPs in genes with putative functions related to siderophore activity increased in frequency over time in monocultures (Wilcoxon rank sum test, $p = 0.0213$, Fig. 9). Siderophore production in *S. aureus* has been positively associated with the virulence of infections as siderophores are a public good that facilitate more iron extraction from hosts and more pathogen growth[44]. Although we did not see changes in siderophore production *in vitro*, these mutations could affect other aspects of siderophore activity and underlie changes in virulence (comparisons in siderophore production among ancestor and treatments: ANOVA, $X^2 = 2.8986$, d.f. = 2, $p = 0.2347$, Fig. 10A; Spearman's rank correlation between host mortality and siderophore production in monocultures, $S = 2019.8$, $rho = 0.122$, $p = 0.5707$, Fig. 10B; Pearson's product-moment correlation in polycultures, $t = -0.328$, d.f. = 4, $cor = -0.162$, $p = 0.759$, Fig. 10B).

Overall, these results highlight that virulence and infection load can evolve independently and differently in homogenous and diverse host populations. Moreover, diversity here does not confer protection against novel pathogens, but may conversely increase susceptibility.

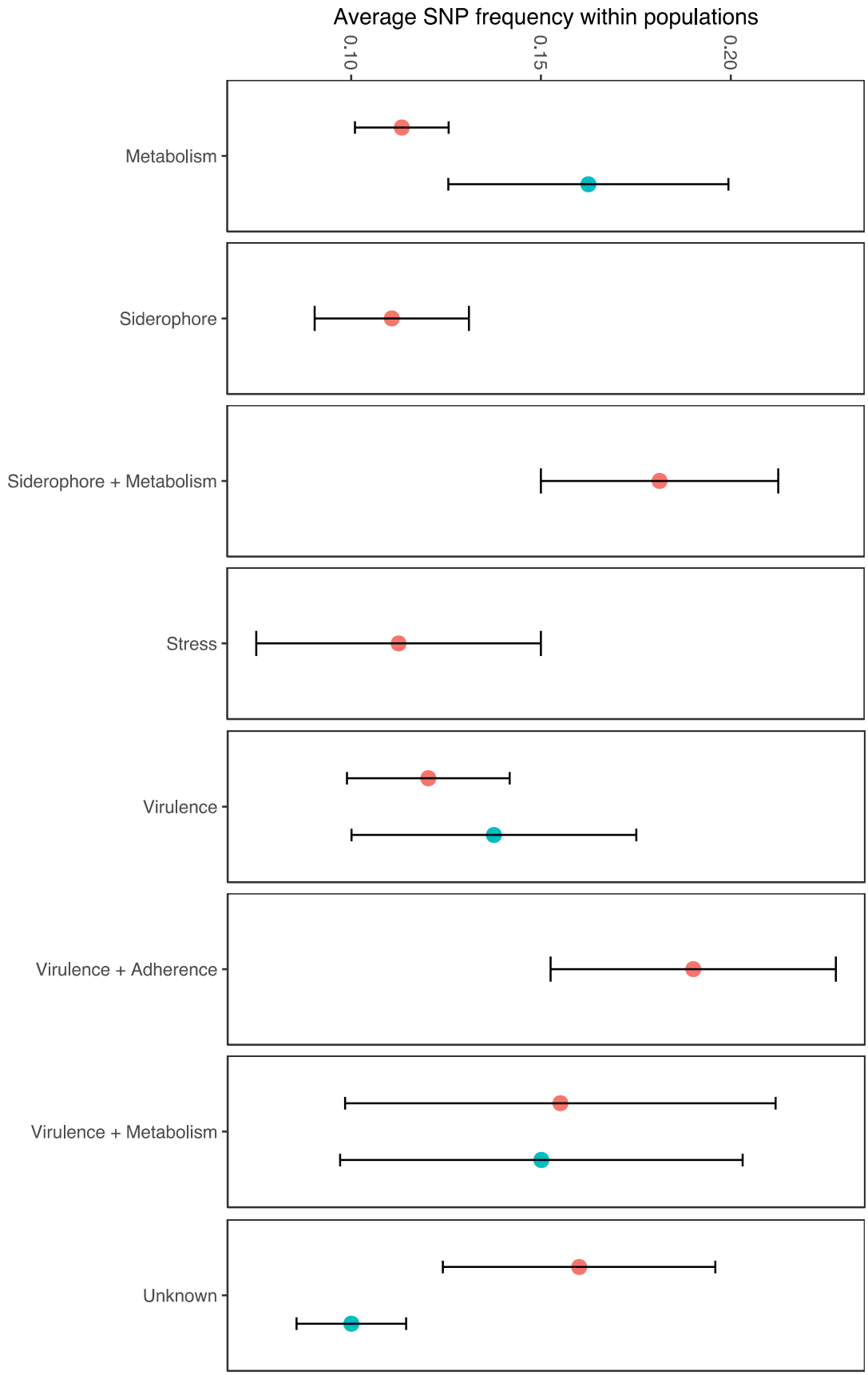

Average SNP frequency within populations
0.10
0.15
0.20
Metabolism
Siderophore
Siderophore + Metabolism
Stress
Virulence
Virulence + Adherence
Virulence + Metabolism
Unknown

**Figure 9**: Within population SNP changes at timepoint 10. Average within population SNP frequency (mean ± SE) of monoculture (red) and polyculture (blue) parasite populations. Within population SNP changes was calculated by adding together SNP frequencies per gene, then dividing by the total number of populations that had that particular SNP change. Gene functions (x-axis) were then determined through a literature search of known genes. We removed functions (Adherence, Adherence + Metabolism, Metabolism + Stress, Virulence + Stress) where only one SNP was found as categories could not be statistically compared. Wilcoxon rank sum tests show no significant differences in SNP acquisition between of monoculture and polyculture for functions; Metabolism ($p = 0.2796$), Virulence ($p = 0.7639$), Virulence + Metabolism ($p = 1$), or Unknown ($p = 0.3653$). Where SNPs were only found in monoculture parasite populations we find that Wilcoxon rank sum test of Siderophore ($p = 0.0213$) is significant from zero, but not for Siderophore + Metabolism ($p = 0.5$), Stress ($p = 0.5$), or Virulence + Adherence ($p = 0.0625$).

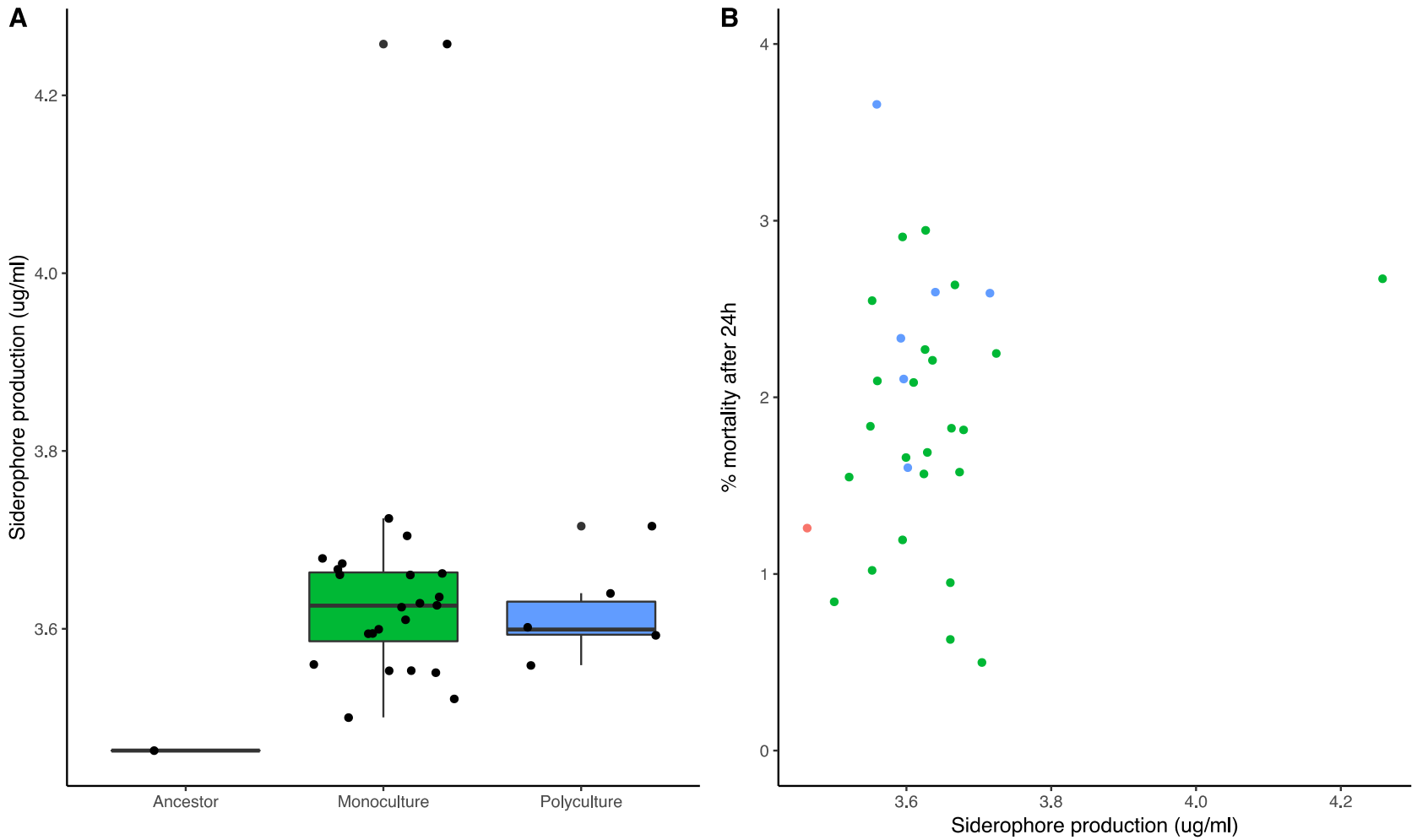



**Figure 10:** Siderophore production. (A) Siderophore concentrations (µg/mL) of ancestral (red), evolved monoculture (green), and evolved polyculture (blue). (B) Host mortality (% dead nematodes) plotted against siderophore production (µg/mL).

## 3.5 Discussion

We observed that evolved genetic distances between *S. aureus* lineages were more similar in closely-related monoculture host genotypes and divergent in the distantly-related. Specifically, after ten passages, variation among pathogen genotypes emerged. These results provide support for the hypothesis that closely related hosts can represent similar selection environments, whether considering jumps between host species[13,14] or evolution within different host genotypes (this study). When we compared patterns of pathogen evolution in closely-related and distantly-related host populations, we found no evidence that host diversity conferred protection (unlike in populations where resistance is already present[26,60,61]). In fact, diversity in these non-resistant host populations may have conversely increased susceptibility. Virulence and infection load (a proxy for transmission potential[62]) predominately increased over time, but evolved separately in both host population types. We thus find that there is independent selection operating on virulence and pathogen load across a range of host population genetic backgrounds in a novel infection.

The dynamics of pathogen virulence evolution is unclear in most emerging disease systems[63]. The 'trade-off' hypothesis assumes that virulence is coupled with transmission because both traits are dependent on host exploitation[30,64]. Theory predicts that early on in the novel interaction when pathogen traits are at non-equilibrium, transmission and virulence can positively covary[12,62], and selection could favour increased virulence[65]. We found evidence to suggest that differences in host traits across isolates, such as metal ion binding, could alter the selection environment in the nematode in ways that shape pathogen virulence evolution. Host metal ion binding can predict the direction of virulence evolution in *S. aureus* ,where iron or zinc limitation[66] results in the production of virulence factors, such as siderophores[57].

However, we found no evidence for a significant association between infection load and host mortality at any point in evolutionary history, suggesting that they evolved separately[67]. Pathogen load and virulence traits have similarly been found to weakly associate and evolve in different directions in natural disease emergence systems[12]. The lack of associations between metrics of virulence and pathogen load in our system suggest that other pathogen traits might better represent transmission potential or virulence, such as within-host growth rate[68] or host reproductive output[69,70], respectively. We additionally show that increases in pathogen load over time in highly related host populations did not depend upon host genetic distance. However, we do find that the pathogen's initial ability to colonise can predict the evolutionary outcome of pathogen load among isolates. Host populations that are easy to exploit (and thus increase transmission potential) may favour faster assimilating pathogens. This result suggests that sampling efforts early in a novel infection could predict the degree of devastation in genetically homogenous host populations.

Transitioning of this bacterium into a novel animal host species and the possibility that its evolutionary dynamics here are short-term and at a non-equilibrium state, may explain the lack of pathogen parallel evolution at the genomic level and why SNPs largely remained at a low frequency. On the other hand, host resistance to emerging pathogens in nature can occur within fewer host generations (e.g., about 20 years for immune adaptation to emerge in Tasmanian devils against facial tumour disease[71] and 12 years for house finches against *Mycoplasma gallisepticum*[72]), suggesting that our timeframe could be a big enough evolutionary window of time. The patterns of genomic evolution could also reflect the occurrence of fluctuating selection or clonal interference we observed in pathogen populations. As mutations arise *de novo*, fluctuating selection can maintain a diversity of

alleles at lower frequencies[73], and clonal interference, a phenomenon common in bacterial evolution, can lead to longer fixation times[74].

Several studies have shown that host diversity limits parasite success[26,61,75] even that of emerging pathogens when standing variation for resistance is likely present[26,68,76]. Conversely, here rather than reducing pathogen colonisation, both the ancestral pathogen and pathogens that evolved in diverse host populations were shown to have higher loads than those evolved in monocultures. If resource competition increases with the relatedness in host populations, as predicted by the niche partitioning theory[77], *C. elegans* stress pathways could be up-regulated in monocultures and strengthen their general defence mechanisms[78]. Alternatively, pooling 24 host isolates might enable those with higher infection loads to super-spread the pathogens in the viscous media environment. A super-spreader host isolate could elevate the pathogen load and mortality rate within the entire replicate host population[79]. Ultimately, aspects of population variation beyond genetic-based resistance could be vital to the transmission and virulence of pathogen infection.

In conclusion, this study reveals that changes in pathogen traits and genomes can be caused by the host population genetic background following emergence. We can expect more infectious disease emergence with global changes driving alterations to habitats and increasing geographic ranges[30,80]. Moreover, the cascading impacts of novel pathogens on ecosystems can be felt when susceptible populations are devastated by infection, sparking losses of both between and within-species diversity along the food chain[81]. Thus, catching the early signs of disease emergence is of paramount importance to reducing the devastating effects on susceptible populations. Our study highlights that within-species host genetic

relatedness warrants consideration as a driver of infection outcomes over time in recent wildlife and human pathogens.

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# Chapter 3:
# Supplementary material

**Table 1:** *C. elegans* strains used in the study. Information obtained from *Caenorhabditis elegans* Natural Diversity Resource (https://www.elegansvariation.org).

| Strain | City, Nation, or State | Isolation Date | Sampled by |
|---|---|---|---|
| **AB1** | Adelaide, Australia | 1983 | D. Riddle and A. Bird |
| **CB4851** | Bergerac, France | Pre-1949 | V. Nigon |
| **CB4853** | Altadena, USA | 1974 | C.D. Johnson |
| **CB4854** | Altadena, USA | 1974 | C.D. Johnson |
| **CB4856** | Oahu, Hawaii | 1972 | L. Hollen |
| **CB4857** | Claremont, USA | 1972 | E.M. Hedgecock |
| **CB4858** | Pasadena, USA | 1973 | E.M. Hedgecock |
| **CX11262** | Los Angeles, USA | 2003 | A. Sivasundar |
| **CX11307** | Los Angeles, USA | 2003 | A. Sivasundar |
| **ED3005** | Edinburgh, United Kingdom | 2004 | A. Cutter |
| **ED3012** | Edinburgh, United Kingdom | 2004 | A. Cutter |
| **ED3017** | Edinburgh, United Kingdom | 2004 | A. Cutter |
| **JU258** | Ribeiro, Madeira | 2001 | M-A. Félix |
| **JU311** | Merlet, France | 2002 | M-A. Félix |
| **JU363** | Franconville, France | 2002 | C. Pieau |
| **JU775** | Lisbon, Portugal | 2005 | M-A. Félix |
| **JU792** | Fréchendets, France | 2005 | M-A. Félix |
| **JU1400** | Sevilla, Spain | 2008 | M-A. Félix |
| **JU1491** | Le Blanc, France | 2008 | M-A. Félix |
| **JU1580** | Orsay, France | 2008 | M-A. Félix |
| **JU1652** | Montevideo, Uruguay | 2009 | NA |
| **LKC34** | Unknown city, Madagascar | 2005 | V. Stowell |
| **N2** | Bristol, UK | 1951 | L.N. Staniland and W. Nicholas |
| **PB306** | NA | 1998 | S. Baird |
| **QX1211** | San Francisco, USA | 2007 | M. Rockman |

**Table 2:** Gene Ontology (GO) term results of *C. elegans* isolate comparisons. Shown are the molecular function comparisons of host isolates varying in genetic relatedness and their infection outcomes from evolved pathogens. *C. elegans* isolate comparisons were selected based on virulence data presented in Fig.2 (main text). For GO term ID, see Supp. Info.2. Highlighted in red are terms that could relate to metal ion binding functions in nematodes.

| **Different virulence in distantly related hosts** | **Different virulence in closely related hosts** | **Similar virulence in distantly related hosts** | **Similar virulence in closely related hosts** |
|---|---|---|---|
| **Comparison 1** | | | |
| **JU775 vs. JU1580** | **JU775 vs. JU1491** | **QX1211 vs. ED3012** | **JU258 vs. JU1580** |
| **cation binding**<br>**transition metal ion binding**<br>**zinc ion binding**<br>**metal ion binding** | G protein-coupled receptor activity<br>**transition metal ion binding**<br>signalling receptor activity<br>transmembrane signalling receptor activity<br>molecular transducer activity | transferase activity, transferring glycosyl groups<br>catalytic activity, acting on RNA<br>transferase activity, transferring hexosyl groups<br>transferase activity<br>catalytic activity | UDP-glycosyltransferase activity<br>transferase activity, transferring glycosyl groups<br>transferase activity, transferring hexosyl groups |

| | | | |
|---|---|---|---|
| | **zinc ion binding**<br>oxidoreductase activity<br>G protein-coupled olfactory receptor activity<br>steroid hydroxylase activity<br>sequence-specific DNA binding<br>DNA-binding transcription factor activity<br>olfactory receptor activity<br>aspartic-type endopeptidase activity<br>aspartic-type peptidase activity<br>G protein-coupled peptide receptor activity<br>**metal ion binding**<br>**cation binding**<br>transcription regulator activity<br>peptide receptor activity | fucosyltransferase activity<br>UDP-glycosyltransferase activity | |

| | | | |
|---|---|---|---|
| **Comparison 2** | | | |
| **JU1400 vs CB4854** | **ED3017 vs N2** | **JU1652 vs JU311** | **JU792 vs LKC34** |
| G protein-coupled olfactory receptor activity<br>G protein-coupled receptor activity<br>olfactory receptor activity<br>acetylgalactosaminyltransferase activity<br>DNA-binding transcription factor activity<br>G protein-coupled peptide receptor activity<br>**transition metal ion binding**<br>sequence-specific DNA binding<br>peptide receptor activity | gated channel activity<br>sequence-specific DNA binding<br>**ion gated channel activity**<br>voltage-gated channel activity<br>**voltage-gated ion channel activity**<br>DNA-binding transcription factor activity<br>**ion channel activity**<br>transcription regulator activity<br>voltage-gated cation channel activity<br>voltage-gated potassium channel activity | ligase activity, forming carbon-nitrogen bonds | carbohydrate binding |

| | | | |
|---|---|---|---|
| transmembrane signalling receptor activity **zinc ion binding** | potassium channel activity **ion transmembrane transporter activity** | | |
| **Comparison 3** | | | |
| **CB4851 vs CB4858** | **CB4858 vs ED3005** | **CB4856 vs AB1** | **CX11307 vs ED3005** |
| **ion transmembrane transporter activity** **ion channel activity** inorganic molecular entity transmembrane transporter activity **protein binding** binding **cation channel activity** small GTPase binding gated channel activity channel activity passive transmembrane transporter activity | G protein-coupled olfactory receptor activity olfactory receptor activity G protein-coupled receptor activity | peptide receptor activity | G protein-coupled receptor activity olfactory receptor activity signalling receptor activity |

| | | | |
|---|---|---|---|
| **ion gated channel activity**<br>Ras GTPase binding | | | |
| **Comparison 4** | | | |
| **ED3017 vs JU1652** | **ED3012 VS JU258** | **CB4854 VS N2** | **CB4856 VS CB4853** |
| steroid hydroxylase activity<br>G protein-coupled peptide receptor activity<br>oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen<br>oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one | sequence-specific DNA binding | anion binding<br>adenyl ribonucleotide binding<br>adenyl nucleotide binding<br>purine ribonucleotide binding<br>purine nucleotide binding<br>ATP binding<br>nucleoside phosphate binding<br>nucleoside phosphate binding<br>nucleotide binding<br>transcription coregulator activity<br>purine ribonucleoside triphosphate binding<br>ribonucleotide binding | DNA-binding transcription factor activity<br>sequence-specific DNA binding<br>G protein-coupled receptor activity<br>peptide receptor activity<br>**G protein-coupled peptide receptor activity**<br>**transition metal ion binding**<br>**transferase activity, transferring glycosyl groups**<br>transcription regulator activity<br>signalling receptor activity |

| | | | |
|---|---|---|---|
| donor, and incorporation of one atom of oxygen<br>peptide receptor activity<br>**transition metal ion binding**<br>DNA-binding transcription factor activity<br>**iron ion binding**<br>sequence-specific DNA binding transcription regulator activity<br>monooxygenase activity | | carbohydrate derivative binding<br>small molecule binding<br>protein binding<br>**ion transmembrane transporter activity**<br>ion gated channel activity<br>drug binding<br>gated channel activity<br>binding<br>voltage-gated channel activity<br>**voltage-gated ion channel activity**<br>inorganic molecular entity<br>transmembrane transporter activity<br>catalytic activity, acting on a protein<br>**ion channel activity** | UDP-glycosyltransferase activity<br>molecular transducer activity<br>oxidoreductase activity<br>**zinc ion binding**<br>steroid hydroxylase activity<br>transmembrane signalling receptor activity<br>transferase activity, transferring hexosyl groups<br>**metal ion binding**<br>**cation binding**<br>oxidoreductase activity<br>carbohydrate binding |

| | | | |
|---|---|---|---|
| | | | |
| **Comparison 5** | | | |
| **CX11262 vs N2** | **QX1211 vs CB4854** | **JU1652 vs JU792** | **JU1491 VS JU360** |
| phosphorus-oxygen lyase activity<br>**voltage-gated ion channel activity**<br>voltage-gated channel activity<br>cyclase activity<br>guanylate cyclase activity<br>binding<br>**protein binding**<br>**voltage-gated cation channel activity** | transferase activity, transferring glycosyl groups<br>UDP-glycosyltransferase activity<br>oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen | DNA-binding transcription factor activity<br>transcription regulator activity | acetylgalactosaminyltransferase activity<br>**ion binding** |

| | | | |
|---|---|---|---|
| potassium ion transmembrane transporter activity<br>gated channel activity | **transition metal ion binding**<br>steroid hydroxylase activity<br>transferase activity, transferring hexosyl groups<br>sequence-specific DNA binding<br>**zinc ion binding**<br>G protein-coupled receptor activity<br>DNA-binding transcription factor activity<br>G protein-coupled olfactory receptor activity<br>glucuronosyltransferase activity | | |

**Table 3:** SNP data of passage 10 with gene function allocation of gene products. Distribution across host monoculture (mono) and polyculture (poly) population is graphically shown in figure 9 (main text).

| Position | Reference | SNP | Gene tag | Gene product | Host population | Function |
|---|---|---|---|---|---|---|
| 534776 | T | G | SAS_RS02555 | 16S ribosomal RNA | Mono | **Metabolism**[1] |
| 1434373 | T | A | SAS_RS07090 | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase | Mono & Poly | **Metabolism**[2] |
| 2116677 | G | A | SAS_RS10675 | 23S ribosomal RNA | Poly | **Metabolism**[3] |
| 2290705 | G | T | SAS_RS11560 | 30S ribosomal protein S5 | Mono | **Metabolism**[4] |
| 508473 | T | G | SAS_RS02380 | 4-diphosphocytidyl-2C-methyl-D-erythritol kinase | Mono | **Metabolism**[5] |
| 2734062 | G | A | SAS_RS13830 | accessory Sec system protein Asp1 | Poly | **Virulence**[6] |
| 252031 | A | G | SAS_RS01080-SAS_RS01085 | acyl CoA:acetate/3-ketoacid CoA transferase - PrsW family intramembrane metalloprotease | Poly | **Virulence + Adherence**[7] |
| 1693527 | C | T | SAS_RS08335 | adenine phosphoribosyltransferase | Mono | **Metabolism**[8] |
| 1362660 | G | A | SAS_RS06750-SAS_RS06755 | amino acid permease - catalase | Mono | **Siderophore**[9] |
| 1595090 | C | G | SAS_RS07805 | aminopeptidase P family protein | Mono | **Metabolism**[10] |
| 384882 | G | A | SAS_RS01735 | aminotransferase class I/II-fold pyridoxal phosphate-dependent enzyme | Poly | **Unknown** |
| 1326110 | C | A | SAS_RS06535 | aquaporin family protein | Mono | **Metabolism**[11] |
| 1431962 | C | T | SAS_RS07075 | aspartate-semialdehyde dehydrogenase | Poly | **Virulence + Metabolism**[12] |
| 1548501 | T | C | SAS_RS07560 | ATP-dependent DNA helicase RecQ - reduces nematode killing | Mono | **Virulence**[13] |
| 1900576 | A | T | SAS_RS09310-SAS_RS09315 | beta-channel forming cytolysin | Mono | **Virulence**[14] |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1059975 | TAGCTTTAGGTGCTGATGTAGTTGC | T | SAS_RS05230 | bifunctional autolysin | Mono | **Adherence + Metabolism**[15] |
| 2703970 | T | C | SAS_RS13725 | carbamate kinase 2 | Mono & Poly | **Metabolism**[16] |
| 304315 | G | T | SAS_RS01330-SAS_RS01335 | CHAP domain-containing protein - WXG100 family type VII secretion effector EsxA | Mono | **Siderophore**[17] |
| 1034531 | A | ATTAT | SAS_RS05095 | CPBP family intramembrane metalloprotease | Mono | **Metabolism**[18] |
| 2317639 | A | T | SAS_RS11750 | cyclic pyranopterin monophosphate synthase | Mono | **Metabolism**[19] |
| 2527945 | G | T | SAS_RS12845-SAS_RS12850 | D-histidine (S)-2-aminobutanoyltransferase CntL - histidine racemase CntK | Mono | **Siderophore**[20] |
| 2195585 | ATTCGC | A | SAS_RS11090-SAS_RS11095 | deoxyribose-phosphate aldolase - purine-nucleoside phosphorylase | Mono | **Metabolism**[21] |
| 1322701 | AGCG | A | SAS_RS06525 | DNA mismatch repair protein MutL | Mono | **Metabolism + Stress**[22] |
| 702734 | G | A | SAS_RS03350 | DNA-binding response regulator | Poly | **Metabolism**[23] |
| 458723 | A | G | SAS_RS02130 | DUF2309 family protein | Poly | **Unknown** |
| 325676 | C | A | SAS_RS01450 | DUF4064 domain-containing protein | Mono | **Unknown** |
| 2308383 | G | A | SAS_RS11705 | efflux RND transporter permease subunit | Mono | **Siderophore**[9] |
| 1083589 | A | C | SAS_RS05345 | energy-coupling factor transporter transmembrane protein EcfT | Mono | **Siderophore**[24] |
| 1658833 | C | A | SAS_RS08165-SAS_RS08170 | enterotoxin - Fic family protein | Mono | **Virulence**[25] |
| 1263454 | GAAATA | G | SAS_RS06275 | FemA/FemB family glycyltransferase FmhC | Mono | **Metabolism**[26] |
| 1163190 | C | T | SAS_RS05760-SAS_RS05765 | fibrinogen-binding protein | Mono | **Virulence + Adherence**[25] |
| 326572 | C | G | SAS_RS01455 | formate/nitrite transporter | Poly | **Metabolism**[27] |
| 1332856 | G | T | SAS_RS06570 | GTPase HflX | Mono | **Metabolism**[28] |
| 1495257 | C | T | SAS_RS07310 | hyperosmolarity resistance protein Ebh | Mono | **Virulence + Metabolism**[29] |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1116202 | G | T | SAS_RS05520 | hypothetical protein | Mono & Poly | **Unknown** |
| 524447 | A | T | SAS_RS02465-SAS_RS02470 | hypoxanthine phosphoribosyltransferase - ATP-dependent metallopeptidase FtsH/Yme1/Tma family protein | Mono | **Unknown** |
| 1682025 | T | A | SAS_RS08275 | LLM class flavin-dependent oxidoreductase | Mono | **Stress**[30] |
| 1578429 | C | A | SAS_RS07715 | M20/M25/M40 family metallo-hydrolase | Mono | **Stress**[31] |
| 2007983 | C | T | SAS_RS10010 | manganese-dependent inorganic pyrophosphatase | Mono | **Virulence + Metabolism**[32] |
| 730082 | C | A | SAS_RS03495-SAS_RS03500 | MarR family transcriptional regulator - GTP-binding protein | Mono | **Virulence**[33] |
| 2220609 | G | A | SAS_RS11185 | methicillin resistance protein FmtB | Poly | **Virulence + Metabolism**[34] |
| 862171 | T | C | SAS_RS04175 | methionine import ATP-binding protein MetN 2 | Poly | **Adherence + Metabolism**[35] |
| 2420676 | C | T | SAS_RS12285 | MFS transporter | Mono | **Siderophore + Metabolism**[9] |
| 601843 | T | C | SAS_RS02815-SAS_RS02820 | MSCRAMM family adhesin SdrD - MSCRAMM family adhesin SdrE | Mono | **Virulence + Adherence**[36] |
| 341627 | C | T | SAS_RS01525 | N-acetylmannosamine-6-phosphate 2-epimerase | Mono | **Adherence**[37] |
| 957468 | G | A | SAS_RS04635 | oligoendopeptidase F | Mono | **Virulence + Stress**[38] |
| 1188175 | C | A | SAS_RS05930 | penicillin-binding protein | Mono | **Virulence + Metabolism**[39] |
| 2027051 | C | A | SAS_RS10135-SAS_RS10140 | peptidoglycan hydrolase | Mono | **Virulence + Metabolism**[40] |
| 197793 | A | G | SAS_RS00855 | permease | Mono | **Siderophore**[9] |
| 1224242 | C | A | SAS_RS06085-SAS_RS06090 | primosomal protein N' | Mono | **Metabolism**[41] |
| 440881 | T | A | SAS_RS02050 | restriction endonuclease subunit S | Poly | **Metabolism**[42] |
| 1098392 | G | A | SAS_RS05420 | ribonuclease J 1 | Poly | **Virulence + Metabolism**[43] |
| 800534 | C | CA | SAS_RS03855- | ribosome-associated translation inhibitor RaiA - preprotein | Mono | **Siderophore**[44] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | SAS_RS03860 | translocase subunit SecA | | |
| 2743155 | C | A | SAS_RS13840 | serine-rich repeat glycoprotein adhesin SasA | Mono | **Virulence + Adherence**[45] |
| 1986449 | G | A | SAS_RS09915 | sodium:proline symporter | Mono | **Siderophore + Metabolism**[9] |
| 2066931 | TGCCAA | T | SAS_RS10420-SAS_RS10425 | staphylococcal enterotoxin type K - site-specific integrase | Mono | **Virulence**[46] |
| 99839 | G | T | SAS_RS00420-SAS_RS14315 | staphylococcal protein A | Mono | **Virulence + Adherence**[47] |
| 445553 | G | A | SAS_RS02070 | tandem-type lipoprotein | Mono & Poly | **Virulence**[48] |
| 848777 | C | A | SAS_RS04065 | thermonuclease - biofilm formation | Mono | **Virulence + Adherence**[49] |
| 1368440 | A | G | SAS_RS06785-SAS_RS06790 | transcriptional repressor LexA - hypothetical protein | Poly | **Metabolism**[50] |
| 635932 | G | A | SAS_RS02985 | transposase | Mono | **Unknown** |
| 1408853 | G | T | SAS_RS06960 | tryptophan synthase subunit alpha | Mono | **Virulence + Metabolism**[51] |
| 1959839 | G | A | SAS_RS09760 | type 1 glutamine amidotransferase | Mono | **Metabolism**[52] |
| 1791153 | TA | T | SAS_RS08805-SAS_RS08810 | tyrosine--tRNA ligase - penicillin-binding protein | Mono | **Virulence**[53] |
| 1545839 | C | T | SAS_RS07545 | YpdA family putative bacillithiol disulfide reductase | Poly | **Virulence + Metabolism**[54] |
| 2402539 | G | A | SAS_RS12205 | zinc ribbon domain-containing protein | Mono | **Unknown** |

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# 4

# Generalism is maintained in a novel host-pathogen interaction

Data presented in this chapter form a manuscript together with data from Chapter 3.

## 4.1 Abstract

Generalist pathogens can infect multiple host species and/or genotypes. One of the benefits of being a generalist pathogen is the increased chance of successful infection of a novel host. However, it is unclear whether generalists could evolve to specialise on a particular host genotypes upon introduction in a novel host population. We performed exposure assays of monoculture-evolved and polyculture-evolved *Staphylococcus aureus* to compare pathogen performance (infection load and host mortality) in sympatric and novel *Caenorhabditis elegans* host genetic backgrounds. We found that, across all treatments and populations, similar pathogen loads and levels of infection-related host mortality were recorded in both sympatric and novel host isolates. Moreover, we found no relationship between pathogen performance and pairwise genetic distance between sympatric and novel host genetic backgrounds, such as would be predicted in a host species shift. Together, these results highlight the effectiveness of emerging pathogens in maintaining their generalism after evolution in a novel host species.

## 4.2 Introduction

Pathogens (and parasites) can vary in the degree to which they infect a diversity of host genotypes and species. Some pathogens have a narrow host range and specialise on particular genotypes of a host species[1,2]. In contrast, some have the ability to infect a wide array of host genotypes within a species[3], multiple host species[4–6], and can also colonise non-host environments[7]. Pathogen specialism can evolve due to aspects of host-pathogen biology, such as when hosts have reduced dispersal[8], hosts are larger[9], or when pathogens have complex life cycles[10]. Indeed, when the interaction between host and pathogen is highly specific, genes involved in resistance in the host are mirrored in the pathogen genes involved in infectivity/virulence[11,12]. Coevolution between these traits have been shown to drive host-pathogen specificity[11,13] and local adaptation[14,15]. To what extent (and when), such specificity can arise in a generalist pathogen in a novel host-pathogen interaction, where the pathogen is the only species allowed to evolve, is unclear.

There are many benefits associated with host generalism, at both the genotype and species levels. The ability to infect multiple hosts could reduce the chance of pathogen eradication in the event of one host species going extinct[16,17]. Nevertheless, infecting novel hosts can come with a cost. Host switching has the potential of reducing pathogen fitness in the original host species due to the loss of adaptation after evolution in the novel host species[18–20]. If these adaptations are associated with pathogen reproduction, continued switching between host species or genotypes could result in pathogen bottlenecks[21] or maladapted virulence[18]. In addition, if diversity was high in a  host population , in terms of genetic variation for resistance, pathogen performance could be inhibited[22–24]. Given the nature of generalist pathogens, it is therefore unclear whether many generations of evolution in homogenous host

populations could promote specialism. Whether evolution in diverse host populations will alternatively maintain pathogen generalism is also untested.

The degree to which a pathogen can successfully infect in a new host can depend on the phylogenetic relatedness between the new and old host[25]. When susceptibility to infection in the natural host species is similar in the novel host species[25–27], pathogens can shift successfully. With increasing genetic distance between two host species, the chance of successful pathogen transmission is reduced[28,29]. Indeed, the importance of host phylogeny in host shifts has been shown in primate lentivirus[30], rabies virus[31], and drosophila RNA virus[25], where the pathogen increases its host range in a pattern according to host phylogenetic relatedness. This close connection between host relatedness and parasite success may explain why emerging pathogens are usually observed to be generalists: pathogens that do not need to evolve different infection strategies or mechanisms to jump into a related host[32]. Whether this pattern is consistent on a smaller phylogenetic scale, between host genetic backgrounds within a species, is unknown.

Here, we tested whether the genetic relatedness between isolates of the nematode *Caenorhabditis elegans* determined the success of a host switch by evolved lines of *Staphylococcus aureus* from the experiment in Chapter 3. By randomly selecting five nematode isolates, with corresponding evolved pathogen strains, we compared pathogen success in terms of virulence (pathogen-induced host mortality) and infection load. We predicted that if monoculture-evolved pathogen strains have adapted to their sympatric host genotype, then pathogen performance should be higher in the sympatric and lower in the novel host. We further predicted that polyculture-evolved pathogens should conversely maintain their generalism, and so pathogen performance should be similar in novel and

sympatric host populations. We finally quantified host genetic distance to see if patterns of pathogen performance before and after a host isolate shift were linked to host relatedness, whereby pathogens are more likely to infect closely related hosts than distantly related hosts, as seen in host shift studies[26,27].

## 4.3 Materials and methods

### 4.3.1 Nematodes and bacterial pathogen

We randomly selected five *Caenorhabditis elegans* isolates from the evolution experiment detailed in Chapter 3 (Supp. Table 1) as our monoculture (high within-population relatedness) host populations. For our polyculture (low within-population relatedness) treatment we used all twenty-four *C. elegans* isolates (Chapter 3, Supp. Table 1). We generated genetically homogenous lines for each isolate, sustaining them on separate nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 in a 20°C incubator. Nematode isolate synchronisation was achieved by harvesting unhatched eggs from the bleaching process, whereby a 1:1 solution of bleach (NaClO) and 5M sodium hydroxide killed bacterial cells and nematodes. The remaining eggs were incubated over night at 20°C on a shaking platform, when upon hatching were plated onto NGM plates seeded with *E. coli* OP50 food.

Frozen (-80°C) stocks of *Staphylococcus aureus* MSSA476 (GenBank: BX571857.1) were made from the endpoint of evolution experiment performed in Chapter 3. These stock populations had undergone the following procedure: *S. aureus* was passaged 10 times under two different levels of host relatedness, high within-population relatedness (24 independent

monoculture nematode host populations) and low within-population relatedness (six pooled replicate polyculture host populations of 24 nematode isolates), where monoculture and polyculture evolved lines were produced. At each passage, nematode populations (containing both alive and dead animals) were removed, washed, and crushed, where 10% of the population was plated onto Mannitol Salt Agar (MSA) selective media to harvest *S. aureus* colonies for the next passage. Pathogen passages were performed in 24-well plates containing viscous media to limit host avoidance behaviours[33,34], containing approximately 500 L4 (larval stage 4) nematodes per well.

For the assays herein, evolved lineages at passage 10 of the evolution experiment were taken from the freezer, inoculated into Tryptone Soy broth (TSB), and grown overnight at 30°C.

### 4.3.2 Phenotypic assays

We first examined whether our pathogen populations had evolved to specialise on host isolates in monoculture and maintain generalism in host polycultures. We exposed sympatric host populations and a novel host isolate (*C. elegans* CB4857) to evolved pathogens (passage 10 of the evolution experiment, chapter 3) from a random selection of five host isolate biological replicates or five polyculture biological replicates (Fig. 1). Each population contained three technical replicates. Using approximately 200 nematodes per replicate population, host populations were incubated at 25°C for 24h in 250 µL viscous media supplemented with 50 µL evolved pathogen culture in 48-well plates. The viscous media solution consisted of TSB media supplemented with 2.3g HPMC cellulose ((Hydroxypropyl) methyl cellulose, Sigma-Aldrich). After 24h, we calculated host mortality and within-host infection load of all treatments. Host mortality was measured by counting the proportion of dead to total nematodes per replicate. Nematodes were considered dead when they did not

respond to the touch of a platinum wire[35]. To measure infection loads we washed 5 randomly selected nematodes in three 10 μL droplets of M9 buffer before adding them into Eppendorf tubes with 100 μL M9. Pathogens were harvested from nematode guts by first adding 0.7 mm Zirconia beads (BioSpec products), then placing the tubes into a mini beadbeater (BeadBug™, Microtube homogenizer) and shaking for 320 rpm for 45 sec, before being plated out onto selective MSA media plates. After overnight growth at 37°C, infection load was estimated by counting the number of colony forming units (cfu)/nematode host. The degree of host specialisation was calculated by subtracting pathogen performance (host mortality caused, pathogen infection load) in the novel host background from the sympatric host background for each replicate population[36,37].

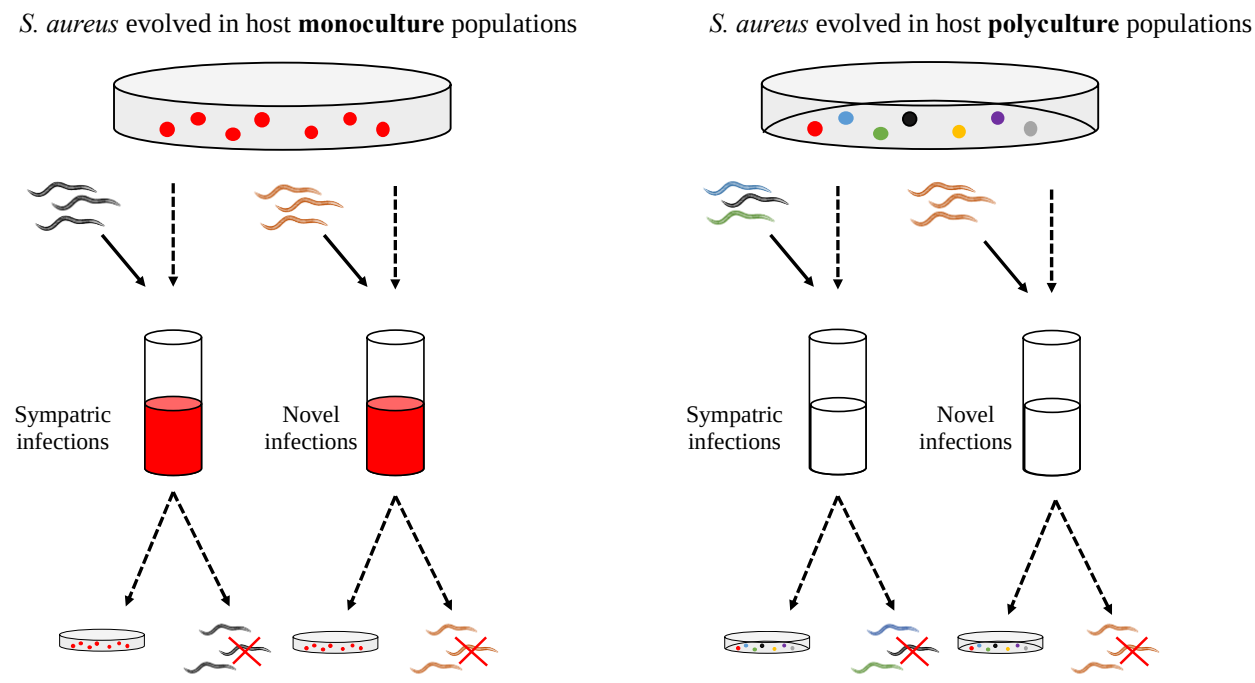



**Figure 1:** Assay design testing for specialism by *S. aureus* lineages. Five biological replicates of monoculture-evolved and polyculture-evolved *S. aureus* strains , each containing three technical replicates, were grown overnight, and then exposed to both sympatric and novel (CB4857) host isolates. After 24h pathogen exposures, host mortality was measured and five nematodes per replicate were extracted, washed, crushed, and plated onto MSA selective media to calculate pathogen infection load.

### 4.3.3 Statistical analysis

Data analysis was conducted in R v 3.6.1. Parametric tests were performed on data that met the assumptions for normality (Shapiro tests) and equal variance (F-tests or Levene's test of more than two groups). As such, a *t*-test was used to examine whether evolved virulence (host mortality) in monocultures was higher in sympatric hosts compared to novel. When assumptions for normality were not met, we used Wilcoxon rank sum tests to determine if high infection loads in monocultures were higher in sympatric hosts compared to novel. To evaluate whether generalism was maintained in polyculture-evolved pathogens, virulence and infection load measures were compared between sympatric and novel host populations through Wilcoxon rank sum tests.

Pearson's product-moment correlation test were conducted to assess if there was a negative linear relationship between pathogen performance and pairwise host genetic distance.

We tested whether the performance of evolved *S. aureus* in novel and sympatric hosts was related to pairwise host genetic distance. We calculated Euclidian distances between a novel host isolate (*C. elegans* CB4857) and a random selection of five sympatric host isolates. To do this, we downloaded the hard-filtered variants of *C. elegans* from the "Caenorhabditis elegans Natural Diversity Resource" (https://www.elegansvariation.org/) and extracted all fixed variants (AF=1) associated with any of the 6 nematode isolates. Next, Euclidean distances between variant frequencies were estimated by creating phylogenetic trees by calculating hierarchical clustering of distances between isolates. Host genetic distance from the novel *C. elegans* isolate could thus predict pathogen success in other host isolates in terms of host mortality and pathogen load.

## 4.4 Results

Given that pathogens in our evolution experiment largely evolved to increase their exploitation of monoculture host populations (Chapter 3), we assessed whether these pathogens had become adapted to their host genetic background. We found that the pathogens did not evolve to specialise in monocultures (infection load: Wilcoxon rank sum test, $V = 52$, $p = 0.679$, Fig. 2A; host mortality: One sample t-test, $t = 1.55$, d.f. $= 14$, $p = 0.143$, Fig. 2B). We further hypothesised that the potential for specialism might be more limited when pathogens evolve in a genetically diverse host population. We found that pathogens evolved in host polycultures were equally able to infect and kill hosts of different genetic backgrounds (Assay design in Fig. 1; infection load: Wilcoxon rank sum test, $V = 85.5$, $p = 0.156$, Fig. 2A; host mortality: Wilcoxon rank sum test, $V = 67$, $p = 0.142$, Fig. 2B). A generalist strategy thus seems to be a conserved outcome of pathogen evolution in this study regardless of the genetic background of the sympatric or novel host population.

We then examined whether pathogen performance in a monoculture host could be predicted by the genetic distance between the sympatric and novel host isolate. However, we found no association between host relatedness and infection load (Pearson's product moment correlation, $R = -0.492$, d.f. $= 13$, $p = 0.062$, Fig. 3A) or with host mortality (Pearson's product moment correlation, $R = -0.103$, d.f. $= 13$, $p = 0.715$, Fig. 3B). These results suggest that host relatedness does not affect the success of a host switch in this host-pathogen relationship. The novel pathogen here maintained a generalist infection strategy, retaining the ability to infect multiple host isolates equally.

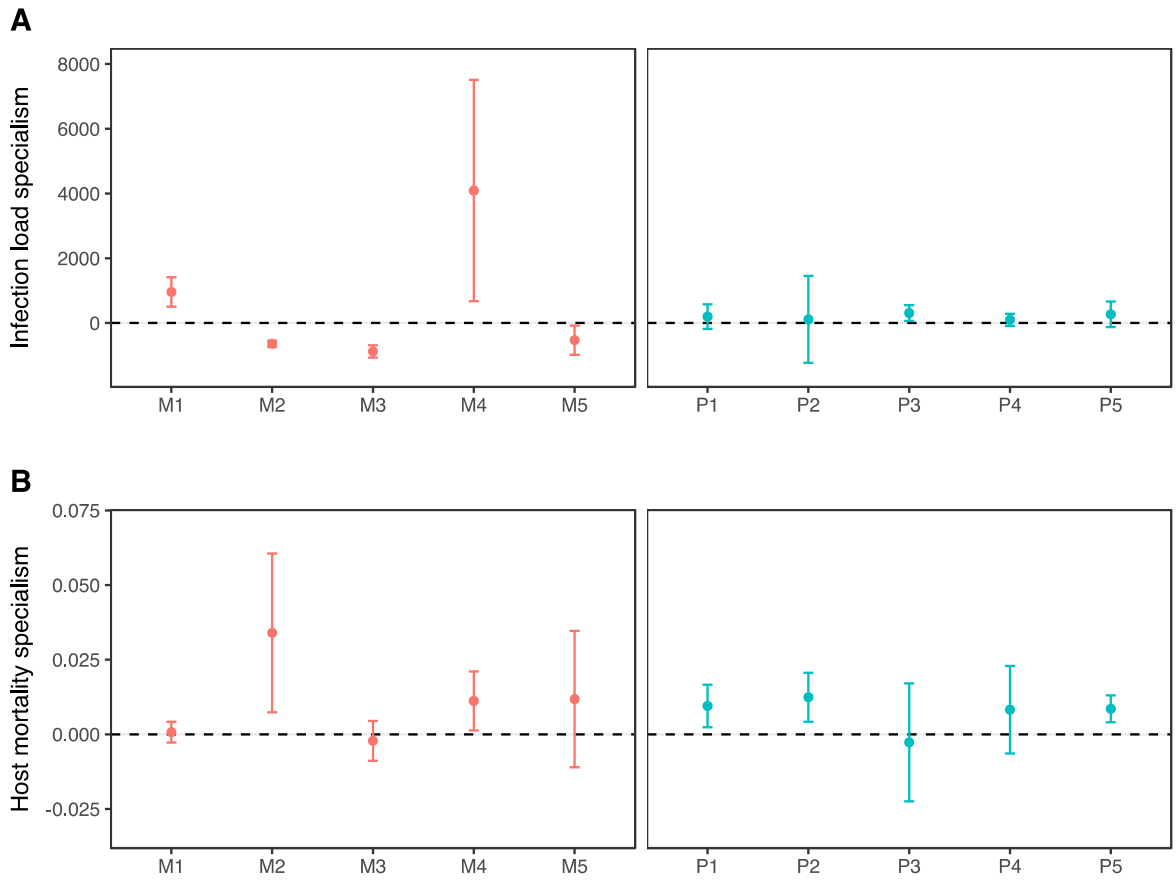



**Figure 2:** Pathogen generalism is maintained across evolved *S. aureus* lineages. Measurements of pathogen performance in host population genetic backgrounds were taken in the form of (A) pathogen infection load (cfu/host) and (B) host mortality (% proportion of dead in population) in monoculture-evolved (Red: M1-M5) and polyculture-evolved (Blue: P1-P5) *S. aureus* separately. Degree of specialism was measured by subtracting pathogen performance in novel (CB4857) host associations from sympatric[36,37], where 0 (dotted line) shows no difference between novel and sympatric *S. aureus* infections. Points show mean ± SE of three technical replicates.

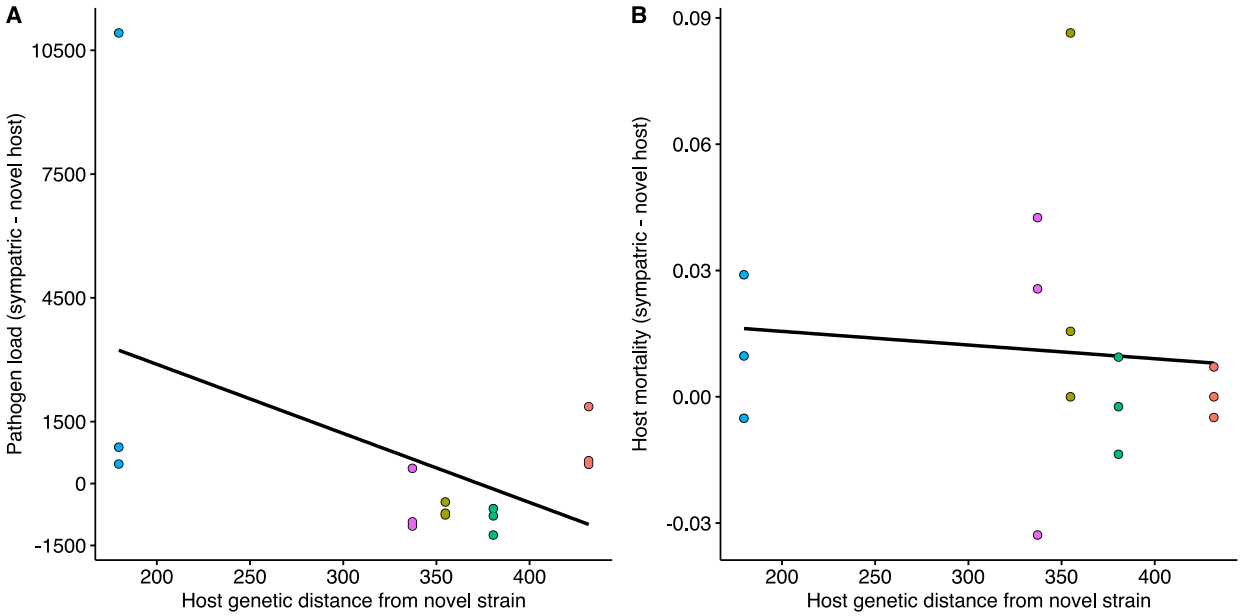



**Figure 3**: Pathogen (A) infection load (cfu/host) and (B) host mortality (% proportion of dead in population) is not a function of genetic distance between sympatric and novel host monocultures. Pairwise host genetic distances were calculated by measuring the Euclidean distances between isolates. Host specialism was calculated by subtracting measures of pathogen performance (within-host infection load and host mortality) in the novel host from values in the sympatric host in which the pathogen evolved for each replicate population (Kawecki & Ebert 2004, Morley *et al.* 2017). Each point colour represents a different pathogen replicate population with three technical replicates for each.

## 4.5 Discussion

We found that *S. aureus* maintained a generalist strategy over evolutionary time. Evolving in a monoculture did not preclude or predict the pathogen's ability to jump into another host population. This result could be explained by the prevalence of host shifts in *S. aureus*[38,39], where its large host range[40–44] has helped it emerge in domesticated bovine[40], poultry[45], and rabbits[42], in addition to being capable of infecting a diversity of invertebrate species including *C. elegans*[46,47]. As a result of their larger host ranges, generalist pathogens are more likely to emerge in other host species than specialist pathogens (i.e., those infect one host species only)[18,48]. Through high mutation rates, generalist pathogens are able to produce a diverse set of genetic variants[16,48]. This diversity increases the chance that one or more variant(s) are capable of avoiding host immune responses and limit specialized host resistance. Thus, the pathogen studied here is a generalist, much like other novel, emerging infections[4,49,50].

Many emerging infections are either highly virulent[51,52] or completely avirulent[53]. We found that *S. aureus* virulence remained relatively low across sympatric and novel host-pathogen associations (~2% mortality). However, low levels of mortality can still cause devastation to host populations[54,55]. Also, minimal reductions in population sizes could have consequences to host social structures. Pathogens (or parasites) only infecting females[56], males[57], or that target the young[58] can completely re-shape populations. For example, parasite pressure in females can reduce offspring quality or reproductive output[59], failing to find a mate due to shortage of males can alter the mating system of a host population[57], and fatalities to young can cause significant reduction in population size and productivity in social insects[58]. Parasite

infection of a set proportion of a host population could therefore have negative cascading effects for the host population as a whole.

With the maintenance of generalism, it is possible for pathogens to jump back into their original host. As new, specific adaptations are not required to successfully infect a novel host, the cost of increasing the range of a generalist pathogen is reduced. For example, in *S. aureus* strains, jumps into domesticated animals are frequent[39,45,60]. As the result of continued contact with farm animals, jumps back into humans from cows have been documented[40]. Although there are cases where *S. aureus* strains has failed to re-infect its original host species. In fact, the loss of function in an adhesion-associated gene in the strain MRSA ST398 meant that the host jump from pig back into one human did not result in continued transmission between humans[61].

In conclusion, this study shows that *S. aureus* causes similar levels of host harm in *C. elegans* populations irrespective of host genetic background. With the ability to infect multiple genotypes, in addition to having the capacity to successfully switch host, this could reduce the potential for coevolution to occur between pathogen and host. As *S. aureus* has the ability to circumvent antibiotic treatment, methods beyond host genetic diversity may need to be considered to reduce this pathogen's infection potential in a novel host population. Additionally, with the increased movement and contact between animals we can expect more emergence of generalist pathogens worldwide[16,49,62].

## 4.6 References


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# Chapter 4:
# Supplementary material

**Table 1:** *C. elegans* strains used in the study. Information obtained from *Caenorhabditis elegans* Natural Diversity Resource (https://www.elegansvariation.org).

| Strain | City, Nation, or State | Isolation Date | Sampled by |
|---|---|---|---|
| **AB1** | Adelaide, Australia | 1983 | D. Riddle and A. Bird |
| **CB4853** | Altadena, USA | 1974 | C.D. Johnson |
| **CB4857** | Claremont, USA | 1972 | E.M. Hedgecock |
| **ED3017** | Edinburgh, United Kingdom | 2004 | A. Cutter |
| **JU258** | Ribeiro, Madeira | 2001 | M-A. Félix |
| **JU1491** | Le Blanc, France | 2008 | M-A. Félix |

# 5

# Thesis discussion

This thesis investigates the prevalence of the monoculture effect across systems in addition to the importance of host relatedness in shaping pathogen evolution and host range. I highlight the main findings of each chapter, followed by a discussion and wider implications of this body of work.

**Chapter 2: Host genetic diversity limits parasite success beyond agricultural systems: a meta-analysis**

In Chapter 2, I looked at the presence of the monoculture effect beyond agricultural studies, the context in which the effect had been traditionally evaluated. The monoculture effect was originally described in agriculture as disease was observed to spread through single cultivar crops better than multiline or multispecies crops[1]. It has therefore long been known that homogenous populations pose the greatest threat to disease risk but a formal assessment across species has not been done until now.

I demonstrated that i) host population genetic diversity limits parasite success across multiple systems, ii) microparasite infection is particularly reduced by high host population genetic diversity, iii) field studies show a stronger effect of host population genetic diversity against parasite success compared to studies in the lab, and iv) parasites with a wide or narrow host range are equally stopped by host diversity. These findings illustrate the widespread benefit of host population genetic diversity in protecting against parasite spread. Indeed, even when considering different host-parasite interactions (genetic-level specialisation of coevolved parasites to emerging infections), genetically homogenous host populations were consistently more susceptible to parasite infection.

This analysis highlighted an important gap in need of further investigation. Novel pathogens infecting diverse host populations, wherein all genotypes are susceptible to infection, may show different pathogen evolutionary trajectories to those pathogens met by resistant genotypes. Yet, the majority of experiments that have advanced our understanding on the protective effects of host genetic diversity are based on host species that share an evolutionary history with the parasite, or at least for which there is likely genetic variation for resistance. However, most novel infections will initially encounter totally susceptible host populations with resistance yet to evolve.

**Chapter 3: Host relatedness shapes the evolution of a novel bacterial infection**

The ability of a pathogen to successfully shift into novel hosts can depend on the phylogenetic relatedness between the ancestral and new host species. This is predicted to be the case as closely-related host species are likely to offer the pathogen similar infection environments[2]. However, it is unclear if this phenomenon is found within-species.

In Chapter 3, I track pathogen evolution under two distinct host treatments: closely-related host populations and distantly-related host populations. I found i) that evolved *S. aureus* monocultures of closely-related *C. elegans* host genotypes show similar levels of virulence but not pathogen load, ii) no association between the evolutionary trajectories of virulence and pathogen load, and iii) diverse host populations were more susceptible to infection relative to homogeneous host populations. Together, these findings reveal the rapid changes in pathogen traits and genomes that can be shaped by the host population genetic background. Additionally, future study into host population variation, beyond genetic-based differences in resistance, could be vital particularly when the whole host population is susceptible to a novel pathogen.

The novel pathogen used in this study is considered a host generalist, but has on occasion evolved host species specialism[3]. It was therefore unclear whether *S. aureus* in our experimental regime would evolve within-host species genotypic specialism or infect all genotypes equally.

**Chapter 4: Generalism is maintained in a novel host-pathogen interaction**

In Chapter 4, I extended the work of Chapter 3 to consider the evolution of host specialism. Infecting a novel host can be costly, even for a generalist pathogen, with the potential of reduced fitness in the original host. As we found that closely-related host genotypes displayed similar levels of pathogen virulence (Chapter 3), we wanted to investigate whether specialism had evolved in our pathogen populations.

I investigated changes in pathogen host range by performing assays using evolved lines of *S. aureus* in closely-related (monoculture) and distantly-related (polyculture) host populations on sympatric and novel hosts. I showed that both monoculture-evolved and polyculture-evolved *S. aureus* performance (pathogen load and virulence) was similar in both sympatric and novel host associations. There was additionally no relationship between host genetic distance and pathogen performance. Combined, these findings show that pathogen generalism was maintained in all host associations over evolutionary time.

## 5.1 General themes and future work

This thesis has explored the impact of host genetic relatedness on the spread and evolution of a novel pathogen. The emerging themes of this thesis are discussed below in addition to suggestions for future work.

### 5.1.1 Host-pathogen specificity and the monoculture effect

This thesis shows that the monoculture effect can occur in interactions lacking in tight host-parasite specificity, where host range was not influenced by host genetic diversity (Chapter 2). As low within-host population diversity allowed for high parasite success, well-adapted parasite and novel parasites performed equally well in these host environments. These results have concerning implications given the current increased opportunity of emerging infectious diseases infecting host populations. With global climate change and the change in habitat structures[4], the maintenance of within-species diversity has never before more important.

Despite conventional wisdom that host genetic diversity reduces disease spread, there is surprisingly little theoretical investigation into the monoculture effect[5–10]. Lively (2010) shows that through high specificity, in a matching alleles framework, host genetic diversity

reduces disease spread. Other models show that varying levels of susceptibility[6], infectivity[8], diversity of resistant genotypes[9], or species richness[10] in host populations reduces the chance of disease emergence and spread in a host population. Future theory could explore the extent to which host-pathogen infection genetic specificity – matching alleles to gene-for-gene as opposite ends of the spectrum – could determine the level of diversity required to stop disease spread ('diversity threshold' as in King & Lively, 2012). For example, a reduction in specificity, such that one pathogen genotype can infect a variety of different host genotypes, should limit the chances a pathogen will encounter a non-susceptible host genotype. Thus, the diversity threshold in the host population would have to be higher[7]. It is possible that knowledge of the degree of genetic specificity in the host-pathogen system could help to determine how much genetic diversity is needed to maintain populations of conserved species.

### 5.1.2 Changing levels of diversity: implications for coevolution and pathogen transmission

There is an interplay between diversity and infection in host-pathogen systems. Future work could examine the dynamics of pathogen evolution and spread under changing levels of host and pathogen diversity.

The monoculture effect predicts that host population diversity limits disease spread[1,5,11], whereas the Red Queen hypothesis posits that parasite infection selects for the maintenance of host population diversity[12–14]. What tips the balance between infection favouring diversity and diversity stopping infection? An experimental evolution approach in a host-pathogen system could be used to answer this question. If host populations are homogeneous, they cannot evolve fast enough to overcome pathogen infection, and the potential for a

coevolutionary race is relatively low[15]. However, an experimenter could introduce new host genotypes to boost host diversity and evolutionary potential, and subsequently track pathogen evolutionary dynamics and rate. With an influx of new genotypes, populations should evolve resistance more rapidly in a genetically homogenous host population. With higher levels of diversity, the potential for host populations to coevolve is heightened as well[16], which would impose reciprocal selection on the pathogen. Thus, increasing levels of host diversity across treatments should result in faster evolution of pathogen infectivity and host resistance. Conversely, immigration of new pathogen genotypes could facilitate pathogen evolution and transmission, and ultimately overwhelming the host population. As more host genotypes are infected, and the resistant pool would be reduced, funnelling the host population into more of a monoculture. Host and pathogen immigration rates could thus tip the balance between coevolution and the monoculture effect, but this hypothesis remains untested.

Introducing new genotypes into populations is a conservation strategy used for at-risk populations. For example, to improve resistance of the Tasmanian Devil (*Sarcophilus harrisii*) to Devil Facial Tumour Disease, individuals that are evolving immune-modulated resistance are being introduced to populations[15]. However, in longer lived animals that are at-risk from infectious disease, genetic management by supplementing population genetic diversity to select for resistance could take too long to stop the spread of infectious disease. Therefore, other approaches are discussed in these species, such as encouraging outbreeding between individuals and quarantining infected individuals[17].

If a bump up in diversity can improve the potential for host resistance, this strategy could be explored in vectors for the control and/or management of vector-borne infectious diseases. A caveat with increasing host diversity is that it may sometimes increase disease risk[10]. Vectors

are usually highly generalist organisms (such as ticks[18], fleas[19], and mosquitoes[20]), capable of feeding off many host species, resulting in more infected host individuals and species. For example, increasing host species richness (i.e. humans and chickens) can sometimes increase vector density (*Triatoma infestans*) leading to higher levels of pathogen transmission (Chagas disease)[21]. Could pathogen transmission to vectors be reduced if vector genetic diversity was increased? The developmental life stages of the pathogen *Trypanosoma cruzi* causing Chagas disease is largely dependent on its Triatmoniae (*Triatoma infestans*) vector[22]. Different *T. cruzi* isolates have been observed to vary in their degree of infectivity and transmissibility after passing through the same vector species[22,23]. Thus, understanding the life-cycle and within-vector growth may help to reduce pathogen proliferation by means of targeting diversity in the vector rather than host.

### 5.1.3 Diversity beyond resistance and its impacts on infection

As mentioned, the majority of experimental and theoretical studies examining the diversity-disease link have focused on genetic variation for resistance in diverse host populations. However, other aspects of host biology which change with levels of population diversity may be playing a role in pathogen infection and evolution. For example, resource competition can vary with relatedness in host populations. Kin selection theory predicts that distantly-related individuals compete more than closely-related individuals as cooperation is weaker between non-relatives[24,25]. In contrast, the niche partitioning theory assumes that related individuals are more likely to compete for the same resource, and will thus diverge to limit within-population competition[26,27]. If competition increases the host contact rate or feeding rate, there could be consequences for disease transmission and spread. Developing theoretical models to vary host relatedness could investigate the effects on competition and pathogen transmission.

A simple model framework for examining the effects of host relatedness on pathogen spread might look like this:

For host (***H***) dynamics, where ***r*** is the birth rate, $\boldsymbol{\beta}$ is the transmission rate and $\boldsymbol{\alpha}$ is the strength of within-species competition:

$$\frac{dH}{dt} = rH - \beta HP - \ln(1 + \alpha H)\, H$$

For pathogen (***P***) dynamics, where $\boldsymbol{\gamma}$ is pathogen yield and $\boldsymbol{\nu}$ is the density-dependent pathogen mortality rate

$$\frac{dP}{dt} = \gamma\beta HP - \nu P$$

Finally, host relatedness (***R***), where $\boldsymbol{\mu}$ is the mutation rate. The first term on the right-hand side (1/***H***) describes the probability that two individuals are siblings and (1- 1/***H***) is the probability that individuals are not siblings but have common ancestors such that these ancestors may have been related (***R***) and neither underwent mutation [$(1 - \boldsymbol{\mu})^2$].

$$\frac{dR}{dt} = \frac{1}{H} - R\left[1 - \left(1 - \frac{1}{H}\right)(1 - \mu)^2\right]$$

This model assumes that the more competitive you are for resources, the more susceptible you are as food consumption and pathogen uptake are linked. Therefore, highly competitive host genotypes will become infected and die faster, thereby reducing host population growth.

Future work could also investigate whether the evolutionary trajectories of present-day emerging infectious diseases are associated with the relatedness in host populations of the novel host species. For example, isolated populations of the Italian agile frog (*Rana latastei*) were found to vary in susceptibility to an emerging strain of *Ranavirus* (frog virus 3)[28], which may be due to variation in within-population genetic diversity[29]. Experimental work investigating the effects of relatedness in these frog populations could inform on the evolution of an emerging pathogen in its natural environment.

### 5.1.4 Concluding remarks

Given the current coronavirus (Covid-19) pandemic, and the persistent spread of White-nose syndrome, Chytrid fungus, and other emerging infectious diseases in novel hosts, this thesis work is very timely. Understanding and addressing the gaps of the effects of host genetic diversity and relatedness on completely novel pathogen associations are of paramount importance as potential driver of infection and pathogen evolution. Future focus should be on experimentation of host-pathogen interactions at the emergence stage to help in understanding why pathogen traits may evolve along different trajectories and how host diversity can be used as a hinderance for infection. The experiments presented in this thesis could help in developing theoretical frameworks as well as expand on future experiments of pathogen evolution and spread to improve global infectious disease management.

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# Appendix

Mutations found in evolved *S. aureus* strains listed by timepoint, treatment, and *C. elegans* host isolate. The position of change on the *S. aureus* genome is indicated under "position", along with the reference amino acid change (Ref) and single nucleotide polymorphism (SNP). The mutation frequency is the proportion of 40 sampled clones that contain the SNP change in the bacterial population. Gene function (Function) has been determined from literature searches when Gene tag and Gene product information are known. Mutation type: Non-synonymous (N), Synonymous (S), Intergenic region (I), Stop gained (S*).

| Mutation frequency of *S. aureus* monocultures at timepoint 5 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Position** | Ref | SNP | Mutation | Gene tag | Gene product | Function | PB306 | N2 | LKC34 | JU792 | JU775 | JU363 | JU311 | JU258 | JU1652 | JU1580 | JU1491 | JU1400 | ED3017 | ED3012 | ED3005 | CX11307 | CX11262 | CB4858 | CB4856 | CB4854 | CB4853 | CB4851 | AB1 | QX1211 |
| **2220609** | G | A | N | SAS_RS11185 | methicillin resistance protein FmtB | Virulence + Metabolism | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0.075** |
| **2220612** | T | A | N | SAS_RS11185 | methicillin resistance protein FmtB | Virulence + Metabolism | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0.075** |

| **1326110** | **2027051** | **1986449** | **1900575** | **1188175** |
|---|---|---|---|---|
| C | C | G | C | C |
| A | A | A | T | A |
| S* | I | N | I | N |
| SAS_RS06535 | SAS_RS10135- | SAS_RS09915 | SAS_RS09310-SAS_RS09315 | SAS_RS05930 |
| aquaporin family protein | peptidoglycan hydrolase | sodium:proline symporter | beta-channel forming cytolysin | penicillin-binding protein |
| Metabolism | Virulence + Metabolism | Siderophore + Metabolism | Virulence | Virulence + Metabolism |
| **0** | **0.1** | **0.175** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0.1** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0.1** | **0.075** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0.3** | **0.1** | **0** |
| **0** | **0** | **0** | **0.1** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0.1** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0.175** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0.1** |
| **0.075** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |

| 1163190 | 1682025 | 748895 | 1548501 | 2317639 |
|---|---|---|---|---|
| C | T | C | T | A |
| T | A | A | C | T |
| I | N | N | N | S* |
| SAS_RS05760- | SAS_RS08275 | SAS_RS03590 | SAS_RS07560 | SAS_RS11750 |
| fibrinogen-binding protein | LLM class flavin-dependent oxidoreductase | glycosyltransferase - cell wall biosynthesis | ATP-dependent DNA | cyclic pyranopterin monophosphate synthase |
| Virulence + Adherence | Stress | Metabolism | Virulence | Metabolism |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0.1** |
| **0** | **0** | **0** | **0.2** | **0** |
| **0** | **0.075** | **0.075** | **0** | **0** |
| **0.075** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0

| 1245336 | 1245335 | 393104 | 640812 | 640810 | 1163192 |
|---|---|---|---|---|---|
| G | G | T | A | C | G |
| A | A | G | T | T | T |
| S | N | N | N | N | I |
| SAS_RS06195 | SAS_RS06195 | SAS_RS14385 | SAS_RS03015 | SAS_RS03015 | SAS_RS05760- |
| ribonuclease 3 | ribonuclease 3 | hypothetical protein | hypothetical protein | hypothetical protein | fibrinogen-binding protein |
| Virulence + Stress | Virulence + Stress | Unknown | Unknown | Unknown | Virulence + Adherence |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0.075** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0.075** | **0.075** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0.1** | **0** | **0** | **0** |
| **0.075** | **0.075** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |

| 845736 | 2664584 | 1245342 | 1245341 |
|---|---|---|---|
| T | T | G | C |
| A | A | A | A |
| I | I | S | N |
| SAS_RS04050-SAS_RS04055 | SAS_RS13525-SAS_RS13530 | SAS_RS06195 | SAS_RS06195 |
| von Willebrand factor binding protein Vwb - extracellular matrix protein-binding | L-lactate dehydrogenase | ribonuclease 3 | ribonuclease 3 |
| Virulence + Adherence | Virulence + Stress | Virulence + Stress | Virulence + Stress |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0.075** | **0.075** | **0.075** |
| **0.075** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |

| 845741 | 845739 | 845738 |
|---|---|---|
| C | T | T |
| A | A | A |
| I | I | I |
| SAS_RS04050-SAS_RS04055 | SAS_RS04050-SAS_RS04055 | SAS_RS04050-SAS_RS04055 |
| von Willebrand factor binding protein Vwb - extracellular matrix protein- | von Willebrand factor binding protein Vwb - extracellular matrix protein- | von Willebrand factor binding protein Vwb - extracellular matrix protein- |
| Virulence + Adherence | Virulence + Adherence | Virulence + Adherence |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0.075** | **0.075** | **0.075** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |

| 1900568 | 341627 | 1332856 | 845742 |
|---|---|---|---|
| C | C | G | G |
| T | T | T | A |
| I | N | N | I |
| SAS_RS09310-SAS_RS09315 | SAS_RS01525 | SAS_RS06570 | SAS_RS04050-SAS_RS04055 |
| beta-channel forming cytolysin | N-acetylmannosamine-6-phosphate 2-epimerase | GTPase HflX | von Willebrand factor binding protein Vwb - extracellular matrix protein-binding adhesin |
| Virulence | Adherence | Metabolism | Virulence + Adherence |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0.075** |
| **0.1** | **0.075** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0.075** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |

| 1178260 | 1595090 | 2071262 | 2559269 | 701982 |
|---|---|---|---|---|
| G | C | C | C | C |
| A | G | A | T | A |
| S | N | I | N | N |
| SAS_RS05870 | SAS_RS07805 | SAS_RS10440-SAS_RS10445 | SAS_RS12995 | SAS_RS03345 |
| hypothetical protein | aminopeptidase P family protein | bi-component leukocidin LukGH subunit H - | LPXTG cell wall anchor domain-containing protein | NAD(P)-dependent oxidoreductase |
| Unknown | Metabolism | Virulence | Adherence | Metabolism |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0.15** | **0.075** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0.1** | **0** | **0** |
| **0** | **0.1** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0.1** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |

| 1525993 | 1434374 | 1434373 | 1332868 |
|---|---|---|---|
| G | G | T | T |
| T | A | A | G |
| N | N | N | N |
| SAS_RS07435 | SAS_RS07090 | SAS_RS07090 | SAS_RS06570 |
| N-acetyl-alpha-D-glucosaminyl L-malate synthase | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N- | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N- | GTPase HflX |
| Stress | Metabolism | Metabolism | Metabolism |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0.075** |
| **0.075** | **0.075** | **0.075** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |

| 1080017 | 937034 | 304315 | 1682026 |
|---|---|---|---|
| C | G | G | G |
| A | A | T | A |
| N | S | I | N |
| SAS_RS05330 | SAS_RS04530 | SAS_RS01330-SAS_RS01335 | SAS_RS08275 |
| phosphoribosylglycinamide formyltransferase | membrane protein | CHAP domain-containing protein - WXG100 family type VII secretion effector | LLM class flavin-dependent oxidoreductase |
| Metabolism | Unknown | Siderophore | Stress |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0**

| 1348272 | 1348271 | 14683 | 1317050 |
|---|---|---|---|
| T | T | C | C |
| A | A | T | A |
| I | I | I | N |
| SAS_RS06670-SAS_RS06675 | SAS_RS06670-SAS_RS06675 | SAS_RS00045-SAS_RS00050 | SAS_RS06505 |
| cardiolipin synthase - ABC transporter ATP-binding protein | cardiolipin synthase - ABC transporter ATP-binding protein | serine--tRNA ligase - AzlC family ABC transporter permease | tRNA (N6-isopentenyl adenosine(37)-C2)-methylthiotransferase MiaB |
| Siderophore | Siderophore | Siderophore | Metabolism |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0**

| 1434359 | 2220611 | 304303 | 1348275 |
|---|---|---|---|
| ACN | CGN | TCAAAAAAAAATGI | GAI |
| SAS_RS07090 | SAS_RS11185 | SAS_RS01330-SAS_RS01335 | SAS_RS06670-SAS_RS06675 |
| 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N- | methicillin resistance protein FmtB | CHAP domain-containing protein - WXG100 family type | cardiolipin synthase - ABC transporter ATP-binding |
| Metabolism | Virulence + Metabolism | Siderophore | Siderophore |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0.05** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0.075** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0.075** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0.1** | **0** | **0** | **0** |
| **0.1** | **0** | **0.075** | **0** |
| **0** | **0** | **0** | **0.075** |
| **0** | **0** | **0** | **0** |
| **0** | **0.075** | **0.1** | **0** |

| 426121 | 2220604 | 1434359 | 1434362 |
|---|---|---|---|
| TCCCCTAAA | CTTTT | ACC | ACTGGT |
| T | C | ATC | A |
| I | N | N | N |
| SAS_RS01965-SAS_RS01970 | SAS_RS11185 | SAS_RS07090 | SAS_RS07090 |
| NYN domain-containing protein - DUF1304 domain- | methicillin resistance protein FmtB | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N- | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate |
| Unknown | Virulence + Metabolism | Metabolism | Metabolism |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0.025** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0.075** | **0** | **0** |
| **0.075** | **0** | **0** | **0** |
| **0** | **0** | **0.025** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0.075** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** |

| 2330963 | 555155 | 1059975 |
|---|---|---|
| AG | ATCCTC | TACGGCCTTTTAAGGTTGGCTTCCGGGGTTGGAAGCCTTGGCC |
| A | A | T |
| N | N | N |
| SAS_RS11835 | SAS_RS02635 | SAS_RS05230 |
| acyl-CoA dehydrogenase | DNA repair protein RadA | bifunctional autolysin |
| Metabolism | Metabolism | Adherence + Metabolism |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0.125** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0.125** | **0.075** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |

| |
|---|
| **2772673** |
| A C T T C |
| A |
| I |
| SAS_RS14000-SAS_RS14005 |
| S-adenosyl-l-methionine hydroxide adenosyltransferase family protein - polyisoprenoid-binding protein |
| Unknown |
| **0** |
| **0** |
| **0** |
| **0** |
| **0** |
| **0** |
| **0** |
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| **0** |
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| **0** |
| **0** |
| **0** |
| **0** |
| **0** |
| **0** |
| **0.075** |
| **0** |
| **0** |
| **0** |

Mutation frequency of monocultures at time point 10

| 1578429 | 1188175 | 508473 | Position |
|---|---|---|---|
| C | C | T | Ref |
| A | A | G | SNP |
| N | N | N | Mutation |
| SAS_RS07715 | SAS_RS05930 | SAS_RS02380 | Gene tag |
| M20/M25/M40 family metallo-hydrolase | penicillin-binding protein | 4-diphosphocytidyl-2C-methyl-D-erythritol kinase | Gene product |
| Stress | Virulence + Metabolis | Metabolism | Function |
| **0** | **0** | **0** | LKC34 |
| **0** | **0** | **0** | JU792 |
| **0** | **0** | **0** | JU775 |
| **0** | **0** | **0** | JU363 |
| **0** | **0** | **0** | JU311 |
| **0** | **0** | **0** | JU258 |
| **0** | **0** | **0** | JU1652 |
| **0** | **0** | **0** | JU1580 |
| **0** | **0** | **0** | JU1491 |
| **0** | **0** | **0** | JU1400 |
| **0** | **0** | **0** | ED3017 |
| **0** | **0** | **0** | ED3012 |
| **0** | **0** | **0** | ED3005 |
| **0** | **0** | **0** | CX11307 |
| **0** | **0** | **0** | CX11262 |
| **0** | **0** | **0** | CB4858 |
| **0** | **0** | **0** | CB4856 |
| **0** | **0** | **0** | CB4854 |
| **0** | **0** | **0** | CB4853 |
| **0** | **0** | **0** | CB4851 |
| **0** | **0** | **0** | AB1 |
| **0.075** | **0.075** | **0.175** | QX1211 |
| **0** | **0** | **0** | PB306 |
| **0.075** | **0** | **0** | N2 |

| **1986449** | **2420676** | **1959839** | **1900576** | **1900575** |
|---|---|---|---|---|
| G | C | G | A | C |
| A | T | A | T | T |
| N | S | N | I | I |
| SAS_RS09915 | SAS_RS12285 | SAS_RS09760 | SAS_RS09310-SAS_RS09315 | SAS_RS09310-SAS_RS09315 |
| sodium:proline symporter | MFS transporter | type 1 glutamine amidotransferase | beta-channel forming cytolysin | beta-channel forming cytolysin |
| Siderophore + Metabolism | Siderophore + Metabolism | Metabolism | Virulence | Virulence |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0.25** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0.075** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0.125** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0.175** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0.15** | **0.075** | **0.075** | **0.075** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |

| **2317639** | **1658833** | **445556** | **1326110** | **1224242** | **2027051** |
|---|---|---|---|---|---|
| A | C | T | C | C | C |
| T | A | A | A | A | A |
| * | S | I | N | * | S | I | I |
| SAS_RS11750 | SAS_RS08165-SAS_RS081 | SAS_RS02070 | SAS_RS06535 | SAS_RS06085-SAS_RS0609 | SAS_RS10135-SAS_RS1014 |
| cyclic pyranopterin monophosphate synthase | enterotoxin - Fic family protein | tandem-type lipoprotein | aquaporin family protein | primosomal protein N' | peptidoglycan hydrolase |
| Metabolism | Virulence | Virulence | Metabolism | Metabolism | Virulence + Metabolism |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0**

| 1682025 | 957468 | 730082 | 1548501 | 2007983 | 1693527 |
|---|---|---|---|---|---|
| T | G | C | T | C | C |
| A | A | A | C | T | T |
| N | * S | I | N | N | N |
| SAS_RS08275 | SAS_RS04635 | SAS_RS03495-SAS_RS03500 | SAS_RS07560 | SAS_RS10010 | SAS_RS08335 |
| LLM class flavin-dependent oxidoreductase | oligoendopeptidase F | MarR family transcriptional regulator - GTP-binding protein | ATP-dependent DNA helicase | manganese-dependent inorganic pyrophosphata | adenine phosphoribosyltransferase |
| Stress | Virulence + Stress | Virulence | Virulence | Virulence + Metabolism | Metabolism |
| **0** | **0** | **0** | **0** | **0.1** | **0.125** |
| **0** | **0.2** | **0.075** | **0.075** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0.1** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0.1** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |

| 1495265 | 1495264 | 1495262 | 1495257 | 524447 |
|---|---|---|---|---|
| C | A | G | C | A |
| T | T | T | T | T |
| N | N | N | S | I |
| SAS_RS07310 | SAS_RS07310 | SAS_RS07310 | SAS_RS07310 | SAS_RS02465-SAS_RS02470 |
| hyperosmolarity resistance protein Ebh | hyperosmolarity resistance protein Ebh | hyperosmolarity resistance protein Ebh | hyperosmolarity resistance protein Ebh | hypoxanthine phosphoribosyltransferase - ATP-dependent metallopeptidase |
| Virulence + Metabolism | Virulence + Metabolism | Virulence + Metabolism | Virulence + Metabolism | Unknown |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0.075** | **0.075** | **0.075** | **0.075** | **0.275** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |

| 1332856 | 197793 | 2743155 | 1362660 | 635932 | 1495266 |
|---|---|---|---|---|---|
| G | A | C | G | G | A |
| T | G | A | A | A | T |
| N | N | N | I | N | N |
| SAS_RS06570 | SAS_RS00855 | SAS_RS13840 | SAS_RS06750-SAS_RS06755 | SAS_RS02985 | SAS_RS07310 |
| GTPase HflX | permease | serine-rich repeat glycoprotein adhesin SasA | amino acid permease - catalase | transposase | hyperosmolarity resistance protein Ebh |
| Metabolism | Siderophore | Virulence + Adherence | Siderophore | Unknown | Virulence + Metabolism |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0.075** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0.2** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0.25** | **0.075** | **0** | **0** |
| **0** | **0.075** | **0** | **0** | **0** | **0** |
| **0.1** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |

| **1682021** | **1682019** | **2402539** | **341627** | **1408853** |
|---|---|---|---|---|
| T | G | G | C | G |
| A | A | A | T | T |
| N | S | N | N | N |
| SAS_RS08275 | SAS_RS08275 | SAS_RS12205 | SAS_RS01525 | SAS_RS06960 |
| LLM class flavin-dependent oxidoreductase | LLM class flavin-dependent oxidoreductase | zinc ribbon domain-containing protein | N-acetylmannosamine-6-phosphate 2-epimerase | tryptophan synthase subunit alpha |
| Stress | Stress | Unknown | Adherence | Virulence + Metabolism |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0.15** |
| **0** | **0** | **0.15** | **0.1** | **0** |
| **0.075** | **0.1** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |

| 848784 | 848783 | 848779 | 848777 | 2704402 | 1188447 |
|---|---|---|---|---|---|
| G | C | C | C | G | A |
| A | A | A | A | T | C |
| S | N | N | N | N | N |
| SAS_RS04065 | SAS_RS04065 | SAS_RS04065 | SAS_RS04065 | SAS_RS13725 | SAS_RS05930 |
| thermonuclease - biofilm formation | thermonuclease - biofilm formation | thermonuclease - biofilm formation | thermonuclease - biofilm formation | carbamate kinase 2 | penicillin-binding protein |
| Virulence + Adherence | Virulence + Adherence | Virulence + Adherence | Virulence + Adherence | Metabolism | Virulence + Metabolis |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0.075** | **0.075** |
| **0.075** | **0.075** | **0.075** | **0.075** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |

| 1658835 | 1658832 | 1658830 | 534776 | 99839 | 1595090 |
|---|---|---|---|---|---|
| G | T | T | T | G | C |
| A | A | A | G | T | G |
| I | I | I | I | I | N |
| SAS_RS08165-SAS_RS0817 | SAS_RS08165-SAS_RS081 | SAS_RS08165-SAS_RS081 | SAS_RS02555 | SAS_RS00420-SAS_RS14315 | SAS_RS07805 |
| enterotoxin - Fic family protein | enterotoxin - Fic family protein | enterotoxin - Fic family protein | 16S ribosomal RNA | staphylococcal protein A | aminopeptidase P family protein |
| Virulence | Virulence | Virulence | Metabolism | Virulence + Adherence | Metabolism |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0.075** | **0.075** | **0.075** | **0.1** | **0.175** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0.1** |
| **0.1** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |

| 601843 | 2527945 | 325676 | 1682026 | 2308383 |
|---|---|---|---|---|
| T | G | C | G | G |
| C | T | A | A | A |
| I | I | N | N | S |
| SAS_RS02815-SAS_RS02820 | SAS_RS12845-SAS_RS12850 | SAS_RS01450 | SAS_RS08275 | SAS_RS11705 |
| MSCRAMM family adhesin SdrD - MSCRAMM family adhesin SdrE | D-histidine (S)-2-aminobutanoyltransferase CntL - histidine racemase CntK | DUF4064 domain-containing protein | LLM class flavin-dependent oxidoreductase | efflux RND transporter permease subunit |
| Virulence + Adherence | Siderophore | Unknown | Stress | Siderophore |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0.25** |
| **0** | **0.125** | **0.1** | **0** | **0.2** |
| **0** | **0** | **0** | **0.075** | **0** |
| **0.1** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |

| 1434362 | 304303 | 1165645 | 2290705 | 1083589 |
|---|---|---|---|---|
| A C T G G T | T G A A A A A A A C T A | A | G | A |
| A | T | C | T | C |
| N | I | I | S | N |
| SAS_RS07090 | SAS_RS01330-SAS_RS01335 | SAS_RS05780-SAS_RS0 | SAS_RS11560 | SAS_RS05345 |
| 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase | CHAP domain-containing protein - WXG100 family type VII secretion effector EsxA | fibrinogen-binding protein | 30S ribosomal protein S5 | energy-coupling factor transporter |
| Metabolism | Siderophore | Virulence + Adherence | Metabolism | Siderophore |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0.125** | **0** | **0** | **0** | **0** |
| **0** | **0.075** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0.075** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0.075** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0.075** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0.175** | **0.1** |
| **0** | **0** | **0.125** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** |

| 1059975 | 1322701 | 2195585 |
|---|---|---|
| CGTTGAATGTAGTCGGTGAATTTCCGATC | CGAGCG | CGTTAC |
| T | A | A |
| N | N | I |
| SAS_RS05230 | SAS_RS06525 | SAS_RS11090-SAS_RS1109 |
| bifunctional autolysin | DNA mismatch repair protein | deoxyribose-phosphate aldolase - purine- |
| Adherence + Metabolism | Metabolism + Stress | Metabolism |
| **0** | **0.075** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0.3** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0.075** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |
| **0** | **0** | **0** |

| 1332872 | 1791153 | 800534 | 139676 | 1263454 | 2066931 |
|---|---|---|---|---|---|
| TCCCCN | TATI | CCAI | CGCTTTTATI | GAAAATAA | TGCCAAA |
| SAS_RS06570 | SAS_RS08805-SAS_RS0881 | SAS_RS03855-SAS_RS0 | SAS_RS00620-SAS_RS00625 | SAS_RS06275 | SAS_RS10420-SAS_RS10425 |
| GTPase HflX | tyrosine--tRNA ligase - penicillin-binding | ribosome-associated translation inhibitor | hypothetical protein | FemA/FemB family glycyltransferase FmhC | staphylococcal enterotoxin type K - site-specific integrase |
| Metabolism | Virulence | Siderophore | Unknown | Metabolism | Virulence |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0.075** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0.075** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0.075** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0.1** | **0** | **0** | **0** |
| **0** | **0.25** | **0** | **0** | **0** | **0** |
| **0** | **0.15** | **0** | **0** | **0** | **0** |
| **0.025** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |
| **0** | **0** | **0** | **0** | **0** | **0** |

| 1034531 | 1332872 | |
|---|---|---|
| A | T | |
| ATTA | ATAAAAAACTTATGCCCGCTC | GTAAAAACCGGAAGT |
| N | N | |
| SAS_RS05095 | SAS_RS06570 | |
| CPBP family intramembrane metalloprotease | GTPase HflX | |
| Metabolism | Metabolism | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0.05** | |
| **0** | **0** | |
| **0.1** | **0** | |
| **0** | **0** | |
| **0** | **0** | |
| **0** | **0** | |

| Mutation frequency at timepoint 5 in polycultures | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Position | Ref | SNP | Mutation | Gene tag | Gene product | Function | M1 | M2 | M3 | M4 | M5 | M6 |
| 1900575 | C | T | I | SAS_RS09310-SAS_RS09315 | beta-channel forming cytolysin | Virulence | 0 | 0 | 0 | 0 | 0.1 | 0.1 |
| 1900576 | A | T | I | SAS_RS09310-SAS_RS09315 | beta-channel forming cytolysin | Virulence | 0 | 0 | 0 | 0 | 0 | 0.1 |
| 1326110 | C | A | S* | SAS_RS06535 | aquaporin family protein | Metabolism | 0 | 0 | 0 | 0 | 0 | 0.1 |
| 384882 | G | A | N | SAS_RS01735 | aminotransferase class I/II-fold pyridoxal phosphate-dependent enzyme | Unknown | 0 | 0 | 0 | 0 | 0 | 0.1 |
| 262373 | T | G | I | SAS_RS01130-SAS_RS01135 | nucleoside hydrolase - transcription antiterminator | Unknown | 0 | 0 | 0 | 0 | 0.125 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1368440 | A | G | I | SAS_RS 06785-SAS_RS 06790 | transcriptional repressor LexA - hypothetical protein | Metabolism | 0 | 0 | 0 | 0 | 0.175 | 0 |
| 2100278 | A | T | I | SAS_RS 10590-SAS_RS 10595 | MutS family DNA mismatch repair protein | Metabolism + Stress | 0 | 0 | 0 | 0 | 0.2 | 0 |
| 984731 | A | T | N | SAS_RS 04765 | XRE family transcriptional regulator | Adherence | 0 | 0 | 0 | 0.075 | 0 | 0 |
| 1044508 | C | A | N | SAS_RS 05170 | 1,4-dihydroxy-2-naphthoate polyprenyltransferase | Adherence | 0 | 0 | 0 | 0.075 | 0 | 0 |
| 1338436 | T | A | I | SAS_RS 06590-SAS_RS 06595 | hypothetical protein | Unknown | 0 | 0 | 0 | 0.1 | 0 | 0 |
| 1431962 | C | T | S | SAS_RS 07075 | aspartate-semialdehyde dehydrogenase | Virulence + Metabolism | 0 | 0 | 0 | 0.2 | 0 | 0 |
| 2247420 | G | A | S | SAS_RS 11315 | iron citrate ABC transporter substrate-binding protein | Siderophore | 0 | 0 | 0 | 0.1 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 820562 | G | A | N | SAS_RS 03945 | sporulation regulator WhiA | Virulence + Metabolism | 0 | 0.075 | 0 | 0 | 0 | 0 |
| 1658833 | C | A | I | SAS_RS 08165- SAS_RS 08170 | enterotoxin - Fic family protein | Virulence | 0 | 0.075 | 0 | 0 | 0 | 0 |
| 1854896 | G | T | I | SAS_RS 09075- SAS_RS 09080 | transaldolase | Metabolism | 0 | 0.075 | 0 | 0 | 0 | 0 |
| 2779691 | G | T | N | SAS_RS 14035 | Cna B-type domain-containing protein | Virulence | 0.1 | 0 | 0 | 0 | 0 | 0 |
| 304303 | TCAAAAAAATAG | T | I | SAS_RS01330- SAS_RS01335 | CHAP domain-containing protein - WXG100 family type VII secretion effector EsxA | Siderophore | 0 | 0 | 0.075 | 0 | 0 | 0 |
| 1434359 | AC | A | N | SAS_RS 07090 | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase | Metabolism | 0 | 0.1 | 0 | 0.025 | 0 | 0 |
| 1434362 | ACTGGT | A | N | SAS_RS 07090 | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase | Metabolism | 0.075 | 0.1 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1434359 | AC | ATC | N | SAS_RS07090 | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase | Metabolism | 0 | 0 | 0 | 0.05 | 0 | 0 |
| 2195585 | ATTCGC | A | I | SAS_RS11090-SAS_RS11095 | deoxyribose-phosphate aldolase - purine-nucleoside phosphorylase | Metabolism | 0 | 0 | 0 | 0.075 | 0 | 0 |

| Mutation frequency at timepoint 10 in polycultures | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Position | Ref | SNP | Mutation | Gene tag | Gene product | Gene Function | M1 | M2 | M3 | M4 | M5 | M6 |
| 384882 | G | A | N | SAS_RS01735 | aminotransferase class I/II-fold pyridoxal phosphate-dependent enzyme | Unknown | 0 | 0 | 0 | 0 | 0 | 0.125 |
| 252031 | A | G | I | SAS_RS01080-SAS_RS01085 | acyl CoA:acetate/3-ketoacid CoA transferase – PrsW family intramembrane metalloprotease | Virulence + Adherence | 0 | 0 | 0 | 0 | 0 | 0.1 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 445553 | G | A | S | SAS_RS 02070 | tandem-type lipoprotein | Virulence | 0 | 0 | 0 | 0 | 0 | 0.075 |
| 445556 | T | A | N | SAS_RS 02070 | tandem-type lipoprotein | Virulence | 0 | 0 | 0 | 0 | 0 | 0.1 |
| 2703970 | T | C | N | SAS_RS 13725 | carbamate kinase 2 | Metabolism | 0 | 0 | 0 | 0 | 0 | 0.125 |
| 1368440 | A | G | I | SAS_RS 06785-SAS_RS 06790 | transcriptional repressor LexA - hypothetical protein | Metabolism | 0 | 0 | 0 | 0 | 0.075 | 0 |
| 458723 | A | G | N | SAS_RS 02130 | DUF2309 family protein | Unknown | 0 | 0 | 0 | 0 | 0.1 | 0 |
| 1431962 | C | T | S | SAS_RS 07075 | aspartate-semialdehyde dehydrogenase | Virulence + Metabolism | 0 | 0 | 0 | 0.3 | 0 | 0 |
| 326572 | C | G | N | SAS_RS 01455 | formate/nitrite transporter | Metabolism | 0 | 0 | 0 | 0.225 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 440881 | T | A | N | SAS_RS 02050 | restriction endonuclease subunit S | Metabolism | 0 | 0 | 0 | 0.075 | 0 | 0 |
| 440882 | T | A | S | SAS_RS 02050 | restriction endonuclease subunit S | Metabolism | 0 | 0 | 0 | 0.075 | 0 | 0 |
| 440886 | T | A | N | SAS_RS 02050 | restriction endonuclease subunit S | Metabolism | 0 | 0 | 0 | 0.1 | 0 | 0 |
| 702734 | G | A | N | SAS_RS 03350 | DNA-binding response regulator | Metabolism | 0 | 0 | 0 | 0.125 | 0 | 0 |
| 1098392 | G | A | N | SAS_RS 05420 | ribonuclease J 1 | Virulence + Metabolism | 0 | 0 | 0 | 0.075 | 0 | 0 |
| 1545839 | C | T | N | SAS_RS 07545 | YpdA family putative bacillithiol disulfide reductase | Virulence + Metabolism | 0 | 0 | 0 | 0.075 | 0 | 0 |
| 862171 | T | C | N | SAS_RS 04175 | methionine import ATP-binding protein MetN 2 | Adherence + Metabolism | 0 | 0 | 0.075 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1116202 | G | T | N | SAS_RS 05520 | hypothetical protein | Unknown | 0 | 0 | 0.075 | 0 | 0 | 0 |
| 2116677 | G | A | I | SAS_RS 10675 | 23S ribosomal RNA | Metabolism | 0 | 0.075 | 0 | 0 | 0 | 0 |
| 2734062 | G | A | N | SAS_RS 13830 | accessory Sec system protein Asp1 | Virulence | 0 | 0.1 | 0 | 0 | 0 | 0 |
| 1880713 | C | T | S | SAS_RS 09215 | restriction endonuclease subunit S | Metabolism | 0.075 | 0 | 0 | 0 | 0 | 0 |
| 1880714 | A | T | S* | SAS_RS 09215 | restriction endonuclease subunit S | Metabolism | 0.075 | 0 | 0 | 0 | 0 | 0 |
| 1880715 | A | T | N | SAS_RS 09215 | restriction endonuclease subunit S | Metabolism | 0.075 | 0 | 0 | 0 | 0 | 0 |
| 1880717 | C | T | N | SAS_RS 09215 | restriction endonuclease subunit S | Metabolism | 0.075 | 0 | 0 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1880719 | A | T | S | SAS_RS09215 | restriction endonuclease subunit S | Metabolism | 0.075 | 0 | 0 | 0 | 0 | 0 |
| 1880720 | A | T | N | SAS_RS09215 | restriction endonuclease subunit S | Metabolism | 0.075 | 0 | 0 | 0 | 0 | 0 |
| 2220611 | C | CG | N | SAS_RS11185 | methicillin resistance protein FmtB | Virulence + Metabolism | 0 | 0.075 | 0 | 0 | 0 | 0 |
| 1434359 | AC | A | N | SAS_RS07090 | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase | Metabolism | 0 | 0 | 0 | 0 | 0.1 | 0.1 |
| 1434362 | ACTGGT | A | N | SAS_RS07090 | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase | Metabolism | 0 | 0 | 0 | 0 | 0 | 0.1 |
| 1434359 | AC | ATC | N | SAS_RS07090 | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase | Metabolism | 0 | 0 | 0 | 0 | 0.025 | 0 |
| 862183 | G | GCCGTATCATTA | N | SAS_RS04175 | methionine import ATP-binding protein MetN 2 | Adherence + Metabolism | 0 | 0 | 0.075 | 0 | 0 | 0 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2220604 | CT TT | C | N | SAS_RS 11185 | methicillin resistance protein FmtB | Virulence + Metabolism | 0 | 0.075 | 0 | 0 | 0 | 0 |