Community dynamics in spatially structured experimental bacterial communities

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Biologia fysiologia ja genetiikka
Pro gradu -tutkielma
Laajuus: 30 op

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28.6.2022
Turku

Pro gradu -tutkielma

Pääaine: Biologia fysiologia ja genetiikka
Tekijä(t): Elizaveta Alitupa
Otsikko: Population dynamics and antibiotic resistance in spatially structured experimental bacterial communities
Ohjaaja(t): Teppo Hiltunen ja Shane Hogle
Sivumäärä: 52 sivua
Päivämäärä: 28.6.2022

Kyseinen Pro gradu -tutkielma koostuu seitsemästä erillisestä osasta. Ensimmäinen osio on johdanto, jonka aikana perehdytän lukijan, aiheen peruskäsitteistöön, esimerkiksi selittämällä mitä on metayhteisö ja metapopulaatio, mitkä ovat niiden yleisimmät mallit ja miksi ne ovat tärkeitä tutkielman aiheen kannalta. Kerron myös antibioottien tuomasta uhasta nykypäivinä ja miksi bakteereiden antibioottiresistenttiyttä olisi tärkeää tutkia. Johdannon lopussa esittelen tärkeimmät tutkimuskysymykset, joihin pyrin tutkielman avulla vastaamaan, sekä kerron keskeisimmät hypoteesini liittyen tutkielmassa tehtyjen kokeiden tuloksiin.

Toisessa osassa esittelen tarkan kuvauksen tutkielman yhteydessä suoritettujen kokeiden menetelmistä ja erilaisista työvaiheista, joiden tarkoituksena oli tutkia bakteereiden migraation vaikutusta bakteeriyhteisöjen rakenteeseen, sekä antibioottiresistenttiyden kehittymistä bakteereilla ja muutoksia populaatiodynamiikassa. Tulokset-osiossa perehdytän lukijan tuloksiin ja analysoin niitä.

Tämän jälkeen esittelen muita tutkimuksia, joita on tehty kyseisestä aiheesta ja vertailen niiden tuloksia omista kokeista saatuihin tuloksiin. Pohdin tulosten tarkoitusta, ja minkälaisia mahdollisia johtopäätöksiä niistä voi tehdä mm. kerron miten tärkeä migraatio on ja millä tavoin sen avulla bakteerit voivat saavuttaa antibioottiresistenttiyden. Pohdinta-osiossa kerron myös mahdollisista virhelähteistä ja tulevaisuuden kokeista, jotka antaisivat lisää informaatiota aiheesta.

Pro gradu -tutkielman viimeiset osiot sisältävät kiitokset, lähdeluettelon, johon on kerätty kaikki tutkielmassa käytetyt lähteet, sekä viimeisenä liitteet, jotka sisältävät vielä tarkemman kuvauksen tutkielman aikana suoritettujen kokeiden työvaiheista.

Avainsanat: Metayhteisö, metapopulaatio, migraatio, antibiootti resistenttiys, bakteerit.

Master's thesis

Subject: Biology physiology and genetics
Author(s): Elizaveta Alitupa
Title: Population dynamics and antibiotic resistance in spatially structured experimental bacterial communities
Supervisor(s): Teppo Hiltunen & Shane Hogle
Number of pages: 52 pages
Date: 28.6.2022

This master's thesis consists of seven separate parts. The first section is an introduction, during which I introduce basic concepts, for example, by explaining what a metacommunity and metapopulation are, what are the most common ecological models and why they are important to the topic of the thesis. I will also talk about the threat, posed by antibiotics and why it is important to study antibiotic resistance in bacteria. At the end of the introduction, I present the main research questions and hypotheses related to the thesis.

In the second part, I present a detailed description of the methods of the experiments and the different work steps to study the effect of bacterial migration on antibiotic resistance, as well as the development of antibiotic resistance in bacteria and changes in population dynamics. In the results section, I present the results from these experiments and analyze them.

After that, I will present results from related studies and compare their results with my own. I consider the purpose of the results, and what kind of possible conclusions can be drawn from them. For example, I will tell you how important migration is according to the results and how it can change community composition. In the section of discussion, I will also talk about possible sources of error and future experiments that would provide more information on the topic.

The last sections of the master's thesis contain acknowledgments, a list of references and final part is the appendices, which contain an even more detailed description of the work steps of the experiments performed during the master’s thesis.

Key words: Metacommunity, metapopulation, migration, antibiotic resistance, bacteria.

Contents

1 Introduction

Here I introduce the metacommunity concept and how it may be applied to bacterial communities. I will also talk about threats of the antibiotics and what consequences it could bring to the health and environment and how antibiotics could disturb a metacommunity. At the end I will present my hypotheses that are related to my experiments that I have done in my master's thesis.

1.1 Metacommunities and metapopulations

According to D.S. Wilson (1992) metacommunity is a set of local communities that are linked by dispersal of multiple potentially interacting species. Basically, this means that in metapopulation we have population that is spatially structed and metacommunities consist of metapopulations. Metapopulation theory is examine what regulates persistence of metapopulation in system that is constructed of connected habitat patches. Otherwise, metacommunity studies examine what regulates coexistence of multiple species in that kind of system. (Hanski & Gaggiotti, 2004, page 134)

Metacommunities combines two common features of many biological systems: first, that species are interacting in complex ways and, second, that spatial heterogeneity and fragmentation lead to patches of suitable habitat in a matrix of nonhabitat. Importantly, species interactions can affect spatial processes and vice versa. During next paragraphs I will explain importance of heterogeneity and trade-off in metacommunity. I also will explain how different spatial dynamics effect metacommunity and what kind of different paradigms of metacommunity exist.

1.1.1 Importance of environmental heterogeneity

Environmental heterogeneity has important role to determines the ecological processes that have effect on diversity in metacommunities. It allows creation of different habitats, which can provide protection for some specialist species and boost diversity of metacommunity (for example, in the case of species-sorting and mass effect paradigms that I describe later). When migration rates are high, populations may be able to colonize and persist in suboptimal patches (Mouquet & Loreau, 2002).

However, environmental heterogeneity between patches can prevent species exclusion at the metacommunity level because species could succeed better in different environment. In both situations, diversity will increase only if patch-type heterogeneity

maintains “source” populations that replenish “sink” populations (see section 1.2.1 for further details). However, very high migration rates can also homogenize the composition of patches (Amarasekare & Nisbet, 2001; Levine & Rees, 2002; Mouquet & Loreau, 2002).

1.1.2 Trade-off in metacommunity

Trade-off in the context of metacommunity dynamics relate to the tension between colonization ability and susceptibility to stochastic extinction. In the patch-dynamics model of metacommunities, species can coexist regionally because competitively weak species have high dispersal rates that increase their probability of dispersing to a new patch, while strong competitors will generally outcompete dispersal specialists locally. In the same way metapopulation theory suggests that trade-offs that are relating to biotic ability and colonization ability in patches can also be significantly important. (Hastings, 1980; Tilman, 1994; Klausmeier, 2001; Yu & Wilson, 2001; Levine & Rees, 2002).

This kind of dynamics affect in two opposite ways to the species-sorting: it could increase competitive ability that is related to increased specialization, in the opposite way high migration decrease intensity about which patches are covered by species that are best specialist in particular conditions (Mouquet et al., 2002).

1.2 Spatial dynamics

In metacommunity ecology it is important to understand how species interact with each other at different spatial scales and how that could be influenced by spatial dynamics. (Holyoak & Ray, 1999).

1.2.1 Source-sink dynamics

Metacommunity patches differ in size and quality. The source-sink dynamics model describes how variation in habitat quality may affect the growth or decline of populations. (Pulliam, 1988). In my thesis for example I use different antibiotic levels to create variation between patches and their quality for bacteria. According to H.R. Pulliam (1988) habitats where reproduction exceeds mortality (sources) support habitats where reproduction is insufficient to balance local mortality (sinks) in metapopulations.

1.2.2 Mass effect

Mass effects allow species to colonize habitats where they could not survive without a recurring influx of immigrants from a nearby patch (Shmida & Wilson, 1985), for example due to high dispersal rates. Mass effects are difficult to prove when we take account complex interactions between different mechanisms shaping biotic communities, however there is some empirical evidence that support their importance in nature. (Heino & Bini, 2021).

1.2.3 Rescue effect

The rescue effect is a mechanism where immigration could prevent local extinction. Prediction of the model according of J.H Brown and Kodric-Brown (1977) "when immigration rates are high relative to extinction rates, turnover rate is directly related to the distance between an island and the source of colonizing species." For example, study where was examined arthropod's distribution among isolated plants supported the mechanism of rescue effect. (J. H. Brown & Kodric-Brown, 1977). Mass effects and rescue effects are both capable of modifying species abundances (Pulliam, 1988) and interactions between species (Holt, 1985).

1.2.4 Dispersal and colonization

Dispersal in metacommunity means individuals movement from a site to another, in other words migration. Dispersal connects patches in the metacommunity. In Fig. 1. we can see the relationship between migration rate and diversity. There is no requirement for the perspective of metacommunity if there is no migration at all. In this situation, the dynamics of local communities are completely independent from each other, and diversity of local communities is low because of the absence of migration. When there are essential levels of migration, we can assume that every species could probably get to every patch, but the effect of colonization and corresponding competitive abilities become meaningful in case of determining structure of local community. If there are opposite circumstances and levels of migration increase until all species can disperse relatively fast to all patches, the local communities are less affected by the order of colonization and typical fugitive species are eliminated by patch-type specialists. If migration rises at a significantly high level, it has different effect. It contributes to local growth rates and allows species that are locally inferior competitors to persist through mass effect and increasing local diversity (Fig. 1.) (Mouquet & Loreau, 2002)

High level of migration is also decrease diversity of the entire metacommunity, with meaningful effect also on local diversity. In extreme case when migration level is so high that population are entirely mixed, metacommunity diversity fall apart in a single closed community. (Hanski & Gaggiotti, 2004, pages 139-140).

In this thesis I examine situation where I have three different level of migration (no, low and high) in bacterial metacommunities and how those different migration levels effect of metacommunities dynamics and diversity.

Another mechanism of spatial dynamics concerning movement of species is colonization. Colonization means that in sites where there were absent of population before, population become established. Changes in colonization dynamics have effect on whole dynamics of metacommunity. For example, according to study of Altermatt et al. (2008) that was done in Helsinki University, climate change is effects on colonization dynamics and thereby change dynamics and composition of entire metacommunity of three Daphnia species. (Altermatt et al., 2008).

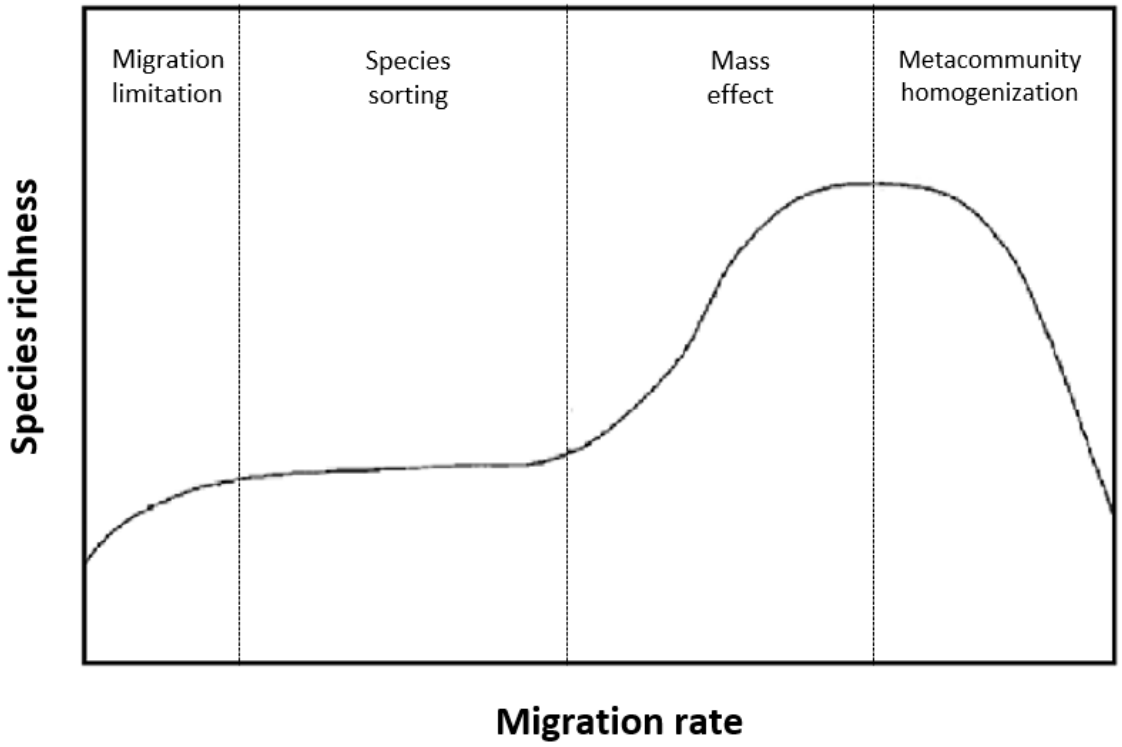


Fig. 1. Theoretical link between migration rate and species richness that illustrate main mechanisms which have effect to community structure (Hanski & Gaggiotti, 2004, page 140). Redraw after (Hanski & Gaggiotti, 2004, page 140).

1.3 Metacommunity frameworks

There are four different metacommunity conceptual frameworks. Leibold et al. (2004) outline paradigms that diminish the range of possible metacommunity categorizations and their corresponding model parameterizations, to a smaller representative subset. These four metacommunity frameworks are species-sorting, mass effects, neutral and patch dynamics and can be used to compare community dynamics and similar patterns from both empirical and theoretical contexts (Leibold et al., 2004).

Differences between the four frameworks include competition-colonization vs. species-sorting dynamics, niche structure vs. species equivalency and finally homogeneous vs. heterogeneous patches. (Leibold et al., 2004) In some cases, empirical data may be explained by equally well by multiple frameworks simultaneously (Debout et al., 2009; Logue et al., 2011a).

1.3.1 Species-sorting and mass effect frameworks

Both the mass effect paradigm and species-sorting framework assume low to moderate species dispersal rates with heterogeneous habitat patches that optimally support different species (Leibold et al., 2004; Loeuille & Leibold, 2008).

In the species-sorting framework dispersal rates are often low but equal between species (Fig. 2.). This leads to high abundance of each species in optimal patches and low abundance in suboptimal environments(Leibold et al., 2004). Species-sorting is explored experimentally for example in case of benthic microalgal communities (Matthiessen & Hillebrand, 2006) and it has been recognized in coral colonies between commensals (Caley et al., 2001).

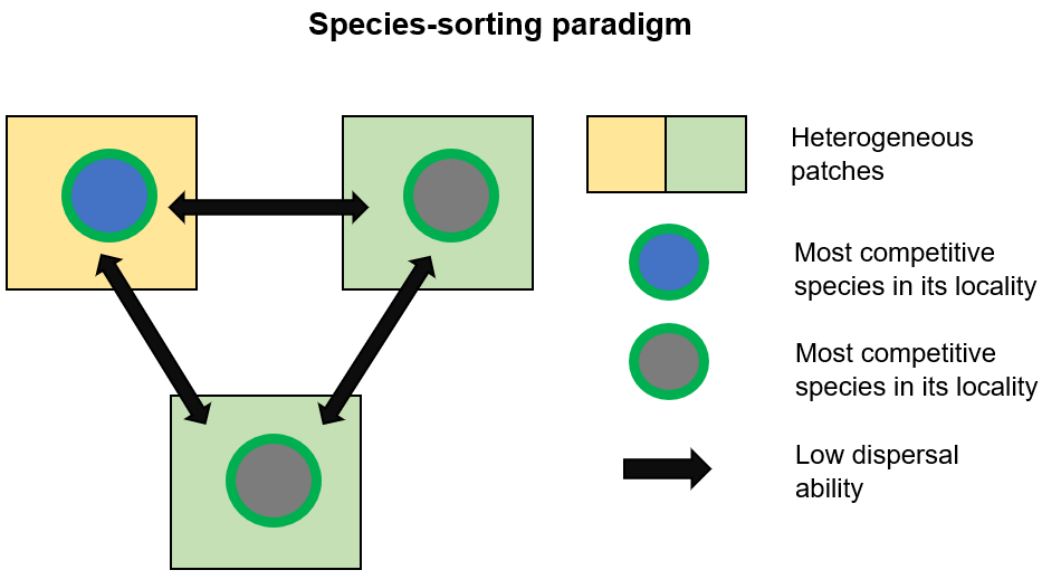


Fig 2. Structure of the species-sorting paradigm. Both grey and blue species occur where they are the best competitors. If the abiotic conditions of the localities change (suddenly or intermittently), a re-component of the metacentre may occur due to the spread of individuals of each species and species could survive. (Métacommunauté — Wikipédia). Redraw after (Métacommunauté — Wikipédia.)

In the mass effect paradigm, dispersal rates are higher than in species sorting but they are still equal between species (Fig. 3.). This kind of situation leads to higher abundance of species, and they form sub-optimal habitats and have source-sink dynamics within the metacommunity. This framework focuses on the effect of emigration and immigration on local population dynamics (Mouquet & Loreau, 2003; Leibold et al., 2004). Mass effect dynamics have been observed in forest fragmentation experiments in southeast Australia (Davies et al., 2005).

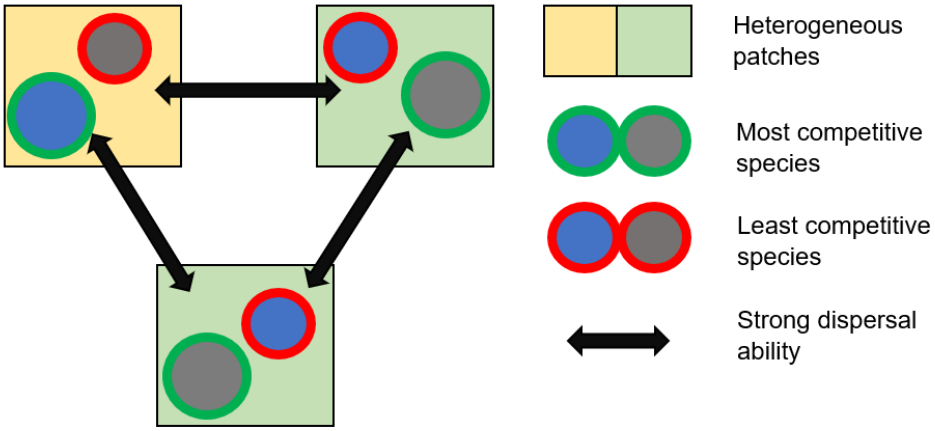


Fig 3. Structure of the mass effect paradigm. Grey and blue species occur mainly in places where they are the best competitors. However, species also occur in places where another species is a better competitor through dispersal. (Métacommunauté — Wikipédia). Redraw after (Métacommunauté — Wikipédia.)

1.3.2 Neutral framework

The neutral paradigm suggest that all species react to the environment in the same way, and they are demographically equivalent (Fig. 4.). In other words, species are identical in their movement, fitness, and competitive competency (Leibold et al., 2004).

Interaction between populations emerge through random walks that change relative frequencies of species. Using expectations of species loss (for example,extinction or emigration) and growth like immigration and speciation there is possibility to clarify dynamics of species diversity (Leibold et al., 2004; Vellend, 2010).

Neutral metacommunity dynamics have been used to explain community dynamics in aquatic microbial communities (Östman et al., 2010) and in Panama's tropical trees (Jabot et al., 2008).

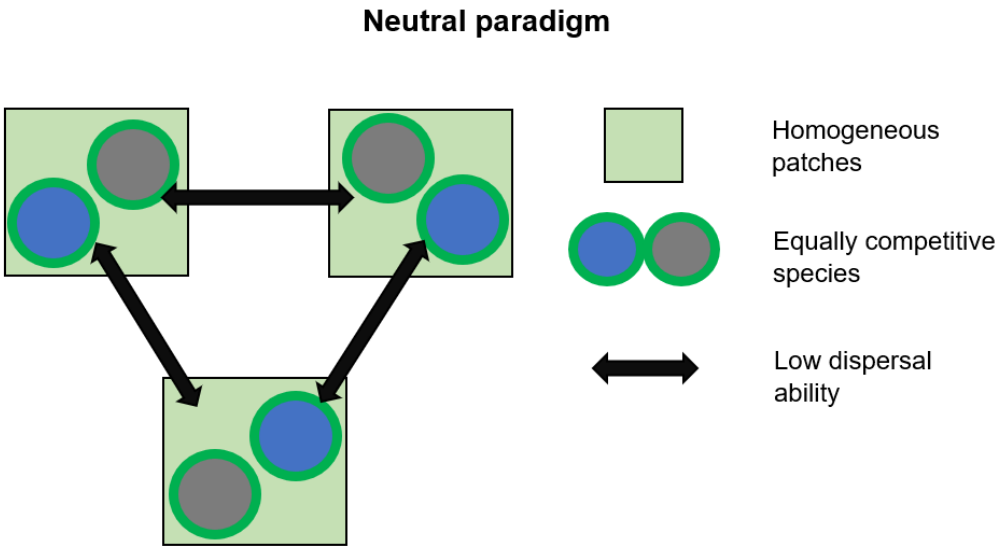


Fig 4. Structure of the neutral paradigm. The grey and blue species are the same in all localities. Local populations may eventually spread to other locations through dispersal. (Métacommunauté — Wikipédia,). Redraw after (Métacommunauté — Wikipédia.)

1.3.3 Patch dynamics framework

Patch dynamics arise from Levins's (1969) metapopulation model broadened to include multiple species. Here patches are homogeneous, can contain populations and experience patch-level disturbance events like competitions (Hastings, 1980; Tilman, 1994). Patches may be occupied, or uncopied and local species diversity is defined by dispersal (Fig. 5.). Local extinction and colonization dominate spatial dynamics (Leibold et al., 2004).

Generally, one species is better adapted at the local scale (meaning patch) and others have a dispersal advantage at the regional scale. This could happen through species' competitive ability (Hastings, 1980; Nee & May, 1992) or fecundity (Yu & Wilson, 2001; Yu et al., 2001). Patch dynamics have been studied experimentally using protozoa, bacteria, and rotifer system (Cadotte, 2006), and pond-breeding mosquitos (Chase & Shulman, 2009).

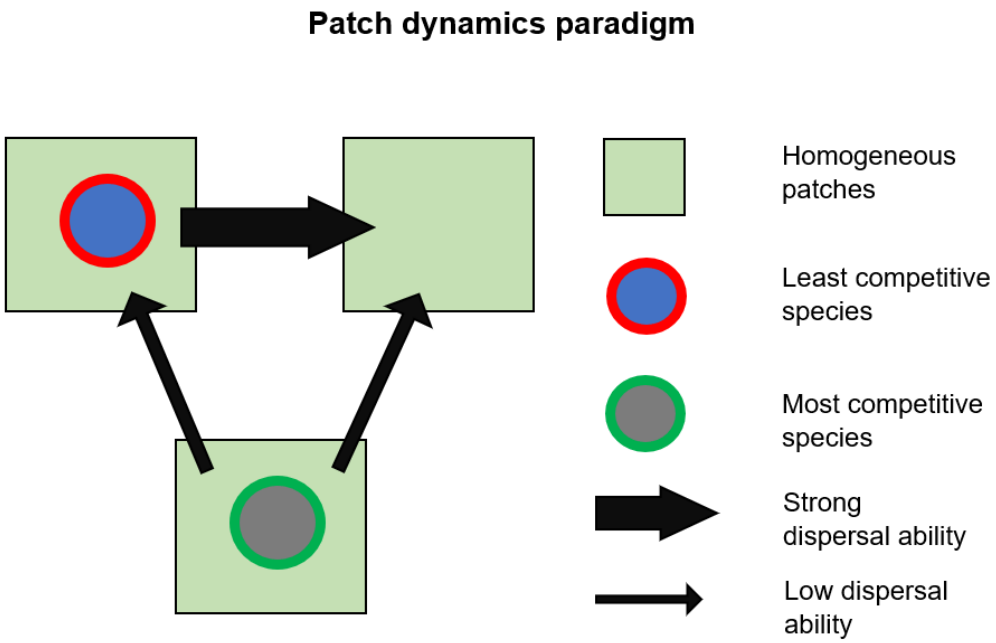


Fig 5. Structure of the patch dynamics paradigm. The best competitor (grey one) excludes a blue species that has died locally during competition. The blue species, which has a higher dispersal capacity, inhabits new localities. (Métacommunauté — Wikipédia.). Redraw after (Métacommunauté — Wikipédia.)

1.3.4 Limitations of the metacommunity frameworks

The four theoretical metacommunity frameworks (species-sorting, mass effects, patch dynamics, and neutral dynamics) offer useful mechanistic explanations for predicting empirical community patterns in nature. However, empirical patterns may be explained equally well by any of these frameworks or a mixture of multiple frameworks operating simultaneously. Ecological processes in metacommunities can be complex, for example because of varying species interaction strengths that are dependent on migration rates. This is possible, for example, because a predator may have sufficiently high migration rate that regulates metapopulation structure and local populations. Predators then interact with prey with significantly lower migration rates and the prey's local population is regulated through local processes (Holt, 1997; van Nouhuys & Hanski, 2002).

Fig. 6. Overview of four metacommunities where their differences are visible through the different axes of heterogeneity of habitat patches, rate of dispersal and of the equivalence among the species (Logue et al., 2011a). Redraw after (Logue et al., 2011b).

Brown B.L. and others (2016) considered problems related to the four metacommunity frameworks or which they named them "Big 4". There is not an issue with the four frameworks which clearly represent theory of metacommunity, but there is problem because many scientists have assumed that "Big 4" represent the entire inference space of metacommunities and it describe all possible metacommunity dynamics. If that assumption would be right, all metacommunities could be categorize in the same paradigm, but clearly the situation is not like this and there are two reasons why (Brown et al., 2016). Firs, the four frameworks are not mutually exclusive (Leibold et al., 2004; Logue et al., 2011a; Winegardner et al., 2012). Second, the four frameworks do not establish all assumptions of metacommunity theory (Brown et al., 2011; Logue et al., 2011; Winegardner et al., 2012). The second problem is illustrated in Fig. 6. where the four frameworks are limited to three different axes: environmental heterogeneity, species equivalence and influence of dispersal (Logue et al., 2011a).

1.4 Microbial metacommunities

Metacommunity theory has been applied to microbiomes, but there are important aspects require adaptation of the theory especially in study of host systems (Koskella et al., 2017). For example, it is important to focus on the properties of the host like a fitness and development if we want to understand microbial community system as a whole (Miller et al., 2018).

Miller et al. (2018) suggest improving metacommunity theory for more accurate study of microbial metacommunities (Fig. 7). They suggest that is it important to incorporate response between the species pool and patches and feedback between microbial community and the patches. They also clear the model of dynamics in the species pool also showing microbes that do not reside in a host patch. That kind of change was done because there are many observations that microbes that live and grow in the neighboring environment are common members of the host microbiome, and they constitute an additional, non-host patch. (Miller et al., 2018).

In their article Miller et al. (2018) also highlight that is important to consider the response between host and their resident microbiota. If we ignore the ways the microbiome can affect the host and what consequence could happen (in for example behavior, evolution, or development), it could lead to the fundamental misunderstanding of host-microbiome biology. (Miller et al., 2018). During next paragraphs I reveal how metacommunity theory has been used to study human microbiomes.

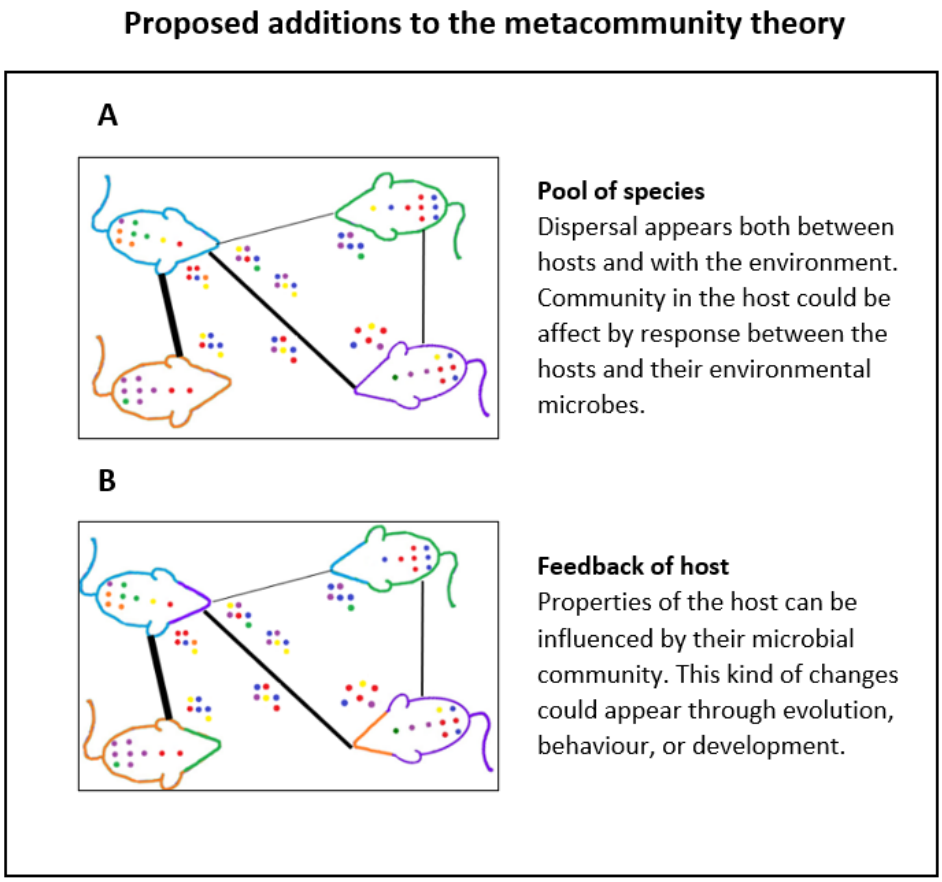


Fig. 7. Expansions of metacommunity theory to applying it to the host–microbiome systems. Mouses of different color are represented different hosts. Dots inside the mice represent microbes inside the host and dots outside the mice represent microbes that living in the external environment. Changes in the colors of host (B), represent changes in the host state due to the community composition. (Miller et al., 2018). Redraw after (Miller et al., 2018).

1.4.1 Metacommunities in human microbiomes

In the context of microbiomes, metacommunity theory conceptualizes human hosts as patches where such that microbial communities can be dispersed between humans (Costello et al., 2012). Metacommunity theory could be helpful to figure out of the relevance of environmental and dispersal selection in shaping host-associated communities that is very important when we are talking about human microbiome (Mihaljevic, 2012).

Metacommunity frameworks that assume patches are homogeneous and dispersal between patches bring variation in community (neutral and patch dynamics paradigms), can be useful for examine populations of closely related hosts. Other metacommunity paradigms assume that patches are heterogeneous and variation in community results at least partly from environmental selection that we can assume in this case could be for example host genetics or diet. (Costello et al., 2012).

High dispersal rates will favor immigration and low dispersal will favor adaptation within a patch. This framework may be useful for understanding responses to antibiotic use. If immigration would lead to antibiotic resistance, it would be better solution to focus on quarantine and hygiene than focus on altering antibiotic dose or duration. (Costello et al., 2012).

Metacommunity theory has been also applied to examine the drivers of non-host-associated microbial community and dynamics (van der Gucht et al., 2007; Ofiţeru et al., 2010). However, metacommunity theory has been rarely used to examine host-associated communities (Sloan et al., 2006; Hovatter et al., 2011). For example, studies about the host selection and its dependence on the age of host or microbial group could give important information about how hosts “filter” microbes and, conversely, how microbes avoid this kind of filtering. This kind of understanding is significant if we want to directly manipulate host-associated communities in clinical field, for example, by creating probiotics that can avoid host filtering and establish within a host (Costello et al., 2012).

1.5 Threats of antibiotics and antibiotic resistance bacteria

Antibiotics have saved many lives, but there are negative indirect consequences from widespread antibiotic use. In many cases we use antibiotics too often or in the wrong way and that spreads antibiotic significantly and lead to emergence of the antibiotic

resistance bacteria. Every single day, approximately from 1% to 3% of the people in the developed world are using pharmacologic doses of antibiotics. (Goossens et al., 2005).

Antibiotic resistant bacteria are a global health threat. The aim of the antibiotic therapy is to achieve enough large concentration of the drug for a sufficient duration in a particular body site so that the elimination of pathogen could happen. However, even if that aim is achieved, different amounts of antibiotics will always be found in different sites of organism depending on the way of administration and its pharmacodynamic properties. The application of antibiotics provides a strong selection pressure for the evolution of antibiotic resistance traits. For example, human oral and gut communities are recognized as places where evolution of antibiotic resistance often happens (Salyers et al., 2004; Smillie et al., 2011; Roberts & Mullany, 2014). Most human associated bacteria are beneficial or neutral for the human host. Antibiotic treatments will also have destroy symbiotic or commensal gut microbiota, and is the most common reason of disturbance in human-microbe associated communities.

Also, antibiotics are released to the environment from pharmaceutical industry, hospitals, and community. In the environment they logically have effect on animal husbandry, plant production and aquaculture. Eventually, antibiotics spread everywhere moving along between different environments, while increase antibiotic resistance level in bacteria. (Andersson & Hughes, 2014)

According to WHO (*World Health Organization 2019b*, n.d.) in 2017 three percent of the newest case of tuberculosis and 18 percent of the older cases were either cause by multiple drug resistant bacteria (resistance at least one antimicrobial drug in three or more antimicrobial categories) or rifampicin resistant bacteria. A total of 558 000 new cases of tuberculosis in these resistance groups were estimated in 2017. Antibiotic resistance to tuberculosis caused 230 000 deaths in that same year mainly in India and China.

1.6 Aim and hypotheses of the thesis

In this master's thesis I examine antibiotic resistance as a spatial process. I also test hypothesis that antibiotics can develop spatially distributed environments where the harshness of the environment and thus also the strength of selections for antimicrobial resistance varies. I also study whether it is possible for bacteria to develop antibiotic

resistance in different metacommunity patches if there is migration between populations and different populations are living in different antibiotic environment.

I want to explore what kind of changes that kind of migration can bring to the bacterial population and how bacterial populations change when they have some strains that have already antibiotic resistance. So, is there any possibility that intermediate patches can act as stepping stones for resistance, if they are connected to high antibiotic patches where selection is stronger but initially lethal for naive bacteria?

Also, I want to know what will happen if I manipulate bacterial communities with an antibiotic, which can be used to model the stress arising from the habitat, that bacteria also experience in the natural habitats. With this modeling, I can study how different species respond to stress and what is the role of evolution in stress tolerance.

So, the aim of the thesis is among other things to investigate how genetic diversity between different populations affects community dynamics, for example, whether bacterial biomass changes during the experiment and how bacterial community's change.

One of the research hypotheses is that genetic diversity must have some effects on community dynamics, but the effects will not be shocking, for example, species that succeeded at the beginning will also succeed at the end of the experiment. I also hypothesized that migration increase antibiotic resistance in population, whereas in communities where no migration has occurred, extinction occurs.

2 Materials and methods

To implement my experiment in master's thesis I used HAMBI-strains that are from Microbial culture collection HAMBI that is situated at Viikki Campus (HAMBI Microbial Culture Collection, University of Helsinki — University of Helsinki n.d.). In the culture collection there are over 3000 different bacterial strains, but in these experiments, I used only 23 strains. In the laboratory group where I did my thesis, they used only particular 23 strains (HAMBI-strains are visible in Table 1) for their different experimental evolution protocols. Those strains have been selected for the purposes and functionality for that laboratory over the years.

During the experiments and tests I used evolved (EVO) strains that were exposed to antibiotic before experiments and ancestor (ANC) strains that were never exposed to antibiotic. Most evolved strains had developed complete resistance.

2.1 Experiments before main experiment

Before the main experiment I had to do few initial experiments that gave me valuable information for the main experiment of the thesis. In the initial experiments I also used HAMBI-strains of which I talked before (Table 1). The first experiment was a streptomycin selection experiment consisting of two different parts. The aim of the first part was create evolved populations that I could use for the main experiment and aim of the second part was create individual evolved clones. I also did minimum inhibitory concentration (MIC) test using ancestral HAMBI-strains and evolved clones from part two of the streptomycin selection experiment. During next paragraphs I will talk more detail about those experiments.

2.1.1 Streptomycin selection experiment part 1

I started this part of the experiment using 23 HAMBI-strains evolved populations (information are visible in Table 1) that were done before through a selection experiment by some other employee of the laboratory, and I also used 23 HAMBI-strains ancestor populations (information are visible in Table 1). After that I combined same EVO-strains with ANC-strains (same HAMBI-code) and grew them in plates in 12 different streptomycin concentrations (0, 1, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 and 5000 µg/ml). Next, I mixed all same HAMBI-strains together and get 23 different evolved populations. More delailed protocol is visible in Appendix 1.

2.1.2 Streptomycin selection experiment part 2

In part 2 of the streptomycin selection experiment, I used evolved populations from part 1. I created dilution series from every 23 HAMBI-strains and grew them in plates. After that I picked up 16 individual evolved clones from every strain and grew them separately in liquid culture in plates. More detailed protocol is visible in Appendix 2.

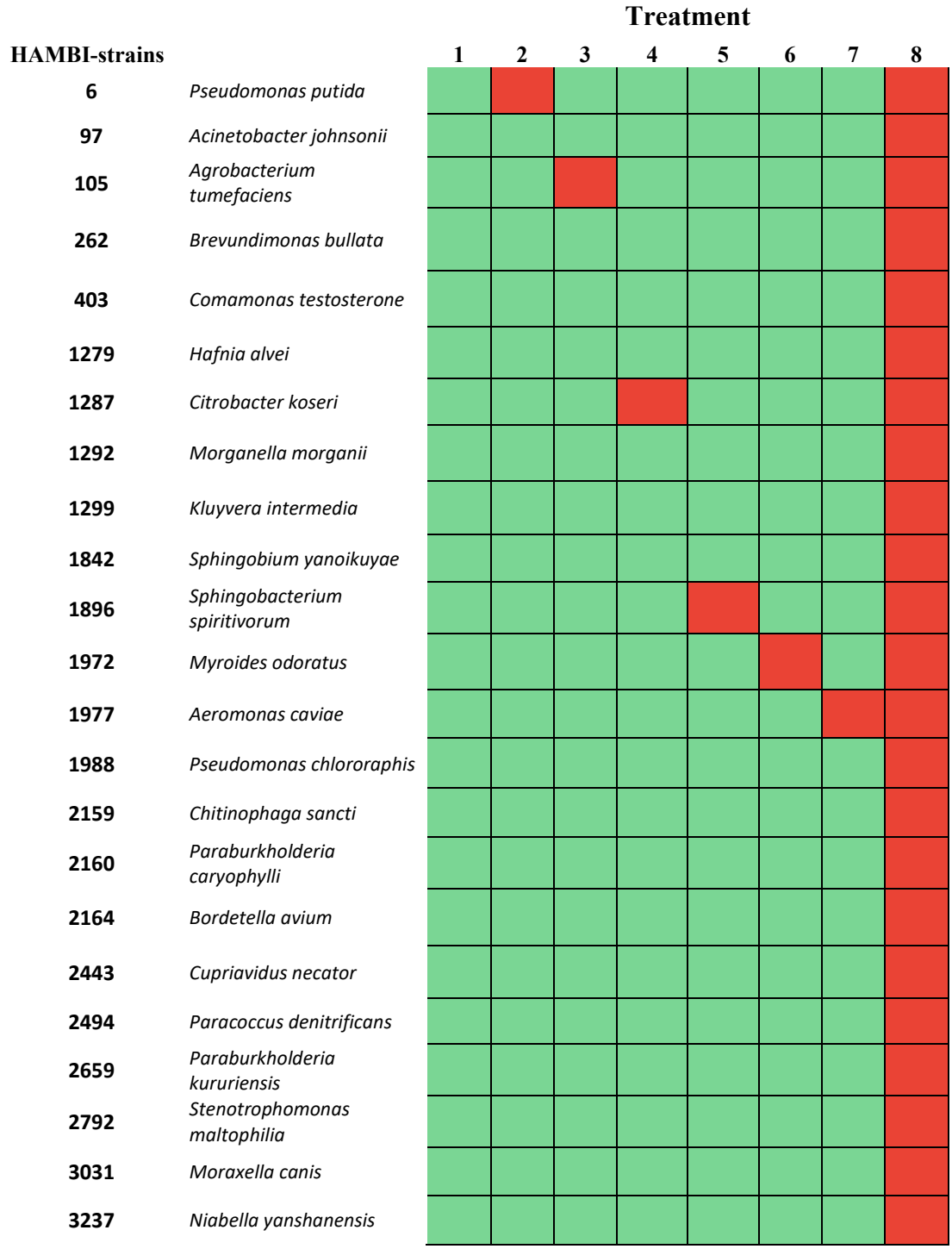
2.1.3 Streptomycin minimal inhibitory concentration (MIC) and dose-response measurements.

I did MIC-test using individual evolved clones from part 2 of streptomycin experiment and ancestor strains. In MIC-test I used only HAMBI-strains 6, 105, 1287, 1972, 1977 and 1896, because information about them was significantly important relative to main experiment and 16 clones of each strain represented population rather well. During MIC-test I used 13 different streptomycin concentration to evaluate concentration that prevents visible growth of bacteria in different strains. From this data, the IC50 value (the concentration of antibiotic that results in a density of half the maximum) for each species was estimated (see data analysis section below). More detailed protocol is visible in Appendix 3.

2.2 Main experiment

In the main experiment I created eight genetic treatment groups using 23 different HAMBI-strains. I had 23 HAMBI-strains that were ANC and the same 23 HAMBI-strains, but they were EVO-populations from part 1 of the streptomycin experiment. Eight different genetic treatments are visible in Table 1. In treatments two through seven, I used HAMBI-strains evolved populations 6, 105, 1287,1972, 1977 and 1896 (visible in red in Table 1) and that is the reason I did MIC-test particularly used those strains.

Table 1. Eight genetic treatments using 23 HAMBI-strains. EVO strains are marked by red colour and ANC strains are marked by green colour.



In the experiment I also used three different antibiotic concentrations (no streptomycin: 0 µg/ml, low streptomycin: 20 µg/ml and high streptomycin: 1000 µg/ml) and I had also three different connectivity levels between patches (no connectivity, low connectivity and high connectivity). Based on that, every treatment (eight of them) has all of three antibiotic concentrations with four replicates of them (look Fig. 7. for more information).

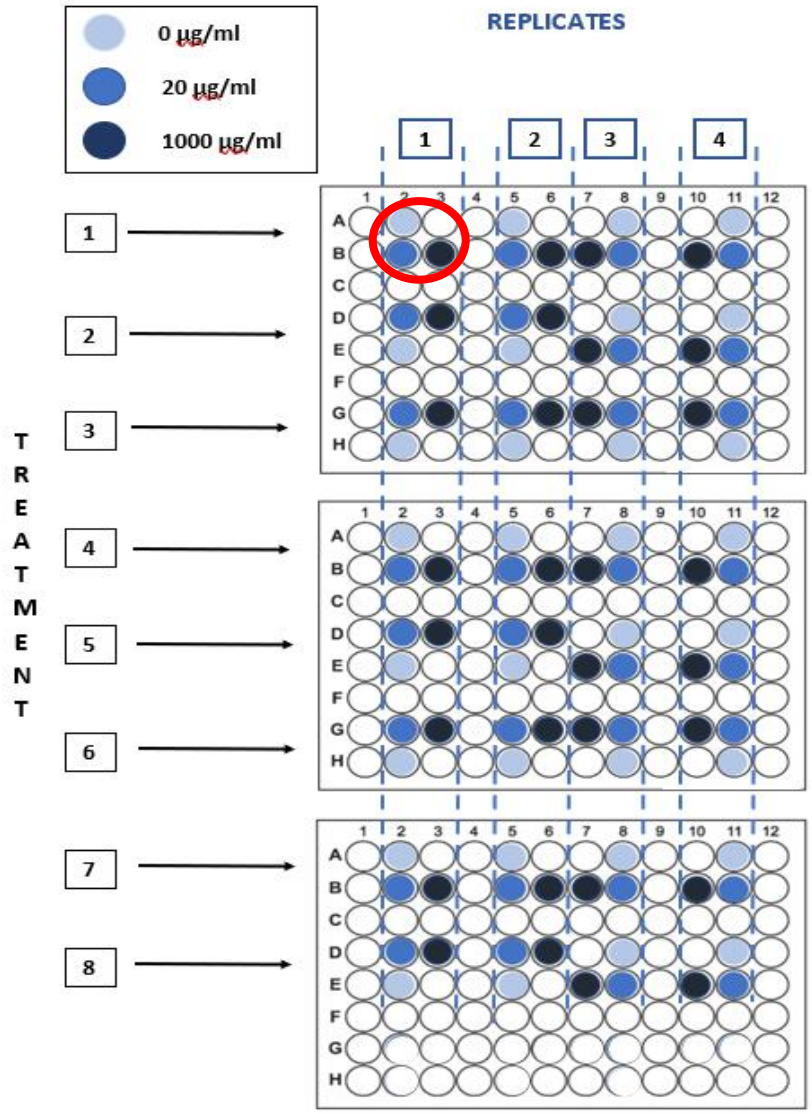


Fig. 7. Exact location of different treatments, their replicates and antibiotic concentration in wells of plates. This is a representation of one connectivity level (altogether three), for example no connectivity. In other words, there are 9 plates in the whole experiment, and one metacommunity is made up of particular treatment and of it's one replicate (three wells with different antibiotic concentrations, example of metacommunity is marked by red circle).

Connectivity level between patches means that based on the current connectivity level I have mixed three wells together from the same treatment and from the same replicate. After that I put the current mix back to grow (see Table 2 for more information).

Table 2. Concept of the connectivity treatment, where timepoints of different connectivity levels between patches are marked by X. According to that high connectivity treatment was always occur every 3rd transfer and low connectivity transfer was occurred every 6th transfer. In no connectivity treatment there was no mixing at all. Timepoints that are marked by orange means that in timepoint there was also plate freezing besides the normal transfer. Timepoint with yellow marks were pointed out for sequencing. Time between transfers was always 48 hours, and in every transfer, I was measure OD (optical density).

Transfer	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
No connectivity																							
High connectivity			X			X			X			X			X			X			X		
Low connectivity						X						X						X					

2.3 Creating and growing treatment groups in the main experiment

I grew all ANC strains (23 strains and 23 EVO populations in microcosm bottles using 100 % R2A medium. Microcosms were kept in incubator at 25 ± 0.1 °C and shaken constantly at 50 r.p.m. I created eight treatment groups by putting 1 ml of the right strain into a 50 ml falcon tuble (according to the Table 1) and mixing them up using vortex (eight falcons at the end). I did four eppendorf tubes of pellets from every mix (1.5 ml of mix pereppendorf tube, centrifuge 10 min (13 000 rpm) and pour the supernatant out) and froze them. This eppendorf tubes with pellets were for sequencing start populations for experiment. I also froze mixes (four eppendorf tubes per every mix) with 85 % glycerol (800 µl of mix in an eppendorf tube and I filled the tube with the same amount of 85 % glycerol) and vortex well.

2.4 Starting the main experiment

I used deep 96 well plates in the experiment. At the beginning I filled the plates (9 plates) with R2A medium and the right antibiotic concentration (900 µl/well) (according to Figure 1) using PlateMaster. After that, I putted 50 µl of the right treatment mix to the right well in the plates (according to the Figure 1). Plates were growing for 48 hours in an incubator (25 °C) and shaken constantly at 1000 r.p.m.

2.5 Transfers in main experiment

After growing plates for 48 hours, I measured OD using normal 96 well plates (transfer 100 µl/well using PlateMaster). According to the Table 2, I did different manipulation in different transfer timepoint.

2.5.1 Serial transfer experiment

In serially transferred culturesusing the PlateMaster to transfer 50 µl/well to the new deep 96 well plates after that I was fill "new" plates with fresh medium with possible antibiotic concentration.

2.5.2 Transfer with freezing (marked by orange in Table 2)

I did the serial transfer and after that I put 100 µl/well of samples to the normal 96 well plate, filled to 200 µl/well with 85 % glycerol and vortexed well. I froze plates that I could use them if something was go wrong during the experiment or in some other future experiments.

2.5.3 Transfer in low connectivity timepoint or in high connectivity timepoint

I took samples 780 µl/well from each high or low connectivity plate using a PlateMaster and transferred them to a 12 well plate. After that in every well of the 12 plate I had a mixed community with cells from the three antibiotic treatments. I vortexed all plates and transferred samples to the right wells.

2.5.4 Transfer with sequencing

First, I did the serial transfer. After that I was use special 96-plates for centrifuge. After centrifuge, I pour the supernatant out and froze plates. Subsequently DNA was isolate from this samples (DNA isolating was done by Sovelluskeskus in University of Turku) and was send for sequencing to FIMM (Suomen molekyylilääketieteen instituutti). Isolated DNA was first amplified with Illumina's 16S universal primers (Forward Primer = 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG, Reverse Primer = 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) following standard Illumina protocol (https://support.illumina.com/downloads/16s_metagenomic_sequencing_library_prepar

ation.html). Sequencing was performed using paired end reads with v3 Chemistry on an Illumina MiSeq sequencer at FIMM.

2.6 Quality controls, sequence trim and mapping

This part of the experiment was done by my supervisor Shane Hogle. First, he did quality control using command line tools BBTools, VSEARCH and FASTP (Chen et al., 2018) on the Puhti scientific compute cluster (*Puhti - Docs CSC*, n.d.). He downloaded sequencing data from FIMM and filtered out known contaminants (Phix for example), removed residual adapter sequences and trimed using BBTools the reads at the ends where they were below Q10 read quality (1:10 error rate or a 90% correctness). Hogle merged overlappingpaired reads using BBMerge (B. Bushnell, J. Rood, E. Singer, BBMerge – Accurate paired shotgun read merging via overlap. PloS One 12, e0185056 (2017).), and used VSEARCH to filter merged reads so , that each read had a maximum of two expected errors (Edgar & Flyvbjerg, 2015). After that he mapped those reads using BBMap to a library of the 16S sequences from the 23 HAMBI species to assign species abundances (*BBMap Guide - DOE Joint Genome Institute*, n.d.)

2.7 Data analysis

For statistical modelling and visualizations of different plots, I used Python 3.9 and its different libraries: matplotlib, openpyxl, sklearn and numpy. I calculated Shannon diversity for the treatment one and treatment eight for learning more about diversity of the community. Besides that, I also calculated Bray-Curtis dissimilarity and examine differences in species population between different replicates and between different streptomycin concentrations in treatments one and eight. Dr. Hogle analyzed minimal inhibitory concentrations (MIC) for each species by fitting a non-linear 4-parameter model:

$$f(x) = c + \frac{d - c}{1 + e^{[b \times (\log x - \log e)]}}$$

where *c* is the lower asymptote (i.e. response variable at complete inhibition), *d* is the upper asymptote (uninhibited growth), *e* is an inflection point equivalent to a half growth inhibition dose (IC50), and *b* is a slope of the curve at the inflection point. Dr. Hogle estimated all parameters separately for each strain by fitting dose response curves using nonlinear least squares in R. For the comparing different treatments and HAMBI-species I calculated p-values using Mann-Whitney U-test and Permanova analysis.

3 Results

In this section I will present all results that I get from the experiments. First, I will talk about results from MIC-test and after that I present all results that I get from main experiment. During the analysis of the main experiment I decided with my supervisors that I would present only results from the treatment one (all ancestral species) and treatment eight (all evolved species) with all connectivity levels (no, low and high).

3.1 Inhibitory concentrations of antibiotics for ancestral and evolved bacteria

Fig.8. shows IC50 values of six different ancestor HAMBI-strains (6, 105, 1287,1972, 1977 and 1896) and 16 different evolved clones of the same six HAMBI-strains (actual name of the strains are evident in Table 1). The IC50 value is the antibiotic concentration that theoretically should inhibit the growth to have the maximum value under the given conditions (see methods). So, IC50 is a quantitative measure that tells how much inhibitory substance is needed to inhibit (in vitro) a particular biological component or process by 50%. (Aykul & Martinez-Hackert, 2016).

In Fig. 8. different ancestor HAMBI-strains are marked by green and 16 evolved clones of HAMBI-strains are marked by red. All ANC-strains and all 16 evolved clones of HAMBI-strains had each four technical replicates. I calculated median of each four technical replicates, and they are visible in Fig. 8. by dots (one ancestor dot and 16 evolved dots per HAMBI-strain).

From Fig. 8. we can see that almost every 16 evolved clones of HAMBI-strains had IC50x value over 2000, exceptions are just HAMBI-0006 and HAMBI-0105 where one evolved clone of 16 had value of IC50x approximately 800 and four evolved clones of HAMBI-strain 1977 had IC50 value approximately 50. In case of ancestor, IC50 values differed from each other. For example, the highest level of IC50 of ancestor was in HAMBI-1287 (approximately 2050) and the lowest value of ancestor was in HAMBI-0006 (approximately 20). While examining the Fig.8. we can say that IC50 values of ancestors were lower (or at least the same) in all HAMBI-strains than values of evolved clones in the same HAMBI-strains. IC50 values of evolved clones were also significantly higher than in case of ancestor. These high IC50 values in the evolved

clones suggest that the evolved populations of these species were almost completely resistant to the antibiotic.

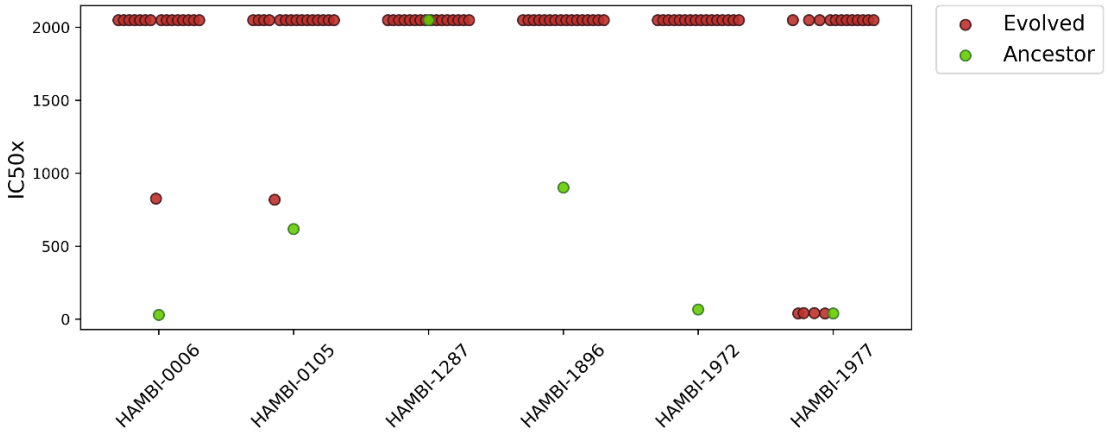


Fig. 8. IC50 values of six ancestor HAMBI-strains and 16 evolved clones of six HAMBI-strains (6, 105, 1287,1972, 1977 and 1896). Evolved clones are marked by red and ancestor are marked by green. Each dot represents median value of four technical replicates.

3.2 Results of the main experiment

During next sections I present results of the main experiment. I will talk about optical density result from all measurement of the main experiment and about sequence results from the end of the main experiment. I present results using different figures and tables.

3.2.1 Optical density results

Fig. 9. presents results of for optical density measurement from all main experiment of treatment one and treatment eight. There were 23 transfers during experiment that are visible in X-axis and in Y-axis there are values of optical density. Results of different migrations levels of both treatments (No, Low and High) are also visible separately. Different concentrations of streptomycin are marked by different colors (green, yellow and red) and one dot is always represent median value of four different replicates.

In treatment one there were less spikes in every migration level group and overall optical density values were lower than in treatment eight. In both treatment optical density was higher at the end of the experiment (started rising approximately after 15th transfer). Treatment one without migration there were significantly fewer spikes and the increases in optical density were much lower at the end than in no migration level of treatment eight. In low migration level of treatment one there was quite higher rise of optical density than in no migration (treatment one), but there were clearly less spikes and rising than in low migration of treatment eight. In case of high migration there was

quite same situation than during low migration. So, in high migration of treatment one there was higher rise than in low migration, but there were less spikes and rising than in high migration of treatment eight. Between different streptomycin concentrations there were quite barely differences, except when examining spikes. In case of spikes there are visible differences between streptomycin concentrations and there were varies which concentration had higher value of optical density. However, usually red dots (1000 µg/ml of streptomycin) were higher than others. The highest value of optical density during experiment was approximately 0,9 and the lowest was approximately 0,3.

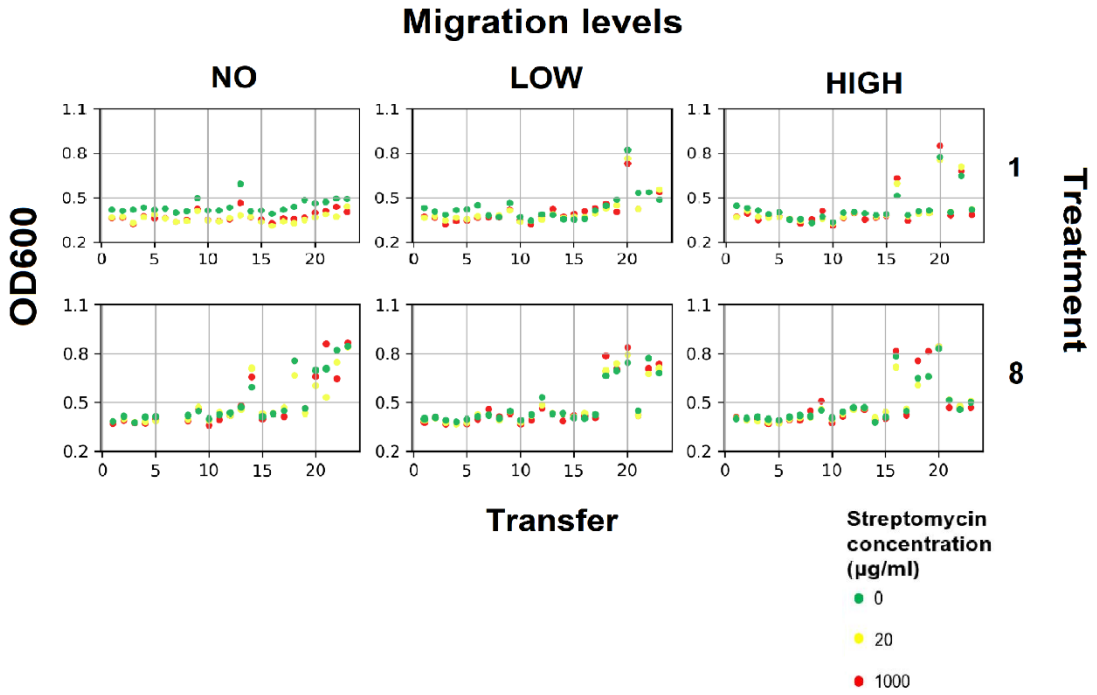


Fig. 9. Optical density results of the treatment one and treatment 8 during whole main experiment (23 transfers). Different migration levels (No, Low and High) of both treatments are plotted separately. Different streptomycin concentrations are marked by dots of different colours (green is 0 µg/ml, yellow is 20 µg/ml and red is 1000 µg/ml)

3.2.2 Sequencing results

There are sequencing results from the start and from the end of the experiment in Fig. 10. In Fig. 10. we can see abundance of different HAMBI-strains that are marked by different colours and different manipulation groups and replicates are visible separately.

We can see that at the start of the experiment in both treatment group there was quite large abundance of HAMBI-1287, approximately 25% (marked by dark green).

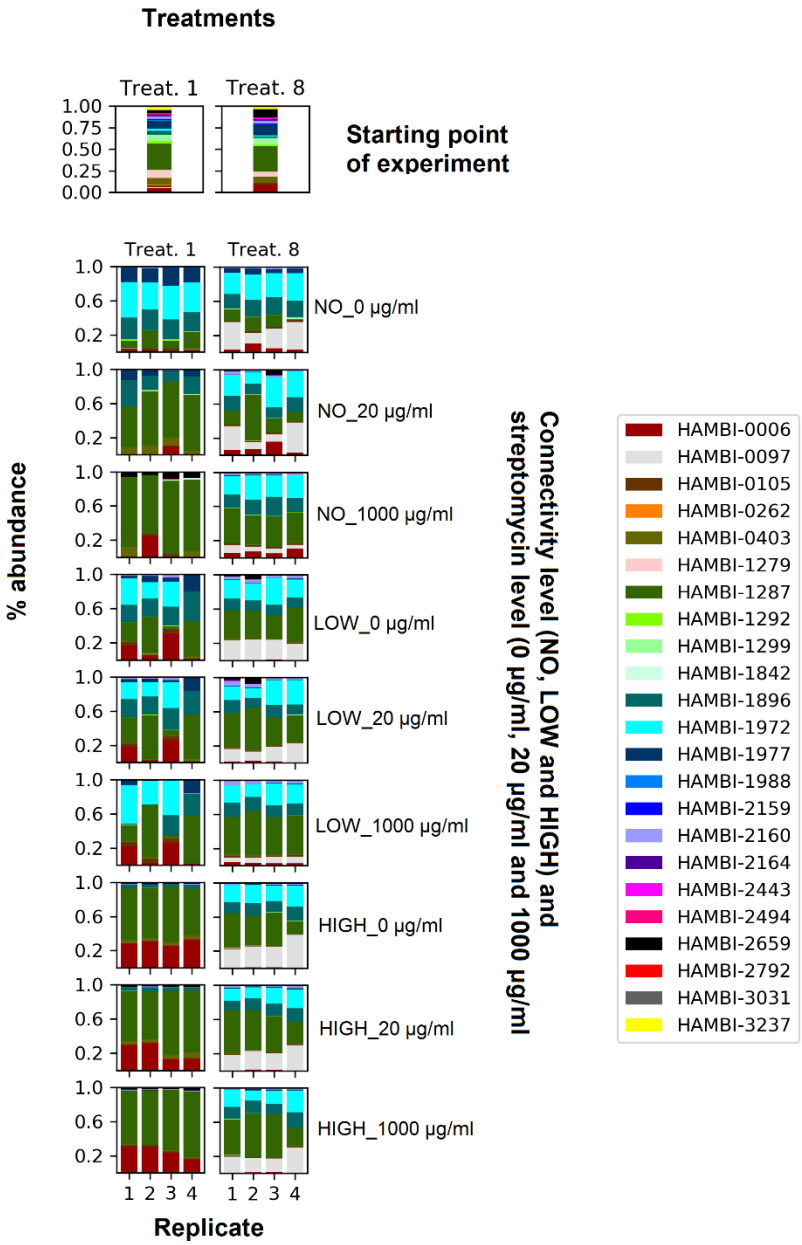


Fig. 10. Sequence result of treatment one and eight of the main experiment from the start and end of the experiment. Different HAMBI-strains are marked by different colour and there are plot using their percentual abundance. Different migration level (No, Low and High) and different concentration of streptomycin (no antibiotic: 0 µg/ml, low concentration: 20 µg/ml and high concentration: 1000 µg/ml) are visible separately.

There were significant differences between treatment groups (Table 3), but also between manipulating groups (Table 5.) and replicates (Fig 12.). Abundance of some of the HAMBI-strains in the end of the experiment were clearly low or lack at all. However, there were some HAMBI-strains (for example, HAMBI-1287 (dark green) that I early mention, HAMBI-1972 (turquoise) or HAMBI-0006 (burgundy)) that had significantly high abundance at the end of the experiment (Fig. 11).

Table 3. Permanova test results between treatment 1 and treatment 8. There is comparison always between same migration group and antibiotic concentration in different treatment. Rows that are marked by green are represent significantly differences between treatment groups (p-value is less than 0,05). P-values were calculated using Permanova analysis.

Permanova test between treatment 1 and treatment 8

Treatment 1	Treatment 8	p-value
NO_0	NO_0	0,023
NO_20	NO_20	0,026
NO_1000	NO_1000	0,018
LOW_0	LOW_0	0,033
LOW_20	LOW_20	0,063
LOW_1000	LOW_1000	0,243
HIGH_0	HIGH_0	0,024
HIGH_20	HIGH_20	0,029
HIGH_1000	HIGH_1000	0,039

There were significantly differences between treatment groups (Table 3). In many cases different HAMBI-strains did well for example in manipulation group where was no migration and no antibiotic (NO_0 µg/ml), in treatment one there was low level or completely abundance of HAMBI-0097 (light grey), but in treatment eight abundance of HAMBI-0097 was approximately 25%. Another example is in manipulation group where was high migration level and high antibiotic concentration level (HIGH_1000 µg/ml), in treatment one there was significant abundance of HAMBI-0006 (burgundy), but in treatment eight abundance of the same strain was really low or completely absent (Fig 11.).

There were visible differences between treatments, for example between different migration groups (Table 3.). There was also the absence of some HAMBI-strains inside the same treatment and same migration group. For example, in treatment one, inside no migration group in no antibiotic group (NO_0 µg/ml) there was quite high abundance of HAMBI-1972 (turquoise), but in others antibiotic levels of the same migration groups there was low or completely absent of abundance of the same strain (Fig 11.).

We can also see that replicates were also quite different from each other in many cases. Especially in low migration level of treatment one replicates were almost completely different (Fig 12.). However, logically replicates of the same metacommunity (similar number of replicate, in the same treatment, in the same migration group, but from the different antibiotic level) were quite similar (Fig 12.).

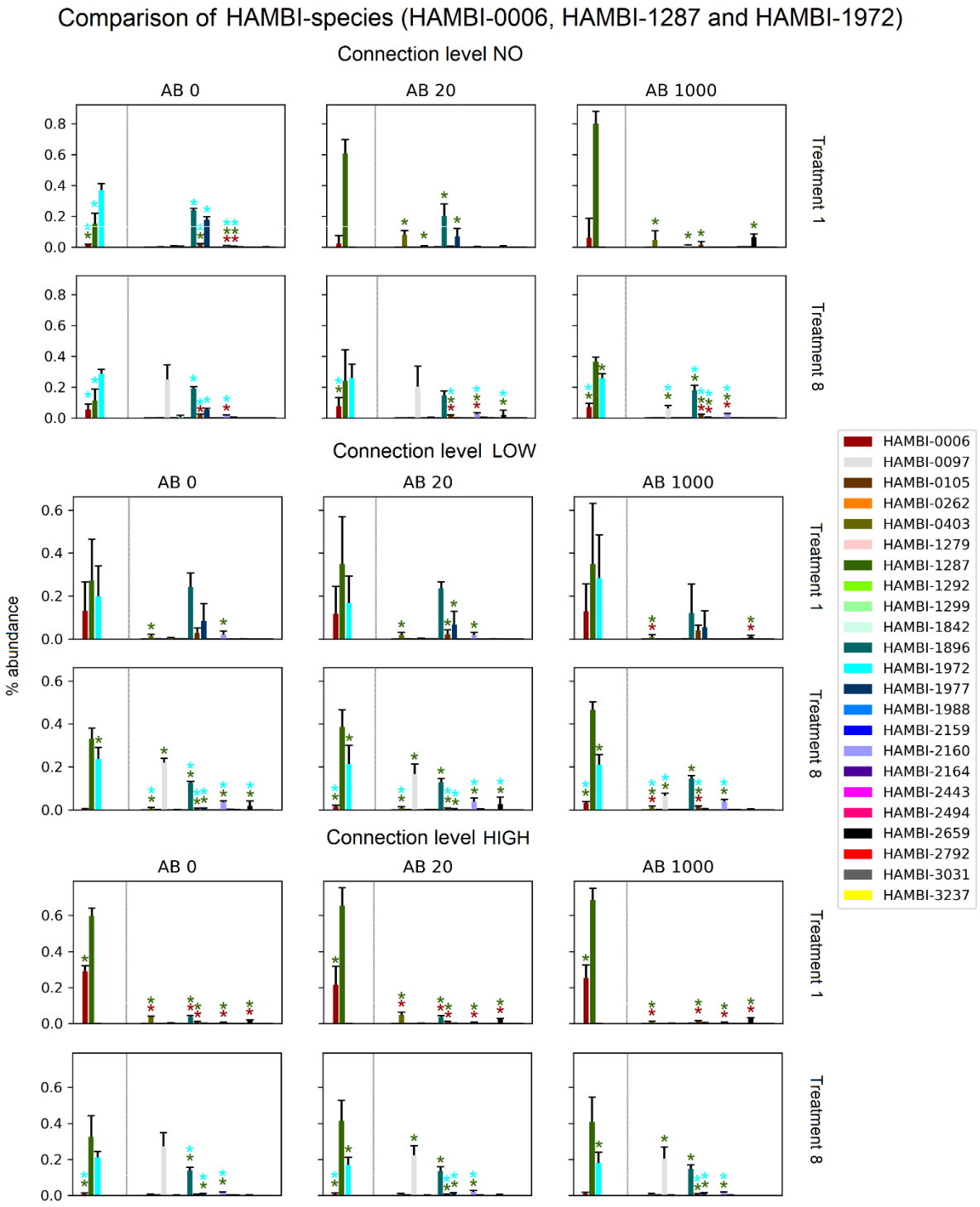


Fig 11. Comparison of HAMBI-1287 (dark green), HAMBI-1972 (turquoise) and HAMBI-0006 (burgundy) with other HAMBI-species and with each other in different treatment groups (1 and 8), connection levels (NO, LOW and HIGH) and streptomycin concentration (0 µg/ml, 20 µg/ml or 1000 µg/ml). Bars are representing percentual abundance of different HAMBI-species (mean was taken from replicates) and asterisks are marked when there were significant differences (p-value is less than 0,05) between species (colour of asterisk represent which species are compared with each other). Above the bars are also marked standard error. Actual p-values are visible in appendix 4. P-values were calculated using Mann-Whitney U-test.

3.2.3 Sequencing results (Shannon diversity)

The Shannon diversity index describes the diversity level of a community and is calculated as: $(H) = -\sum_{i=1}^{s} p_i \ln p_i$

Where p is the proportion (n/N) of individuals of one species i, observed (n_i) divided by the total number of individuals observed (N_i) summed over s total species. The higher the value of index, the higher the diversity is. In Table 4. I present sequencing results of main experiment using Shannon diversity. Shannon index was always calculated using median of four replicates of the particular treatment and manipulation group.

From the Shannon indexes we can see that diversity level was higher in all manipulation groups in treatment eight than in treatment one. In low migration level there was no significant differences between treatments, but in no and high migration level diversity was clearly higher in treatment eight than in treatment one.

When considering diversity level inside the treatment group between different manipulation groups, we notice that in low migration there was the highest diversity, and in high migration the lowest. In case of streptomycin concentrations, inside the same migration level and treatment, the lowest diversity was always in high concentration of antibiotic (1000 µg/ml of streptomycin) and the highest diversity was in no antibiotic level (0 µg/ml of streptomycin).

Table 4. Calculations of Shannon diversity of different manipulation groups of treatment one and treatment eight. There are nine different manipulation groups of both treatments: Three different migration levels (No, Low and High) with always three different streptomycin concentrations (no antibiotic: 0 µg/ml, low concentration: 20 µg/ml and high concentration: 1000 µg/ml). One Shannon index was always calculated using median of four different replicates of particular treatment and manipulation group.

Migration level _AB	Median of Shannon indexes in treatment 1	Median of Shannon indexes in treatment 8
NO_0	1,58	1,77
NO_20	1,12	1,69
NO_1000	0,60	1,66
LOW_0	1,63	1,63
LOW_20	1,52	1,63
LOW_1000	1,26	1,57
HIGH_0	1,09	1,53
HIGH_20	1,05	1,49
HIGH_1000	0,87	1,51

3.2.4 Sequencing results (Bray-Curtis dissimilarity)

Bray-Curtis dissimilarity helps to examine the dissimilarity between different treatment groups. In Fig. 12. there are represent dissimilarity between different replicates of the same treatment and the same manipulation groups (same migration level and same antibiotic concentration). Fig. 12. was done using multi-dimensional scaling (MDS) that based on Bray-Curtis indexes between replicates in same treatment, connectivity level and streptomycin level. Samples that are further apart are relatively more different in terms of species community composition than samples that are closer together. In Fig. 12. different replicates are marked by different green colors.

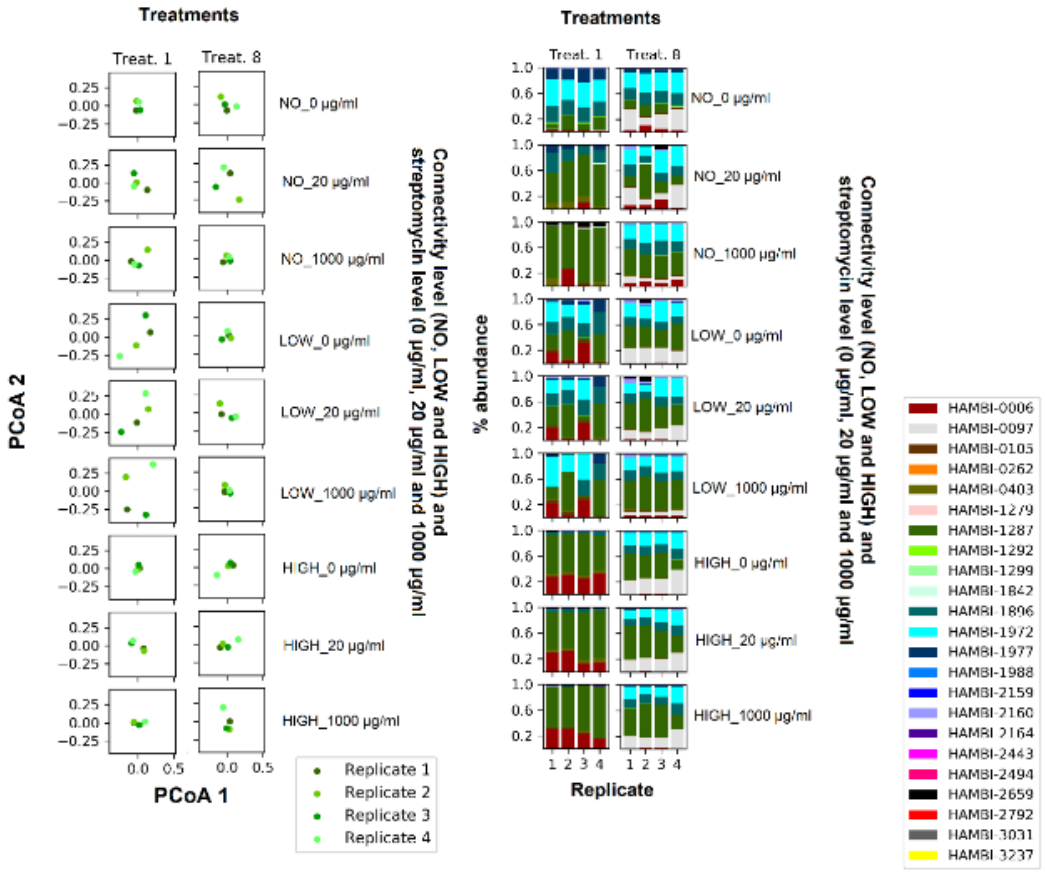


Fig. 12. Bray-Curtis dissimilarity between replicates of the same treatment and same manipulation level (same migration level and same streptomycin concentration) that was done using multi-dimensional scaling (MDS). Different replicates are marked by different green colours. In figure we can see also same information of sequence results as in Fig. 10.

Using Bray-Curtis dissimilarity we can see clearly how different replicates were in low migration in treatment one. When we compare that observation to the treatment eight in the same migration level, we can see that replicates were quite similar in case of treatment eight (Fig 12.). In treatment eight replicates are also quite similar in all manipulation groups, except in no migration and in low streptomycin concentration

level (NO_20 μg/ml), where replicates differ more from each other (Fig 12.). In high migration, replicates were the most similar in both treatment group (Fig 12.). In no migration level, replicates were a little bit more different from each other if comparing to the high migration (Fig 12.).

In Fig. 13. I present dissimilarity between different streptomycin concentrations of the same treatment and the same migration groups using Bray-Curtis dissimilarity same way as in Fig. 12. Different streptomycin concentrations are marked by different colours of the dots (green is 0 μg/ml, yellow is 20 μg/ml and red is 1000 μg/ml).

From the Fig. 13 and Table 5 we can cee that the highest differences between different antibiotic concentration levels were in no migration, especially in case of treatment one. The lowest differences were again in high migration (Table 5). In treatment eight antibiotic concentrations were more similar than in treatment one in all migration levels except the low migration, where level of similarity was more similar in treatment 1 (Table 5.).

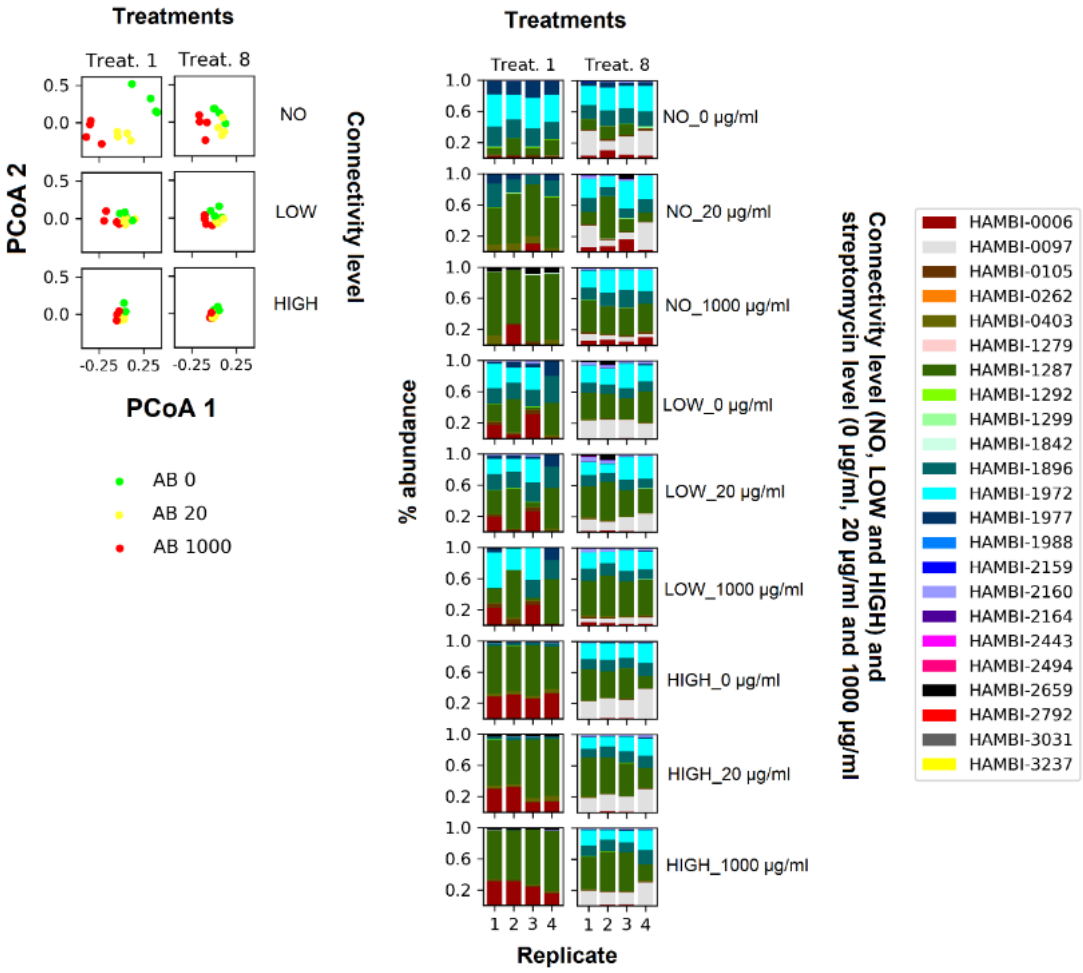


Fig. 13. Bray-Curtis dissimilarity between streptomycin concentrations of the same treatment and same migration level) that was done using multi-dimensional scaling (MDS). Different antibiotic concentrations are marked by different colors (green is 0 µg/ml, yellow is 20 µg/ml and red is 1000 µg/ml). In figure we can see also same information of sequence results as in Fig. 10.

Table 5. Permanova test results between different streptomycin concentrations (0 µg/ml, 20 µg/ml 1000 µg/ml) in treatment 1 and in treatment 8. There is comparison always in same migration group and treatment group between different antibiotic concentrations. Rows that are marked by green are represent significantly differences between antibiotic levels (p-value is less than 0,05). P-values were calculated using Permanova analysis.

Permanova test between different antibiotic concentrations

Connection level/treatment	Antibiotic level comparing (µg/ml)	p-value
NO_1	0 - 20	0,03
LOW_1	0 - 20	0,799
HIGH_1	0 - 20	0,292
NO_8	0 - 20	0,15
LOW_8	0 - 20	0,306
HIGH_8	0 - 20	0,302
NO_1	0 - 1000	0,034
LOW_1	0 - 1000	0,557
HIGH_1	0 - 1000	0,032
NO_8	0 - 1000	0,036
LOW_8	0 - 1000	0,025
HIGH_8	0 - 1000	0,34
NO_1	20 - 1000	0,032
LOW_1	20 - 1000	0,527
HIGH_1	20 - 1000	0,223
NO_8	20 - 1000	0,143
LOW_8	20 - 1000	0,15
HIGH_8	20 - 1000	0,9

3.2.5 Summary of results

When we examine Fig. 12. that show the dissimilarity between replicates, we can see that high and no connectivity treatments are quite consistent in community composition between independent replicates. With the low connectivity this is not the case, and we can see that the replicates are often quite different.

When we examine what happened when we increased streptomycin level in no migration shifts the community composition predictably across the 4 replicates. The evolution treatment changes the abundance of rarer strains, but generally the main trend is for HAMBI-1287 (that mark on dark green) to dominate with increasing streptomycin concentration in all evolution treatments (Fig. 11.) and we saw early (Fig. 8.) that same

HAMBI-strain had significantly high IC50 values in case of both ancestor and evolved strains.

Increased streptomycin level (from no to high) in high migration has little effect on differences between antibiotic concentrations (Fig. 13.) Also, increased streptomycin levels (from no to high) in high migration seemed to have little effect on the differences in the four independent replicates (quite similar with each other) (Fig. 13.). However, we can see differences on which species dominates during high immigration, especially when we examine treatment one. In case of high migration in treatment one there were just couple HAMBI-species (HAMBI-1287 and HAMBI-0006) that were clearly dominated and had high abundance (Fig. 10). And in case of treatment eight there were different HAMBI-species in high migration that dominated than in treatment one (if we do not take account of HAMBI-1287) that is also important. (Fig. 11.)

The most dynamic result was in the low migration. Here increasing streptomycin (from no to high) also has little effect. However, the four independent replicates were quite different from each other (for example in treatment 1) (Fig. 13.)

4 Discussion

During next sections I will compare my observations of the experiment to the results from the previous studies. I try to explain role of migration and changes that happened during manipulation with different concentration of antibiotic in my experiment. At the end I will summarize my conclusions of the experiment and what new I have discovered during master's thesis.

4.1 Role of migration

The aim of the thesis was investigating what effect migration have to the bacterial metacommunity and why it is so important. In my experiments I used streptomycin as a stress to the bacterial metacommunity and I also tried to demonstrate how different migration rates impact metacommunity composition.

Previous study has demonstrated that high immigration has effect on antibiotic adaptation of bacteria by increasing genetic variation in metacommunity (Perron et al., 2008). Furthermore, another study suggests that migration between bacterial populations, helps with adaptation in different environment condition and spreading new mutation that enable to avoid extinctions (Friman et al., 2015). However, even if migration can help with adaptation by increasing sizes of populations and spreading profitable mutations, at the same time migration could inhibit adaptation by producing mismatches between subpopulations that are locally adapted (Lenormand, 2002).

Cairns et al. (2020) had experiment where they used different antibiotic pulses and tested how immigration effect on metacommunity. Their discovery during study proved the importance of immigration and species-sorting for bacteria, when there is manipulation with antibiotics. However, they did not test different levels of migration, but only absence or presence. In the study they suggest that higher immigration levels could provoke the role of evolution and replicate communities deviate more during antibiotic pulse. They suggest that this kind of effect would occur because of species contain random adaptive mutations or another reason could be that there was difference between communities in timing of the occurrence of those mutations. (Cairns et al., 2020).

According to the results of my thesis, I can say that migration has significantly important role in dynamic in metacommunity as in previous studies. In case of low

migration level, we can see that immigration has clear effect on rates of diversity and antibiotic resistance of bacteria. However, if dispersal is too high (high migration level) it is leading to the situation where community composition of three antibiotic patches is homogenized and, in the process decreases community diversity. Previous studies did not report about situation where communities were homogenized, but I think that they did not use so high migration manipulation.

4.2 Antibiotic resistance in metacommunity

Previous antibiotic studies have demonstrated that the rate of bacterial adaptation during antibiotic treatment is dependent on how quick antibiotic has released and final concentration of antibiotic (Perron et al., 2008; Lindsey et al., 2013). De Visser et al. (1999) have shown that the size of population and through that, the supply of valuable mutations be caused by variation of antibiotic concentrations, because of the resistance development.

Previous studies have also shown that sizes of population have tendency to be lower in variable antibiotic environments versus stable, even if populations would have the same mean of antibiotic concentration. This kind of phenomena is happened, because unfavorable antibiotic conditions decrease population density above what can be compensated during suitable conditions. (Lawton, 1988). Also, variation of antibiotic's amplitude has effect on sizes of population even if the mean of antibiotic would be stable all the time. This is happening because variation of amplitude is anyway expose populations to ultimate conditions. (Menges, 1992). Furthermore, stage of troublesome conditions is commonly longer in autocorrelated environments. In this kind of environment conditions are more similar in time and apparently it will lead to population of lower sizes. (Gonzalez & Holt, 2002).

Friman et al. (2015) have shown in their study that migration improve growth of bacteria especially in the high antibiotic environment. Results pointed out that bacteria developed highest resistance to antibiotics in the environment with the high antibiotic level, and this kind of adaptation was possible only because of migration, even if during experiment bacteria were also manipulated with different predators and study their influence on bacterial adaptations in environment where are antibiotics. (Friman et al., 2015).

In their study they also surprisingly noticed that adaptation in the high antibiotic environment was led to the comparably lower growth cost than in case of low and intermediate antibiotic environment. Results has also shown that antibiotic's concentration increasing destabilized population dynamics of all species, in this case only migration was able to stabilize densities of bacterial population. Furthermore, bacteria evolved to grow better in the environments where there were antibiotics in all different concentration, even though at the beginning of experiment increasing antibiotic concentration increased the mortality of bacteria. All in all, results suggest that migration and mean antibiotic concentration had strongly effect on bacterial evolution and temporal antibiotic fluctuations had effect primarily on dynamics of species' population. (Friman et al., 2015).

There are multiple explanations for this kind of results. In the high antibiotic environment bacteria had better opportunity to get compensatory mutations, because in this kind of environment bacterial could reach relatively highest population densities and also significant variation of mutations. (Friman et al., 2015). Next important thing is that bacteria clearly have different degrees of pleiotropic growth cost and reason for that are antibiotic resistance mechanisms that could works differently (Proctor et al., 2006). Finally, previous study has demonstrated that pleiotropic costs caused by bacterial hypermutator phenotypes are relatively small, so probably bacteria favour hypermutator phenotypes and eventually it leads to the resistance in the absence of antibiotic selection (Perron et al., 2010). Other studies did interesting observation and showed that formation of biofilm can directly expand bacterial resistance to antibiotics (Hoffman et al., 2005; Jones et al., 2013).

According to my experiment, there were not so many differences between environments where were different concentration of antibiotics. In case of ancestor bacteria, I noticed that diversity was lower in high antibiotic level than in others, even if there was also migration. Reason for that could be that antibiotic level was so high that even the migration couldn't compensate so significant stress. However, my results also suggested that migration have significant role in antibiotic resistance.

4.3 Summary of discussion and future studies

Previous study and my own experiment have clearly shown that immigration rates have significantly strong effect on metacommunity composition. Immigration can change dynamic of populations and even help populations escape from extinction. However,

too much dispersal led to the increasing the relative cost of resistance and diversity of population turn lower.

My experiment with previous study also proved that antibiotics could develop spatially distributed environments where the harshness of the environment and thus also the strength of selections for antimicrobial resistance varies. Also, genetic modification had effects on community dynamics, and in some cases the effect was significant. Diversity of populations was quite different in some cases.

During my experiment different replicates gave an idea of the reproducibility of the findings and, also, they told how different the combinations of connectivity, evolution, and streptomycin are. So different combinations clearly have effect on outcome of experiment. Also, I demonstrated that immigration that is high enough, clearly change replicates from each other and in that kind of case different species start dominating in community. So basically, we can say that migration have significant role also in the population dynamic. However, it also have to be some greater role for some random processes in that kind of case and I think there is needed more studies to understand more about this kind of phenomena.

It would be interesting to study more closely what changes happen in the genetic level during antibiotic resistance and migration. How genome of bacteria changes during manipulation with antibiotic if bacteria have opportunity to get antibiotic resistance and is there some characteristic that help bacteria to develop antibiotic resistance. For example, previous studies showed that hypermutator phenotypes are important in this case (Perron et al., 2010). I am interesting that are there some more different phenotypes that have significant advantages already at the start.

4.4 Sources of error

During experiment I got significantly high optical density measurements. We compared them to our previous experiments and noticed that there were too high especially in case with highest antibiotic concentration. However, I assume that we can explain rapid spikes by some internal dynamics and transfer to the fresh medium.

All transfers during experiment were done using PlateMaster. Unfortunately, sometimes it did not work as it should be. For example, it leaved some tips empty or took too much liquid. Usually, I noticed mistakes but of course I can’t guarantee that during whole experiment there wasn’t any mistake. However, results seemed to be quite logical and

there were not any surprising differences between them, so I assume that during experiment there were not happened any meaningful mistakes.

5 Acknowledgements

I would like to warmly thank all people that helped with this thesis. Of course, firstly my supervisors Teppo Hiltunen and Shane Hogle that helped me out through all the process that sometimes was not easy. Secondly, I want to thank my co-workers: Ida-Marija Hyvönen, Tiia Korpi, Inga-Katariina Aapalampi and Olli Pitkänen, that helped me in the lab and support me. Finally, I want to thank my husband, parents and friends that gave me so much mental support and forces to continue whenever it was difficult.

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7 Appendices

In this section are collected all appendices of master's thesis. Appendices contain precise protocols of experiments that I was done before the main experiment.

7.1 Appendix 1: Protocol of streptomycin selection experiment part 1

The aim of the first part of the streptomycin selection experiment is growth the ancestor HAMBI-strains (23 different strains) and evolved HAMBI-strains populations (23 different strains) together in different streptomycin concentrations. At the end I get evolved populations that have as much as possible genetic variation.

Working stages:

- All evolved populations (23 HAMBI-strains) that came from previous selection experiment (from Helsinki) are grow first 96 hours in separate microcosmos bottles in temperature of 28ºC (shaking 50 r.p.m.), using R2A-culture medium. Pipette 50 µl from freezing tube of each evolved strains to the microcosmos bottles where are 6 ml of R2A-culture medium. Same procedure is done to the ancestor HAMBI-strains.

- After grow, evolved strains and ancestor strains are mix (always same strains of both evolved and ancestor in the same amount).

- Use mix of each strain and transfer 20 µl to the deep 96-plates where are 12 different streptomycin concentrations (0, 1, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 and 5000 µg/ml). Every strain is grown in 2 X 12 wells (576 wells of all and six 96-plates). Grow in temperature of 28ºC (shaking 1000 r.p.m.)

- Transfer 20µl every 48 hours to the new 96-plates where are same concentrations of antibiotic as in original plate. If there is no growth, transfer from lower concentration to higher (try couple times, but if it is not working, empty wells could be at the end).

- Take 100 µl from every 24 wells of the same strain in the falcon and mix using vortex.

- Freeze with glycerol, six Eppendorf-tubes (2 ml).

7.2 Appendix 2: Protocol of streptomycin selection experiment part 2

The aim of the second part of streptomycin selection experiment is create and pick up independent evolved clones.

Working stages:

- Using evolved populations from first part of experiment, pipette always 100 µl to the 9,9 ml of M9-medium (use 15 ml falcons). Do necessary dilution series 1:5 (1 ml to the 4 ml of M9-medium and so on.) to all evolved populations (23 strains at total). Vortex always between transfer to the new falcon tube.
- Transfer 100 µl to the R2A-growing plate and grow 96 hours in temperature of 28ºC. Try different dilutions.
- Pick 16 random clones from each evolved population and transfer clones separately to the 96-deep plates where is R2A-culture medium. Grow 96 hours in temperature of 28ºC (shaking 1000 r.p.m.).
- Freeze evolved clones with glycerol in 96-plates.

7.3 Appendix 3: Protocol of MIC-test

The aim of the MIC-test is clarified IC50 values for each strain. I use evolved clones (that were done in streptomycin selection experiment part 2) from strains 6, 105, 1287,1972, 1977 and 1896 and the same ancestor HAMBI-strains. All in all, two plates (one of evolved clones and one of ancestor)

Working stages:

- Transfer 10 µl from frozen plates to the deep 96-plates where is 600 µl R2A-culture medium per well. Grow 48 hours in temperature of 28ºC (shaking 1000 r.p.m.).
- Do 1:100 dilution using 96-deep plates where is M9-medium. Mix wells using PlateMaster.
- Prepare streptomycin plates (13 different concentrations on antibiotic in R2A-culture medium)

- Pipette dilution 1:100 of both plates (ancestor and evolved clones) so that you have ancestor dilution in 13 different streptomycin concentrations and evolved clones' dilution, so that you have four replicates of every 13 different streptomycin concentrations.

- Grow 48 hours in temperature of 28ºC (shaking 1000 r.p.m.), and measure optical density using spectrophotometer.

13 different concentrations of streptomycin:

0 µg/ml
1 µg/ml
2 µg/ml
4 µg/ml
8 µg/ml
16 µg/ml
32 µg/ml
64 µg/ml
128 µg/ml
256 µg/ml
512 µg/ml
1024 µg/ml
2048 µg/

7.4 Appendix 4: p-value of HAMBI-species comparison

Table 4. p-value of comparison HAMBI-0006, HAMBI-1287 and HAMBI-1972 with other HAMBI-species. P-values were calculated using Mann-Whitney U-test.

Comparison of HAMBI-species (HAMBI-0006, HAMBI-1287 and HAMBI-1972)

Connection level/Streptomycin level/Treatment group

NO_0, Treat 1

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-1896	HAMBI-0105	HAMBI-1977	HAMBI-2160	HAMBI-2164
HAMBI-0006		1	1	1	0,971	1	0,014	0,014
HAMBI-1287	0,014		1	1	0,014	0,657	0,014	0,014
HAMBI-1972	0,014	0,014		0,014	0,014	0,014	0,014	0,014

NO_0, Treat 8

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0097	HAMBI-1896	HAMBI-0105	HAMBI-1977	HAMBI-2160
HAMBI-0006		0,9	1	1	1	0,014	0,829	0,014
HAMBI-1287	0,171		1	0,943	1	0,171	0,171	0,171
HAMBI-1972	0,014	0,014		0,557	0,014	0,014	0,014	0,014

NO_20, Treat 1

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0403	HAMBI-1292	HAMBI-1896	HAMBI-1977
HAMBI-0006							
HAMBI-1287				0,014	0,014	0,014	0,014
HAMBI-1972							

NO_20, Treat 8

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0097	HAMBI-1896	HAMBI-0105	HAMBI-2160	HAMBI-2659
HAMBI-0006		0,986	0,986	0,971	0,971	0,014	0,029	0,057
HAMBI-1287	0,029		0,757	0,343	0,557	0,014	0,014	0,014
HAMBI-1972	0,029	0,343		0,243	0,057	0,014	0,014	0,014

NO_1000, Treat 1

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0403	HAMBI-1842	HAMBI-0105	HAMBI-2659
HAMBI-0006							
HAMBI-1287				0,015	0,014	0,015	0,014
HAMBI-1972							

NO_1000, Treat 8

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0097	HAMBI-1896	HAMBI-0105	HAMBI-1977	HAMBI-2160
HAMBI-0006		1	1	0,243	1	0,014	0,014	0,014
HAMBI-1287	0,014		0,014	0,014	0,014	0,014	0,014	0,014
HAMBI-1972	0,014	1		0,014	0,029	0,014	0,014	0,014

LOW_0, Treat 1

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0403	HAMBI-1896	HAMBI-0105	HAMBI-1977	HAMBI-2160
HAMBI-0006		0,9	0,757	0,057	0,943	0,171	0,557	0,1
HAMBI-1287	0,171		0,243	0,029	0,243	0,057	0,1	0,029
HAMBI-1972	0,343	0,829		0,171	0,757	0,171	0,243	0,171

LOW_0, Treat 8

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0403	HAMBI-0097	HAMBI-1896	HAMBI-0105	HAMBI-1977	HAMBI-2160	HAMBI-2659
HAMBI-0006										
HAMBI-1287			0,029	0,014	0,014	0,014	0,014	0,014	0,014	0,014
HAMBI-1972		0,986		0,014	0,657	0,014	0,014	0,014	0,014	0,014

LOW_20, Treat 1

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0403	HAMBI-1896	HAMBI-0105	HAMBI-1977	HAMBI-2160
HAMBI-0006		0,971	0,757	0,243	0,943	0,343	0,557	0,443
HAMBI-1287	0,057		0,171	0,014	0,171	0,014	0,029	0,014
HAMBI-1972	0,343	0,9		0,171	0,9	0,171	0,171	0,171

LOW_20, Treat 8

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0403	HAMBI-0097	HAMBI-1896	HAMBI-0105	HAMBI-2160	HAMBI-2659
HAMBI-0006		1	1	0,057	1	1	0,1	1	0,557
HAMBI-1287	0,014		0,014	0,014	0,014	0,014	0,014	0,014	0,014
HAMBI-1972	0,014	1		0,014	0,243	0,1	0,014	0,014	0,014

LOW_1000, Treat 1

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0403	HAMBI-1896	HAMBI-0105	HAMBI-1977	HAMBI-2659
HAMBI-0006		0,9	0,9	0,029	0,343	0,343	0,171	0,029
HAMBI-1287	0,171		0,343	0,014	0,171	0,1	0,057	0,014
HAMBI-1972	0,171	0,757		0,1	0,1	0,1	0,1	0,1

LOW_1000, Treat 8

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0403	HAMBI-0097	HAMBI-1896	HAMBI-0105	HAMBI-2160
HAMBI-0006		1	1	0,014	1	1	0,014	0,9
HAMBI-1287	0,014		0,014	0,014	0,014	0,014	0,014	0,014
HAMBI-1972	0,014	1		0,014	0,014	0,057	0,014	0,014

HIGH_0, Treat 1

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0403	HAMBI-1896	HAMBI-0105	HAMBI-2160	HAMBI-2659
HAMBI-0006		1		0,014	0,014	0,014	0,014	0,014
HAMBI-1287	0,014			0,014	0,014	0,014	0,014	0,014
HAMBI-1972								

HIGH_0, Treat 8

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0097	HAMBI-1896	HAMBI-1977	HAMBI-2160
HAMBI-0006		1	1	1	1	0,557	1
HAMBI-1287	0,014		0,171	0,243	0,029	0,014	0,014
HAMBI-1972	0,014	0,9		0,943	0,014	0,014	0,014

HIGH_20, Treat 1

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0403	HAMBI-1896	HAMBI-0105	HAMBI-2160	HAMBI-2659
HAMBI-0006		1		0,014	0,014	0,014	0,014	0,014
HAMBI-1287	0,014			0,014	0,014	0,014	0,014	0,014
HAMBI-1972								

HIGH_20, Treat 8

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0097	HAMBI-1896	HAMBI-0105	HAMBI-1977	HAMBI-2160
HAMBI-0006		1	1	1	1	0,557	0,829	0,986
HAMBI-1287	0,014		0,014	0,029	0,014	0,014	0,014	0,014
HAMBI-1972	0,014	1		0,9	0,243	0,014	0,014	0,014

HIGH_1000, Treat 1

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0403	HAMBI-0105	HAMBI-2160	HAMBI-2659
HAMBI-0006		1		0,014	0,014	0,014	0,014
HAMBI-1287	0,014			0,014	0,014	0,014	0,014
HAMBI-1972							

HIGH_1000, Treat 8

	HAMBI-0006	HAMBI-1287	HAMBI-1972	HAMBI-0097	HAMBI-1896	HAMBI-0105	HAMBI-1977	HAMBI-2160
HAMBI-0006		1	1	1	1	0,443	0,657	0,943
HAMBI-1287	0,014		0,029	0,029	0,014	0,014	0,014	0,014
HAMBI-1972	0,014	0,986		0,757	0,243	0,014	0,014	0,014