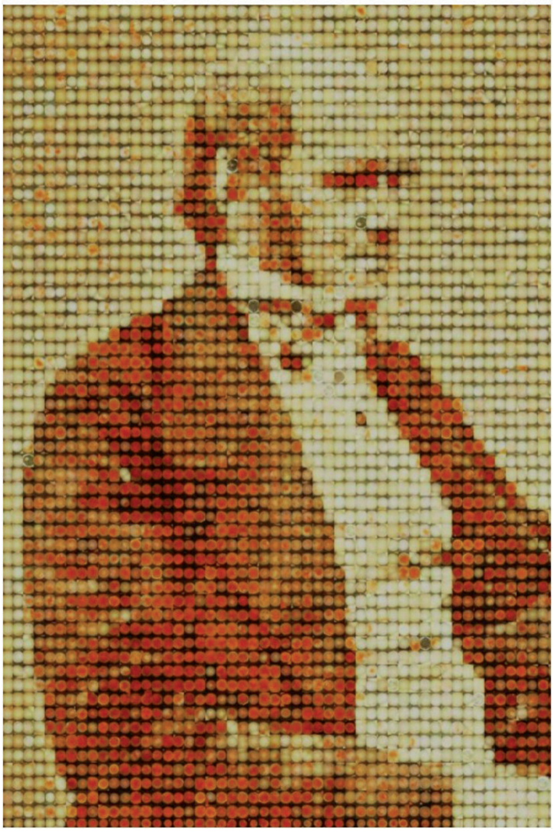
JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
311

Kati Saarinen

The Evolution of Temperature Tolerance and Invasiveness in a Fluctuating Thermal Environment



JYVÄSKYLÄN YLIOPISTO

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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston vanhassa juhlasalissa S212,
tammikuun 8. päivänä 2016 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Science of the University of Jyväskylä,
in building Seminarium, auditorium S212, on January 8, 2016 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2016

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Pekka Olsbo, Ville Korkiakangas
Publishing Unit, University Library of Jyväskylä

Cover illustration by Tarmo Ketola (Charles Darwin,
picture assembled from *Serratia marcescens* bacterial colonies).

URN:ISBN:978-951-39-6459-7
ISBN 978-951-39-6459-7 (PDF)

ISBN 978-951-39-6458-0 (nid.)
ISSN 1456-9701

Jyväskylä University Printing House, Jyväskylä 2016

Vaihtelu virkistää.

-Suomalainen sananlasku

ABSTRACT

Saarinen, Kati
The evolution of temperature tolerance and invasiveness in a fluctuating thermal environment
Jyväskylä: University of Jyväskylä, 2016, 34 p.
(Jyväskylä Studies in Biological and Environmental Science
ISSN 1456-9701; 311)
ISBN 978-951-39-6458-0 (nid.)
ISBN 978-951-39-6459-7 (PDF)
Yhteenveto: Lämpötilatoleranssin ja invaasiokyvyn evoluutio lämpötilaltaan vaihtelevassa ympäristössä

The consequences of the climate change on species are still uncertain, despite of intensive research. Currently, rising temperature is not the only concern, since the climate change scenarios also predict increases in the amount of disturbances, such as storms, floods, and thermal fluctuations. Disturbances have also been shown to affect species' evolution, for example by selecting for traits that are advantageous in fluctuating environments but are also facilitating invasiveness. In this thesis, I study the consequences of evolving in a fluctuating thermal environment by utilizing bacterial microcosms. First I tested the effects of fluctuating vs. constant temperature on the evolution of temperature tolerance, both in one-species setting (I) and with multiple species (II). Then I studied how pre-adaptations to fluctuating vs. constant temperature, together with environmental conditions, affect invasion success (III). Additionally I also tested the effects of propagule pressure and genetic diversity on invasion success (IV). The results of the studies I and II show that constant and fluctuating thermal environments require different kinds of adaptations, as measurements taken in constant environments (traditional tolerance curve approach) did not reveal the adaptations to fluctuating environment. In study III, I found that fluctuations during invasion as well as pre-adaptations to fluctuating environment increased the invasion success of the invader. However, in study IV, I did not find any clear evidence that high propagule pressure or high genetic variance would have increased invasion success. To conclude, the results in this thesis demonstrate that adaptations that aid species to cope with disturbed environments can also lead to increased invasiveness. Furthermore, these adaptations might not be detectable using traditional measurement methods, which could, in the worst case, lead to incorrect conclusions and management actions, when considering climate-change driven extinction risks, or the effects of invasive species on natural environments.

Keywords: Adaptation; bacteria; disturbed environment; experimental evolution; invasion; temperature fluctuation; tolerance curve.

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LIST OF ORIGINAL PUBLICATIONS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I – IV.

I Ketola, T., Saarinen, K. 2015. Experimental evolution in fluctuating environments: tolerance measurements at constant temperatures incorrectly predict the ability to tolerate fluctuating temperatures. *Journal of Evolutionary Biology* 28: 800-806.

II Saarinen, K., Laakso, J., Lindström, L., Ketola, T. Constant and fluctuating thermal environments require different adaptations: evolution experiments with nine bacterial species. Submitted manuscript.

III Saarinen, K., Lindström, L., Ketola, T. Double-trouble with the climate change? Environmental fluctuations increase invasion success and select for more invasive genotypes. Manuscript.

IV Saarinen, K., Lindström, L., Ketola, T. Experimental invasions in bacterial microcosms: no effect of propagule pressure or genotypic diversity on invasion success. Manuscript.

The contributions of the authors to the original papers.

	I	**II**	**III**	**IV**
Original idea	TK	TK, KS	KS, TK	KS, TK
Experimental design	TK	KS, TK	KS, TK, LL	KS, TK, LL
Data collection	KS, TK	KS, TK	KS	KS
Data analysis	TK	KS, TK	KS	KS
Writing	TK, KS	KS, TK, LL, JL	KS, TK, LL	KS, TK, LL

KS = Kati Saarinen, TK = Tarmo Ketola, LL = Leena Lindström, JL = Jouni Laakso

1 INTRODUCTION

1.1 Evolutionary adaptation: mutations, variation and natural selection

The unifying theme of my thesis is evolutionary adaptation and its consequences – how species adapt to their environment through natural selection and how pre-adaptations enable successful function also in novel environments. In this thesis, the main focus is on thermal adaptation and especially on adapting to rapid, within generation fluctuating thermal environment.

Mutations in the genes of organisms provide the raw material that enables individual variation, and variation enables natural selection: only those individuals that adapt to their environment and succeed to reproduce pass their genes forward. Mutations arise spontaneously and inevitably in all organisms. Most mutations are neutral in the sense that they do not change the function (protein synthesis) of genes, and a proportion of mutations are deleterious, because they prevent or radically alter the normal functioning of genes. However, rare beneficial mutations can quickly spread in populations, if a mutation induces such a change in the gene functioning that it causes a strong fitness advantage for the individuals who carry the mutation (Bell 2015).

In clonal organisms, like bacteria, mutations play a bigger role in creating variation within populations than in sexual organisms, in which recombination of alleles also creates variation (Bell 2015). However, evolutionary adaptation in bacteria does not exclusively depend on the rate of occurring mutations, since bacteria can also exchange genetic material via horizontal gene transfer (Soucy *et al.* 2015). It has also been shown that evolutionary adaptation in bacteria can be accelerated in stressful (e.g. variable/fluctuating) environments, when natural selection starts to favour the so called mutator alleles that increase random mutation rates. In constant, non-stressful environments mutators are usually selected against, since bacteria are already well adapted to their environment (Galhardo *et al.* 2007).

1.2 Temperature, thermal adaptation and tolerance curves

Temperature is among the major physical environmental variables determining species richness and distribution over all taxa. Organisms can adapt to different thermal environments either by regulating and maintaining their body temperature constant via different cellular and metabolic mechanisms, or by changing the body temperature along the environmental temperature. In the latter case, mobile organisms can in a sense regulate their body/cell temperature by seeking suitable thermal habitats (Hochachka and Somero 2002). Every organism has a species-specific temperature tolerance range, from a minimum temperature which is the lowest possible temperature allowing growth, to a maximum temperature, after which growth stops (because cellular proteins start to unfold and become damaged). In the between of the extremes (closer to the maximum than the minimum) is an optimum temperature at which growth is fastest (Madigan *et al.* 2000).

The thermal tolerances of individual species are usually presented as tolerance curves, which consist of measurements of performance/tolerance taken in several constant temperatures (Huey and Kingsolver 1989, 1993). Species that are generalists in their thermal tolerance have elevated and broad tolerance curves (high overall tolerance and also relatively high tolerance of extreme temperatures), whereas specialists have narrow tolerance curves where high tolerance is visible only at optimum temperatures. Fluctuating temperature should select for generalist genotypes with elevated and broad tolerance (Levins 1968). However, it is not completely understood whether selection in fluctuation actually targets the individuals that cope well with all the constant temperatures of the selection range, or does fluctuation favour individuals that have other means of adapting to the fluctuating temperature. These other kinds of adaptations to fluctuation could be for example increased reversible phenotypic plasticity (Hughes *et al.* 2007, Bennett and Hughes 2009), for example in expressing heat shock proteins (Sorensen *et al.* 2003, Ketola *et al.* 2004) or reversible plasticity in maximizing reproduction during short periods of optimum conditions (Gilchrist 1995, New *et al.* 2014). Tolerance curves are very widely used in evaluating species performance, for example in breeding genetics (Falconer and Mackay 1996), in assessing the impacts of climate change on species (Deutsch *et al.* 2008, Huey *et al.* 2012), or in predicting the potential emergence of invasive species (Lee and Gelembiuk 2008). Thus, if tolerance curves are not necessarily reliable tools to predict species performance in fluctuating environments, there is a danger that the possible false predictions can lead to erroneous management actions.

1.3 Climate change and disturbed environments

The latest studies on global climate change predict ever increasing mean temperatures and show current alarming news about the melting ice covers of the North Pole and Antarctica. However, the change in the mean temperature is not the only worrying piece of news, as the studies also predict increases in the number and magnitude of extreme climatic events. Depending on the geographical location, different kinds of disturbances will become more and more common: peaks of extremely low and high temperatures, thermal fluctuations, heatwaves, droughts, storms and floods (Stocker *et al.* 2014). This will inevitably lead to immense changes in ecosystems all around the world, forcing species to find new suitable habitat areas or to adapt to the new conditions, to avoid extinction. Unfortunately the rate of extinctions will probably become even faster in the near future (Barnosky *et al.* 2011), since many species cannot change their location or evolve fast enough to keep pace with the global change.

Besides, human influence has already caused significant amount of disturbance in natural environments (habitat destruction, pollution, fragmentation etc.), and disturbed environments are also more prone to the spread of invasive species (Davis 2009). Based on the above-mentioned facts and scenarios, it is not surprising that the global change has been hypothesized to promote the range expansions of invasive species (Clements and Ditommaso 2011). Hence, to be able to predict the extent of changes in natural environments in the near future, currently it would be extremely important to better understand how organisms adapt to rapid changes. Furthermore, it would be essential to recognize what kind of consequences those adaptations have, regarding species' competitive and invasive abilities.

1.4 Invasions

1.4.1 Invasive species

An invasive species can be defined as a human-introduced organism that has established and spread outside its native range, and has a negative impact on the native species and the ecosystems of the new area. Nevertheless, most invasive species are not invasive everywhere, but instead only certain populations become invasive under suitable conditions (Colautti and MacIsaac 2004, Davis 2009). Invasions of alien species are, after habitat destruction and fragmentation, the second most important cause of species extinctions. Invaders can cause local extinctions of the native species either through direct competition and predation, or via pathogens they carry with them (Cox 2004). In addition to the adverse effects on natural environments, invasive species also have a huge negative impact on agriculture, causing enormous economic costs worldwide (Pimentel *et al.* 2001). Nowadays invasive species are able to spread to new locations more easily than ever, because of human influence. In addition to intentional species introductions and transportation of living organisms, also the unintentional spread of invasive species is on the increase. People travel and transport all kinds of materials all around the world more than ever. Within the huge amounts of cargo in trains, airplanes and ships, and within ship ballast waters, invasive species can "hitch-hike" to new locations they would never reach without the aid of human-invented means of transportation (Cox 2004).

1.4.2 Evolutionary history of the invader and the receiving community

Evolving in a disturbed environment can select for traits that are associated with increased competitive ability, such as fast growth, plasticity and persistence (Lee and Gelembiuk 2008), especially if the continual disturbances/fluctuations occur within generations (Levins 1968, Turelli and Barton 2004, Meyers *et al.* 2005, Lee and Gelembiuk 2008). These kinds of adaptations enable species to cope with the changing environment and to avoid extinction. However, selection in disturbance can also lead to the emergence of new invasive species, as the same adaptations that help species to survive, can also be invasive traits. The invasiveness does not usually occur in the native area of the invaders, but problems appear if the invaders spread to new locations, where they can occupy empty niches, and in the worst case take over and cause extinctions within the native communities of the new area. However, the role of pre-adaptations (caused by evolutionary history) in invasions is still a lot less studied subject than the on-going rapid evolution of invasive species during/after establishment. Also, whether the evolutionary history of the species in the invaded community influences invasibility is especially poorly understood.

1.4.3 Genetic variability of the invader

In genetically diverse populations natural selection has a big pool of variants to choose from, and therefore variable populations adapt to new environments more easily than populations with only few genetic variants. Genetically diverse populations also have a good chance of having pre-adapted genotypes. This is why genetically variable populations could also have a high invasion success, which has been a common finding in experimental studies (Forsman 2014). Nevertheless, invasive populations are usually not as genetically diverse as their native populations (Dlugosch and Parker 2008), and in the new areas more variability is gained from other, previously allopatric populations (Kolbe *et al.* 2004, Roman and Darling 2007). It has also been shown that even clonal populations can be highly invasive (Burrell *et al.* 2015). The amount of genetic variance in itself is not necessarily the only factor inducing high invasiveness, but the causes can be more complex, depending on the detailed adaptations of different variants (Dlugosch et al 2015).

1.4.4 Propagule pressure of the invader

Propagule pressure as a term is used in two meanings (and often simultaneously): it can mean the number of individuals or "propagules" (populations or smaller groups) in one dispersal, or the number of repeated dispersals during invasion (Lockwood *et al.* 2005). Usually the invasion success becomes the higher, the larger the propagule pressure is, and this has been a common finding also in experimental studies (Memmott et al 2005, Colautti *et al.* 2006, Davis 2009, Lockwood *et al.* 2009, Simberloff 2009, Blackburn et al 2011, Wittmann *et al.* 2014, but see Gilbert and Lechowicz 2005, Chytrý et al 2008), during every stage of invasion from dispersal to spread (Blackburn *et al.* 2015). The genetic variance of the invading population is usually positively correlated to the magnitude of the propagule pressure, and for that reason it can be difficult to disentangle the relative impacts of genetic variability vs. propagule pressure on invasion success, without a manipulative approach (Bock et al 2015).

1.4.5 The role of environment in invasions

In principle all environments can be invaded, but there are differences in the level of invasibility of different environments. Most importantly, disturbed and heterogeneous environments have been shown to be more vulnerable to invasions than constant and uniform environments (Burke and Grime 1996, Davis *et al.* 2000, Davies *et al.* 2005, Melbourne *et al.* 2007, Liu *et al.* 2012). The significance of resource fluctuation promoting invasions is well known (Davis *et al.* 2000, Li and Stevens 2012). Fluctuation/disturbance usually leads to the death of individuals or whole populations in a community, which releases free niche space and resources for the potential invaders (Davis *et al.* 2000). It is also possible that fluctuating resources cause fluctuations in competition among species, which prevents competitive exclusion (Davis 2009). Despite the vast research on the effects of resource fluctuations on invasions, studies on the effects of other kinds of fluctuations in the physical environment on invasions are scarce (Kreyling *et al.* 2008).

1.5 Aims of the thesis

The ultimate goals of my thesis are to experimentally test i) does evolving in a constant vs. fluctuating environment induce differences in temperature tolerance, ii) does evolving in a fluctuating environment improve the invasiveness of the invader and cause changes in the invasibility of the community, and iii) what is the relative importance of genetic variability vs. propagule pressure in invasions. Below are the titles of original publications and manuscripts, and the more detailed study questions within each paper.

I Experimental evolution in fluctuating environments: tolerance measurements at constant temperatures incorrectly predict the ability to tolerate fluctuating temperatures.

Do fluctuating environments select for changes in tolerance curves measured in constant environments?

II Constant and fluctuating thermal environments require different adaptations: evolution experiments with nine bacterial species.

a. Does evolution under fluctuating temperature lead to broader or more elevated tolerance curves?
b. Do certain temperatures of the selection range exert more selection on tolerance curves than other temperatures?
c. Do measurements performed in fluctuating temperature add more to our understanding of adaptation to fluctuating temperature?

III Double-trouble with the climate change? Environmental fluctuations increased invasion success as well as selected for more invasive genotypes.

a. Are fluctuating environments more prone to invasions than constant environments?
b. Do invaders that have evolved in a fluctuating environment have higher invasion success than invaders evolved in a constant environment?
c. Are community species that have evolved in a fluctuating environment more resistant to invasions than the ones evolved in a constant environment?

IV Experimental invasions in bacterial microcosms: no effect of propagule pressure or genotypic diversity on invasion success.

a. Does the high propagule pressure of the invader lead to high invasion success?
b. Does the high genetic variance of the invader lead to high invasion success?
c. Is highest invasion success achieved, when the invader has a high genetic variance and a large propagule?

2 METHODS

2.1 Experimental evolution

Studying ongoing evolution of higher organisms in natural populations, or experimentally, is difficult for obvious reasons. In natural environments it is hard to manipulate the system in a way that one would really know which environmental factors are affecting the evolution. In laboratory, greenhouse etc. it is possible to manipulate the environmental conditions, but there are still many problems. For example, the time span between generations is long and finding out the effects of evolutionary history (even the near past) of the study organisms can be impossible. For these reasons, many experimental evolution studies utilise well-studied organisms that have short generation times, such as certain insect and plant species, and especially micro-organisms: protozoa, fungi and bacteria. When studying selection and adaptation in long-term evolution experiments (thousands of generations), the common bacterial species, like *Escherichia coli*, are the most convenient choice. Microcosm experiments, especially serial transfer experiments, are relatively easy and inexpensive to conduct. They also enable very good control over the environment and high replication number of individuals and populations. Furthermore, they provide the possibility of taking into account the effects of the past evolutionary history on the ongoing evolution (Warren *et al.* 2006, Kassen 2014).

2.2 Study species

The study species (Table 1) were originally chosen for study II, based on their abilities to grow well in the same medium and to tolerate the rapidly fluctuating temperature range (20 - 30 - 40 °C) of the experiment. The measured/estimated optimum temperatures of each species and the phylogeny of the study species can be found in the supporting information of study II (II, Fig. S1 and S2).

TABLE 1 The study species, references of origin where the ancestor strains of the bacteria were obtained from, and the studies of this thesis where each species was utilized. ATCC is the American Type Culture Collection.

Species	**Origin/Reference**	**Study**
Enterobacter aerogenes	ATCC® 13048™	II, III, IV
Leclercia adecarboxylata	ATCC® 23216™	II, III, IV
Serratia marcescens ssp. marcescens	ATCC® 13880™	II, III, IV
Serratia marcescens DB11 strain	Flyg *et al.* 1980	I, II
Escherichia coli	ATCC® 11775™	II
Pseudomonas putida	ATCC® 12633™	II
Pseudomonas fluorescens	ATCC® 13525™	II
Pseudomonas chlororaphis	ATCC® 17418™	II, III, IV
Novosphingobium capsulatum	ATCC® 14666™	II

2.3 Evolution of thermal adaptation in *S. marcescens* DB11 (I) and in 9 different bacterial species (II)

The goal of the thermal adaptation studies (I and II) was to determine, whether adapting to rapidly (within generation) fluctuating temperature happens by adapting to the specific constant temperatures of the fluctuating temperature range (changes in the shape or in the elevation of the tolerance curve) or by adapting by other means. In study I, *S. marcescens* DB11 bacteria populations were allowed to evolve for 54 days at constant 31 °C or in 2 different rapidly fluctuating temperatures: 24 - 31 - 38 °C (smooth fluctuations) or 24 - 38 °C (abrupt fluctuations). After the evolution treatment, I isolated clones from the populations and measured maximum growth rate and yield (biomass) of the evolved bacterial clones at 7 constant temperatures (20.5, 24, 27.5, 31, 34.5, 38 and 41.5 °C) and in fluctuating 24 - 38 °C to see, if the clones from the different evolution treatments had diverged in their response to constant and fluctuating temperatures (Fig. 1).

In study II, I tested the generalizability of the results from study I by expanding the number of the study species to 9. I allowed populations of each of the 9 species (*E. aerogenes*, *L. adecarboxylata*, *S. marcescens ssp. marcescens*, *S. marcescens DB11*, *E. coli*, *P. putida*, *P. fluorescens*, *P. chlororaphis* and *N. capsulatum*) to evolve separately for 79 days at constant 30 °C and in rapidly fluctuating 20 - 30 - 40 °C. After the evolution treatment, I isolated clones from the populations and measured maximum growth rate and yield (biomass) of the evolved bacterial clones at 3 constant temperatures (20, 30 and 40 °C) and in fluctuating 20 - 40 °C (Fig. 2).

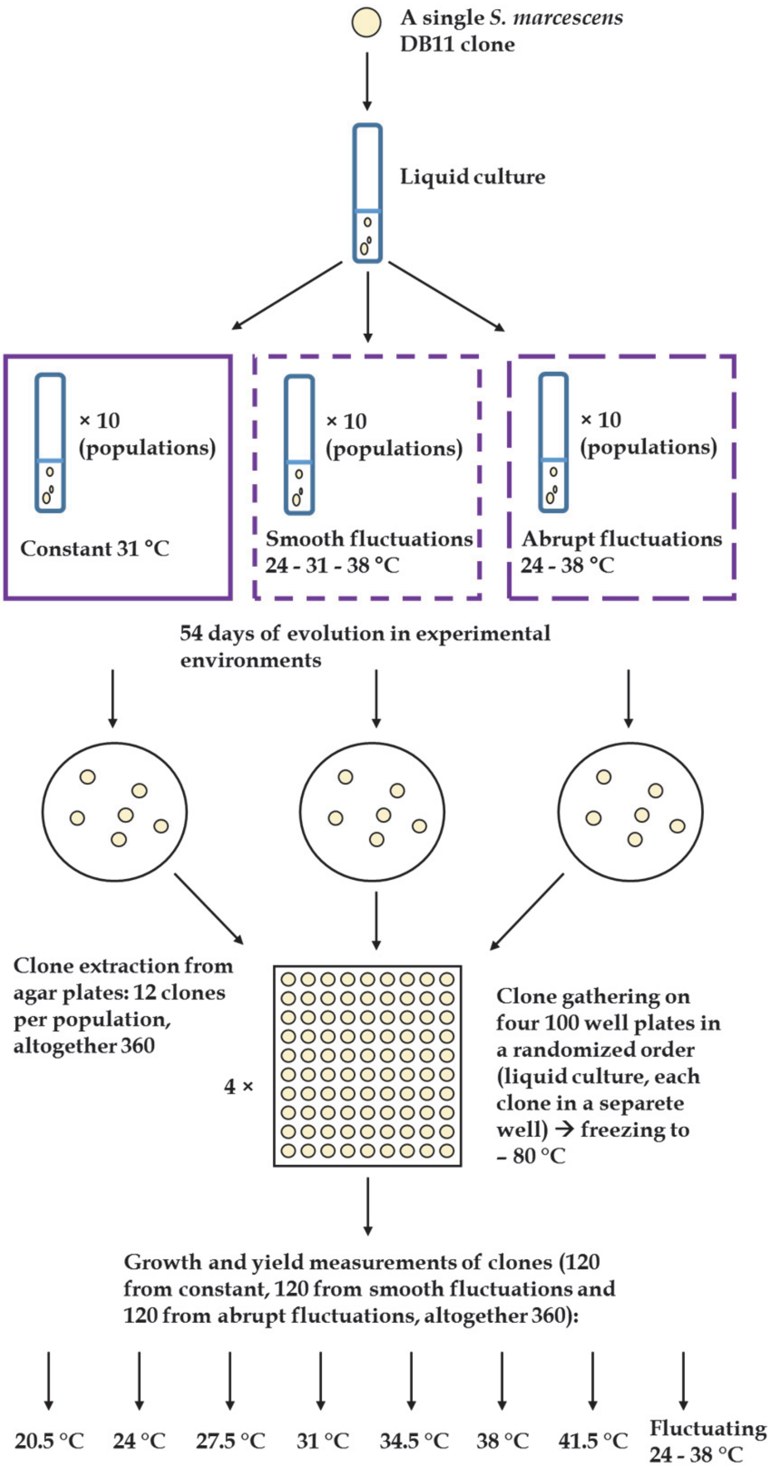

FIGURE 1 The experimental design of study I.

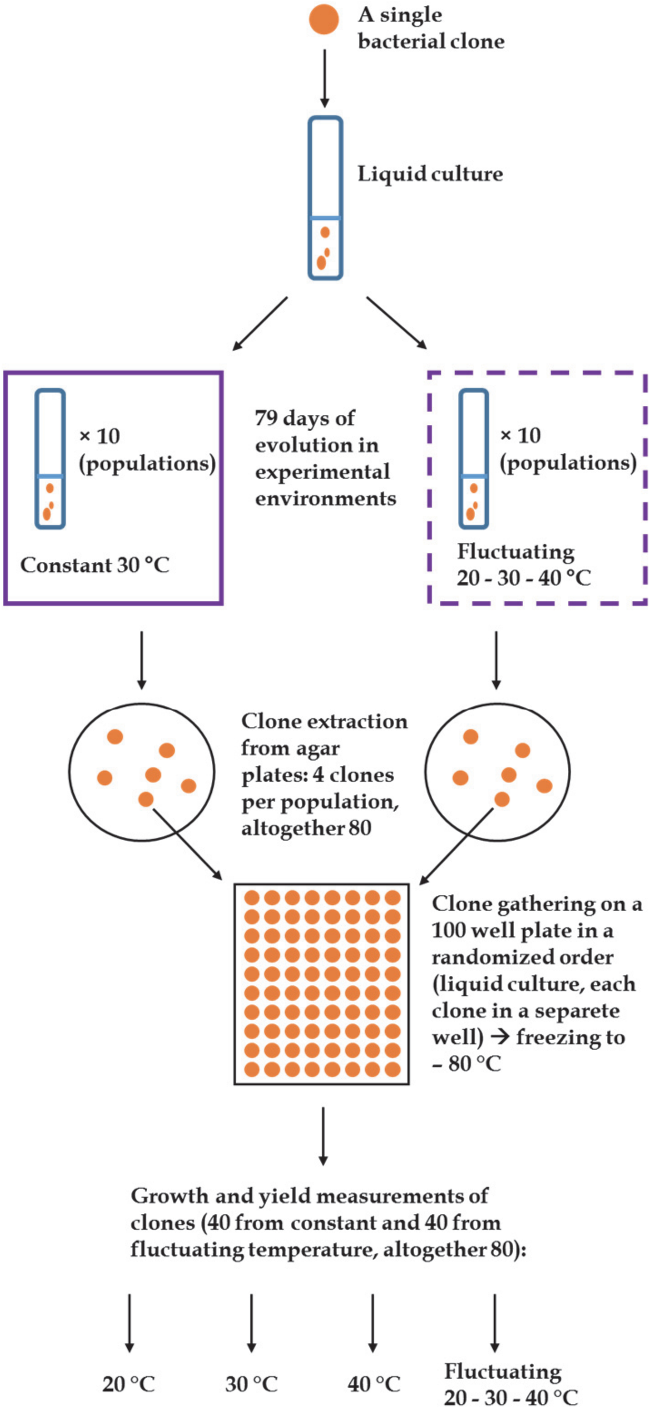

FIGURE 2 The experimental design of study II. The procedure was repeated for all of the nine study species, hence we gained altogether 9 × 80 = 720 bacterial clones.

2.4 Experimental design of the invasion studies (III and IV)

In study III, the aim was to use the evolved bacterial clones from study II, to test in one experiment, if evolutionary histories of both the invader (*S. marcescens*) and the community species (*E. aerogenes*, *L. adecarboxylata*, *P. chlororaphis*) together with the experimental environment have an effect on the invasion success of the invader. Since the invader and the community species had evolved in 2 different thermal regimes (constant 30 °C and fluctuating 20 - 30 - 40 °C) and I used the same thermal regimes as experimental environments, I gained 8 different environment - evolution history combinations (Fig. 3).

In study IV, I used bacterial clones from the constant evolution treatment of study II, to test the effects of genetic variability and propagule pressure of the invader on invasion success (independent of the evolutionary history or the experimental environment). I used 2 different propagule pressures (4 and 12 % of community volume) and 2 levels of genetic variability (clones vs. populations).

In both invasion studies, the basic experimental set-up was the same (Fig. 4). The 3 community species were allowed to establish their populations in replicated communities for 3 days, after which I renewed the communities into fresh medium and added the invader into each community. The invader was added only once. After invasion, I followed the population growth of the invader in the communities for 12 days. During this time period I renewed and took samples from each community, as well as added a small amounts of gene flow from the community species (to mimic a gene flow from adjacent populations) every third day (3, 6, 9 and 12 days after invasion). To find out the invasion success in the communities after the experiment, I plated all the samples from each time point to DNase test agar that allows easy recognition of *S. marcescens* from the 3 community species. Unlike the community species in the experiment, *S. marcescens* has the ability to break down DNA enzymatically, which appears as a clear halo around the *S. marcescens* colonies on the blue-green agar plates (Smith *et al.* 1969). After 2 days of propagation, the ratio of *S. marcescens* colonies vs. other species' colonies on the plates was counted.

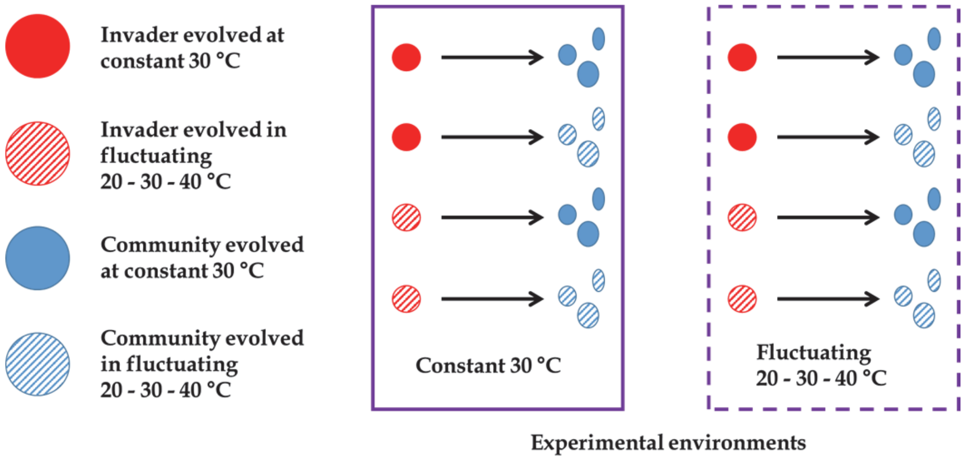

FIGURE 3 The experimental design of study III. The effects of the evolutionary histories of both the invader and the community species together with the effect of the environment on invasion success were tested in the same experimental settings.

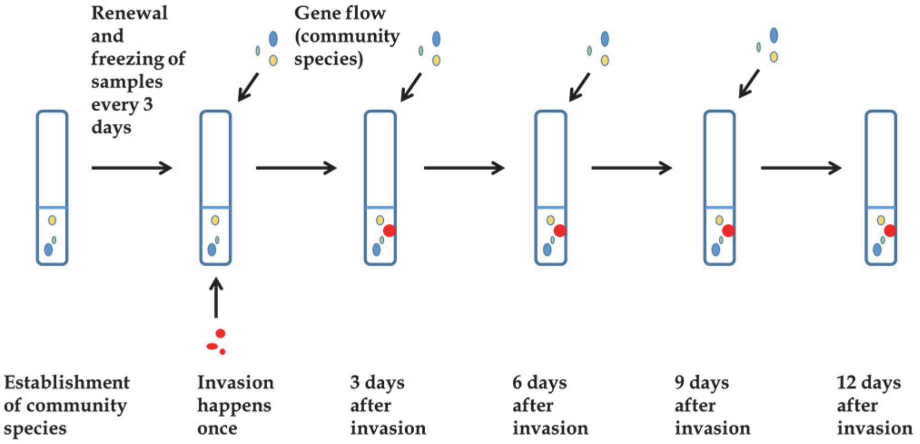

FIGURE 4 The experimental set-up of studies III and IV.

3 RESULTS AND DISCUSSION

3.1 Adaptations to fluctuating temperature (I, II)

Tolerance curves that are based on measurements taken in constant environments should reveal adaptations to fluctuating environments (Levins 1968). In the case of adapting to rapidly fluctuating temperature, there are two common predictions: species either become generalists that cope well with all the experienced temperatures (high elevation of the tolerance curve), or they improve their tolerance at the extreme ends of the fluctuating range, generating broad or flat tolerance curves (Levins 1968, Lynch and Gabriel 1987). Specialists should instead evolve in constant environments (Kassen 2002). I tested whether adaptation to rapidly fluctuating temperature is visible in tolerance curves in the thermal adaptation study I, where I cultured *S. marcescens* bacteria for 54 days at constant 31 °C or in two different rapid temperature fluctuations (between 24 and 38 °C) and in study II, where I cultured nine bacterial species for 79 days at constant 30 °C or in rapidly fluctuating temperature between 20 and 40 °C.

In study I, *S. marcescens* clones evolved in either fluctuating range (smooth or abrupt fluctuations) grew slower at constant optimum temperatures, thus the shape of the tolerance curve differed between the treatments. However, the overall elevation of the growth rate tolerance curve did not differ between the treatments (I, Fig. 1). The clones evolved in either fluctuating range also gained a lower biomass yield over the whole measured range of constant temperatures (lower elevation of the tolerance curve), compared to the clones evolved at constant temperature. The shape of the tolerance curve did not differ between the treatments (I, Fig. 1). Based on tolerance curves, and conventional wisdom, one would have dictated strains from fluctuating environment to be badly adapted to fluctuations. Still, when growth rate was measured in rapidly fluctuating temperature, the clones evolved in fluctuations grew clearly faster than clones evolved at constant temperature (I, Fig. 2).

In study II, where thermal tolerance was tested over 9 bacterial species, clones adapted to fluctuation had a lower growth rate at optimum temperature than clones evolved at constant temperature. Thus, the shape of the tolerance curve differed between the treatments, but the elevation of the tolerance curve was comparable between the treatments. When thermal tolerance was tested as biomass yield over all the species, both the shape and the elevation of the tolerance curves were comparable between treatments (II, Fig. 1). However, the fluctuation adapted clones reached a significantly higher biomass yield than clones from constant temperature, when yield was measured under rapid fluctuation (II, Fig. 2).

The results of the thermal adaptation studies (I and II) lead to a consistent finding: adaptations to rapidly fluctuating temperature were not visible in thermal tolerance curves. In other words the tolerance curves of the fluctuation adapted clones did not have higher elevation or broader shape than the tolerance curves of the clones adapted to constant temperature. Quite the contrary, when looking at the tolerance curves, the fluctuation adapted clones had equal or even worse tolerance at constant temperatures than clones adapted to constant temperature. In accordance with theories, this would indicate that the clones adapted to fluctuation would also have low tolerance of fluctuating temperature. Instead, in both studies, the high tolerance of fluctuation adapted clones to fluctuating environment (local adaptation) came apparent when measurements were conducted in fluctuating environments. This is in line with previous findings suggesting the existence of adaptation mechanisms not detectable in tolerance curves (Schulte *et al.* 2011, Ketola *et al.* 2014). Hypothetically, tolerance to rapidly fluctuating environment could have evolved for example by reversible phenotypic plasticity via increased heat-shock protein expression (Sorensen *et al.* 2003, Ketola *et al.* 2004), by low responsiveness to extreme environments (Suiter *et al.* 2003) or by better exploiting the short-time optimal conditions during fluctuations (Gilchrist 1995, New *et al.* 2014).

In summary, the results of the studies I and II did not support the theories and previous empirical evidence suggesting that generalism or high tolerance of extremes should evolve in fluctuating environments (Levins 1968, Lynch and Gabriel 1987, Leroi *et al.* 1994, Scheiner and Yampolsky 1998, Hughes *et al.* 2007, Ketola *et al.* 2013, Condon *et al.* 2014). Instead, I found that measurements conducted in fluctuating environments can reveal adaptations that are not visible in the tolerance curves. However, it must be taken into account that the previous experimental studies on tolerance to fluctuating environments have mainly concentrated on testing the consequences of slow, between-generation fluctuations.

3.2 Evolutionary history of the invader and the community species and the environment during invasion: effects on invasion success (III)

Theories and previous studies in the invasion literature have shown that disturbed and/or heterogeneous environments are vulnerable to invasions (Elton 1958, Burke and Grime 1996, Davis *et al.* 2000, Davis 2009). It has also been argued that the evolutionary history of the invader in a disturbed environment could play a role in invasion success (Lee and Gelembiuk 2008, Hufbauer *et al.* 2013, Ketola *et al.* 2013). In study III, I tested the environmental effect during invasion and the effects of evolutionary histories of both the invader and the receiving community on invasion success. I found that *S. marcescens* clones that had evolved in the fluctuating temperature were significantly better invaders than the clones evolved at the constant temperature, especially in the later phases of invasion (III, Fig. 2). Interestingly, evolving in the fluctuating environment was also advantageous for the 3 bacterial community species, since fluctuation adapted communities resisted the invasion better than communities evolved at constant temperature, when the invasion took place in the fluctuating environment (III, Fig. 1). Thermal fluctuations in the environment during invasion also enhanced the invasion success at the first stages (III, Fig. 1).

The results of the study III are in line with theories and previous findings concerning the invasiveness of disturbance-adapted invaders and the invasibility of disturbed environments. In addition to theoretical expectations (Lee and Gelembiuk 2008), the high invasion success of invaders from disturbed environments has been shown in recent empirical studies (Foucaud *et al.* 2010). Findings from plant invasion studies (Burke and Grime 1996, Davis *et al.* 2000) and microbial invasions (Liu *et al.* 2012, Li and Stevens 2012) support the theories of high invasibility of disturbed environments (Elton 1958, Davis 2009). Since in study III the invasion proceeded at the first stages most easily in the fluctuating environment, when the community species had evolved in a constant environment, my results show that the vulnerability to invasions can also depend on the evolutionary history of the community species. When the community is locally adapted to the environment, resistance against invasions can be higher than in an environment that does not match the adaptations of the community. This kind of scenario could take place, if the local climate conditions change. However, in my results, the local adaptations of the community to the environment mattered only in the fluctuating environment, whereas in the constant environment communities with either evolutionary history were equally vulnerable or resistant to invasion.

3.3 Effects of propagule pressure and genetic variability of the invader on invasion success (IV)

It has been shown that both high propagule pressure and high genetic variability of the invader can increase the invasion success (Williamson 1996, Roman and Darling 2007, Bell and Gonzalez 2009, Davis 2009, Lockwood *et al.* 2009, Simberloff 2009, Wittmann *et al.* 2014, Forsman 2014). However, since genetic diversity is usually the higher, the higher the propagule pressure is (Robertson 1960, Falconer and Mackay 1996, Davis 2009, Blackburn *et al.* 2015), the relative importance of the two factors in invasions is still quite poorly understood (Bock et al 2015). I tested these ideas in study IV, where I manipulated both the propagule pressure and the genetic variance of *S. marcescens* simultaneously in the same setting. However, my results did not give support for the theories or the previous findings, since I found no effect of either the propagule pressure or the genetic variability of the invader on the invasion success. The invasiveness was equal between the 2 propagule pressure treatments, as well as between low-diversity clones and high-diversity populations (IV, Fig. 1). My results might be explained by the assumed high numbers of individuals in the both propagule pressure levels, which could mask the effects of genetic variability between the treatments (Robertson 1960, Falconer and Mackay 1996). Alternatively, the results can be explained by the probable high phenotypic plasticity of *S. marcescens*, since phenotypically plastic individuals can be able to respond very fast to environmental changes, despite of low genetic variability (Ackermann 2015). It is also possible that interference competition among the clones within the diverse *S. marcescens* populations plays a role, by masking the positive effects of genetic diversity on invasion success (Garbutt *et al.* 2011). The non-significant results of the study IV call for further testing in an experimental setting that has a wider range between the studied propagule pressures, to test whether the findings are actually relevant or simply caused by the too small differences between the treatments.

4 CONCLUSIONS

The results of this thesis, which is based on the experimental thermal adaptation and invasion studies I – IV, show that adaptations that enable species to tolerate thermal fluctuations can also cause increased invasiveness.

First of all, evolving in a constant thermal environment vs. within-generation fluctuating thermal environment did indeed induce differences in thermal adaptation (I, II). The 2 different environments clearly required different kinds of adaptations, but these differences were not visible in thermal tolerance curves. The thermal tolerance curves showed equal or lower tolerance of the clones from fluctuating environment at constant temperatures, which should, according to theories, also predict low tolerance of fluctuating temperature. However, the superior tolerance of the bacterial clones from fluctuating environment was clear when measurements were taken in fluctuating environments. Thus, based on my results, it is not a good idea to jump to conclusions when species performance in fluctuating/disturbed environments is predicted from tolerance curves. In the worst case scenario, the species' response to disturbance might be completely opposite than what is deduced from performance in constant environments (study I). This is certainly not a trivial problem, since tolerance curves are very widely used in predicting species' performance, for example in breeding genetics and environmental management, which are important fields of applied biological sciences. Therefore, I suggest that measurements should also be taken directly in fluctuating environments, whenever possible. In addition to being valuable in comparison with the tolerance curves, the measurements in fluctuation can indeed be the only fitness measures that reveal local adaptations to fluctuating environments. Generally, I think that it is always a good idea to measure fitness or fitness components directly in the particular environment that is under examination, when studying adaptations. With this approach, the risks of drawing false conclusions of species' adaptations can be minimized, which would be essential for example when predicting extinction risks.

In study III, invasiveness of the invader was promoted by evolving in a fluctuating environment, regardless of the environment where the invasion took place. Also, the invasion happened more easily, if the invasion took place in the fluctuating environment. The community species were also more resistant to the invasion, when they had evolved in the fluctuating environment and when the invasion took place in the fluctuating environment. Hence, the good news is that local adaptations to disturbed environments can aid communities in resisting invasions. But then again, if the local environment changes from constant to fluctuating, the community's adaptation to the constant environment makes it more vulnerable to invasions. It is also somewhat worrying that based on my results, evolving in a fluctuating environment generated a universal invader that thrived equally well in both constant and fluctuating environments. To conclude, in addition to the direct negative impacts on biota, the ongoing climate change can, based on my results, also have two-way indirect negative effects. In the worst case scenario the increasing number and magnitude of disturbances that are repeated within generations can increase the invasibility of environments, and through pre-adaptations new invasive species can also emerge from the disturbed areas.

Acknowledgements

First, thanks to my supervisors! It's been great working with you and I appreciate that you all have a great sense of humour. Leena, in addition to being a brilliant scientist, you always give good advice, you know how "the system" (the university bureaucracy) works and how things should be done in practice. Jone, you really are an Original Personality in capital letters. We've definitely had some of the craziest conversations of my life (after "a few" drinks, of course). And last but definitely not least, Tarmo. You have the gift of making people feel better and crack up laughing even at their darkest moment. So don't ever stop making bad jokes! If I hadn't decided to take the course "Experimental population ecology" in the fall 2009, I probably would have never ended up working in your laboratory. Honestly, I don't know where I would be if I had never met you. Maybe I would still be in the misery of finding my place in the scientific world, trying to figure out what is "my thing" after all. At least I'm pretty sure that without your support and belief and trust to my skills I wouldn't have gone this far in the academic life yet. It's been a great six years and the experimental evolution group has been a true dream-team for me. So…thank you, for everything. I hope we'll have plenty of chances to collaborate in the future.

Big thanks to the professors (especially Jonna), the exp.-evo group (Lauri, Rogi, Ilkka & Matthieu), office roommates (Aigi, Hanna & Rogi: you can do it!), the whole eco section and all the people I know from other sections (especially PhD students)! I've spent all my study years (since 2004!) in Jyväskylä, and the biology department really feels like home. I'm quite a nocturnal animal and sometimes a bit anti-social (because I need to be alone to recover from stress), and especially this year I haven't been that much around. But every time I have managed to drag myself to the office (during "office hours"), it has been worth it, thanks to fellow biologists. Luckily, there's a trick to wake up my social side: parties. So especial thanks to all fellow party-animals! All the work-related conferences, meetings and parties have been fabulous, every time. Big thanks goes to the floorball team! Who would have thought that I'd actually like playing floorball (since it always sucked at school), but now I really do. Thanks also to the trainees who helped in the lab: Elina, Jari, Jatta & Krista.

Special thanks to my opponent Jacintha Ellers and to the pre-examiners Alexandre Jousset & Marjo Saastamoinen. Big thanks to my support group Teppo & Lotta-Riina and scientific editor Jari Haimi. Also, thanks to the funders: Biological Interactions Graduate School, Doctoral Programme in Biological and Environmental Science and Centre of Excellence in Biological Interactions.

Huge thanks to all my friends outside work! I met most of you during the study years, and what a misery it would have been without you. Sini, we have been friends for…24 years, how great is that! KWP ry, especially Hellu, Kati M S, Lepsu, Pia, Piste, Päxxä, Ritu & Terhi, thanks to you I can say I have found "my people". There's nothing better than knowing I have friends for life.

Finally, also huge thanks to my family and relatives! Dad, I still miss you every now and then. You know I don't believe in afterlife, but still, if you would be somewhere out there, I know you'd keep thumbs up for me. Mum, you're the one I can tell everything. We have always talked about everything and talked and laughed a lot. Picking up the phone and calling you makes the best moments even better and the worst moments to disappear. Kiitos, äiti.

YHTEENVETO (RÉSUMÉ IN FINNISH)

Lämpötilatoleranssin ja invaasiokyvyn evoluutio lämpötilaltaan vaihtelevassa ympäristössä

Intensiivisestä tutkimuksesta huolimatta kaikkia ilmastonmuutoksen vaikutuksia eliölajeihin ei vielä tunneta. Tällä hetkellä maailmanlaajuinen keskilämpötilan nousu ei ole ainoa huolenaihe, sillä uusimmat ilmastomallit ennustavat myös entistä enemmän ilmaston häiriöitä ja ääri-ilmiöitä, kuten myrskyjä, tulvia ja lämpötilan vaihtelua. Ilmastonmuutos voi vaikuttaa myös eliöiden evoluutioon. Vaihtelevassa ympäristössä luonnonvalinta voi suosia ominaisuuksia, jotka ovat hyödyllisiä vaihtelevaan ympäristöön sopeutumisessa, mutta toisaalta voivat myös lisätä lajien invaasiokykyä. Tällaisia ominaisuuksia ovat esimerkiksi nopea lisääntyminen, kestävyys ja muovautuvuus. Tulevaisuudessa lajeja myös todennäköisesti kuolee sukupuuttoon entistä nopeammalla tahdilla, koska monet lajit eivät kykene siirtymään uusille alueille tai sopeutumaan muuttuneeseen ilmastoon tarpeeksi nopeasti. Ihmistoiminta on myös aiheuttanut luonnonympäristöissä runsaasti häiriöitä (esimerkiksi elinympäristöjen saastumista, tuhoutumista ja pirstoutumista) jo entuudestaan, ja häirittyjen elinympäristöjen on osoitettu olevan erityisen alttiita vieraslajien invaasioille. Näistä syistä olisi todella tärkeää ymmärtää paremmin, miten eliöt sopeutuvat nopeasti muuttuvaan ja vaihtelevaan elinympäristöön ja millaisia seurauksia näillä sopeumilla voi olla uusissa ympäristöissä, koskien erityisesti lajien kilpailu- ja invaasiokykyä.

Eliöiden lämpötilasopeumia – myös vaihtelevan lämpötilan sietoa – on perinteisesti tutkittu mittaamalla eliön kelpoisuutta ilmentäviä ominaisuuksia (esimerkiksi kasvu- tai lisääntymisnopeutta) tasaisissa lämpötiloissa. Mittaustuloksista on koostettu niin sanottu toleranssikäyrä, josta käyvät ilmi ainakin kyseisen eliön optimilämpötila sekä sietokyvyn ääripäät: minimi- ja maksimilämpötila. Vielä ei kuitenkaan ole varmuutta siitä, riittääkö pelkkä toleranssikäyrien tutkiminen vaihtelevan lämpötilan sietokyvyn ennustamisessa. Toleranssikäyriä käytetään laajalti, muun muassa jalostamisessa lajien ominaisuuksien arviointiin, invaasiolajien leviämisen ja mahdollisten uusien invaasiolajien ilmaantumisen tutkimiseen, sekä ennustettaessa ilmastonmuutoksen vaikutuksia eliölajeihin. Näin ollen olisi erittäin tärkeää selvittää, voidaanko toleranssikäyriin todella luottaa, kun tutkitaan lajien menestymistä vaihtelevissa ympäristöissä, vai tarvitaanko tutkimuksessa myös muita menetelmiä. Muutoin pelkkien toleranssikäyrien perusteella tehdyt johtopäätökset voivat pahimmillaan johtaa vääränlaisiin käytännön toimenpiteisiin.

Väitöskirjani kokoava teema on evolutiiviset sopeumat ja niiden seuraukset – miten lajit sopeutuvat luonnonvalinnan kautta ympäristöönsä ja miten kyseiset sopeumat voivat mahdollistaa menestymisen myös uusissa ympäristöissä. Pääasiassa keskityn lämpötilasopeumiin ja erityisesti nopeaan lämpötilanvaihteluun: aiheuttaako vaihtelevassa lämpötilassa kasvaminen erilaisia lämpötilasopeumia verrattuna tasaisessa lämpötilassa kasvamiseen, ja onko kyseisillä sopeumilla vaikutusta invaasiolajin invaasiokykyyn tai vastaanottavan eliöyhteisön invaasioalttiuteen. Lisäksi tutkin invaasiolajin populaatiokoon ja geneettisen monimuotoisuuden vaikutusta invaasiokykyyn. Tutkimus on toteutettu kokeellisen evoluution keinoin käyttämällä tutkimuslajeina useita eri bakteerilajeja.

Väitöskirjani kaksi ensimmäistä artikkelia keskittyvät bakteerien lämpötilatoleranssin evoluutioon vaihtelevassa lämpötilassa. Ensimmäinen tutkimus (I) toteutettiin yhdellä bakteerilajilla, ja toisessa artikkelissa (II) tutkimusta laajennettiin samankaltaisessa asetelmassa käyttämällä yhdeksää bakteerilajia. Ensimmäisessä tutkimuksessa *Serratia marcescens* DB11 -bakteereja kasvatettiin kolmessa eri lämpökäsittelyssä (tasainen 31 °C, vaihteleva 24 - 31 - 38 °C ja vaihteleva 24 - 38 °C) 54 päivän ajan. Evoluutiokäsittelyn jälkeen bakteerikloonien lämpötilatoleranssia testattiin mittaamalla kasvunopeutta ja biomassan määrää (kantokykyä) seitsemässä eri tasaisessa lämpötilassa (20,5; 24; 27,5; 31; 34,5; 38 ja 41,5 °C) sekä vaihtelevassa lämpötilassa (24 - 38 °C). Toisessa tutkimuksessa yhdeksää eri bakteerilajia kasvatettiin tasaisessa 30 °C:n sekä vaihtelevassa 20 - 30 - 40 °C:n lämpötilassa 79 päivää, jonka jälkeen lämpötilatoleranssia testattiin jälleen kasvunopeudella ja biomassan määrällä tasaisissa 20, 30 ja 40 °C:ssa sekä vaihtelevassa 20 - 40 °C:ssa. Molempien tutkimusten tulokset johtivat samaan johtopäätökseen: tasaiseen ja vaihtelevaan lämpötilaan sopeutuminen tapahtuu eri tavoilla, sillä kummassakaan tutkimuksessa toleranssikäyrät eivät paljastaneet bakteerien sopeutumista vaihtelevaan lämpötilaan. Ainoastaan mittaukset vaihtelevassa lämpötilassa osoittivat, että vaihtelevassa lämpötilassa kasvaneet bakteerit todella sopeutuivat lämmönvaihteluun paremmin kuin tasaisessa lämpötilassa kasvaneet.

Kolmannessa artikkelissani tutkin lämpötilasopeumien ja ympäristön yhteisvaikutusta *S. marcescens* -bakteerin invaasiokykyyn sekä kolmen bakteerilajin yhteisöjen alttiuteen invaasiolle (III). Tutkimuksessa testattiin kaikki yhdistelmät asetelmassa, jossa sekä invaasion tekijä että vastaanottava yhteisö olivat sopeutuneet joko tasaiseen tai vaihtelevaan lämpötilaan, ja invaasio tapahtui joko lämpötilaltaan tasaisessa tai vaihtelevassa ympäristössä. Tulokset olivat mielenkiintoisia ja pitkälti hypoteesien mukaisia. Alkuvaiheessa invaasiomenestys oli keskimäärin korkeampaa vaihtelevassa lämpötilassa. Kaikkein menestyksekkäintä invaasio oli silloin, kun vastaanottava yhteisö oli sopeutunut tasaiseen lämpötilaan, mutta eli invaasion tapahtuessa vaihtelevassa lämpötilassa. Loppuvaiheessa vaihtelevaan lämpötilaan sopeutuneet *S. marcescens* -bakteerit olivat aina invaasiokyvyltään parempia kuin tasaiseen lämpötilaan sopeutuneet, riippumatta yhteisön sopeumasta tai ympäristöstä, jossa invaasio tapahtui.

Neljäs tutkimus käsittelee invaasion tekevän *S. marcescens* -populaation koon ja geneettisen monimuotoisuuden vaikutusta invaasiomenestykseen (IV). Teorioiden ja aikaisemman tutkimuksen perusteella invaasiomenestys on yleensä sitä parempaa, mitä suurempi ja geneettisesti monimuotoisempi invaasion tekevä joukko on. Näiden kahden tekijän vaikutusta on kuitenkin ollut vaikea erottaa toisistaan, sillä keskimäärin geneettinen monimuotoisuus kasvaa aina joukon kasvaessa. Viimeisessä osatyössä testasin geneettisen monimuotoisuuden ja populaationkoon suhteellista vaikutusta kahden faktorin koeasetelmassa, jossa invaasion tekeviä *S. marcescens* -populaatioita oli sekä yksilömäärältään että geneettiseltä monimuotoisuudeltaan kahta eri luokkaa. Tulokset kuitenkin poikkesivat odotetuista: sen paremmin populaation koolla kuin monimuotoisuudellakaan ei ollut merkitystä invaasion onnistumisen kannalta.

Yhteenvetona lämpötilatoleranssi- ja invaasiokokeiden tuloksista voidaan todeta, että sopeumat, jotka mahdollistavat menestymisen vaihtelevassa ympäristössä, voivat todella johtaa myös invaasiokyvyn kasvuun. Näitä sopeumia ei myöskään välttämättä voi havaita tutkimalla vain tasaisissa ympäristöissä mitattuja toleranssikäyriä. Toleranssikäyrien antamat tulokset voivat olla jopa täysin päinvastaisia verrattuna vaihtelevassa ympäristössä tehtyjen mittausten tuloksiin. Näin ollen on mahdollista, että toleranssikäyrien perusteella tehdyt päätelmät eliöiden menestyksestä vaihtelevassa ympäristössä voivat olla virheellisiä ja johtaa vääränlaisiin toimenpiteisiin. Olisikin suositeltavaa, että vaihtelevaan ympäristöön sopeutumista tutkittaessa eliöiden kelpoisuutta ilmentäviä ominaisuuksia mitattaisiin aina myös suoraan vaihtelevassa ympäristössä, mikäli mahdollista. Lisäksi invaasiokokeiden tulosten perusteella vaihtelevat ympäristöt ovat alttiimpia invaasioille, ja myös vastaanottavan yhteisön sopeumilla on merkitystä invaasion onnistumisessa. Väitöskirjani tuloksiin perustuen on siis mahdollista, että ilmastonmuutoksella voi olla suorien negatiivisten vaikutusten lisäksi myös kahdenlaisia epäsuoria negatiivisia vaikutuksia luonnonympäristöissä: nopeasti vaihtelevat ilmasto-olot voivat sekä tehdä elinympäristöistä alttiimpia invaasioille että edistää uusien invaasiolajien kehittymistä.

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