JYU DISSERTATIONS 822

Emmi Räsänen

Adaptation to Fluctuating and Extreme Temperatures

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF MATHEMATICS
AND SCIENCE

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ABSTRACT

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Climate change forces species to tolerate heat and faster fluctuations in temperature. The speed of thermal change is expected to affect species ability to adapt, as there should be fitness trade-offs and evolutionary constraints between constant and fluctuating temperatures. However, there is no certainty about the factors that could facilitate or limit adaptation to temperature at different timescales. In this thesis, my aim was to investigate the trade-offs in adaptation to constant mean and extreme temperatures, and fluctuations of varying speed. My study species were fungi and bacteria. First, I used quantitative genetics and association mapping to study if different genes affect thermal tolerance at constant and fluctuating temperatures. Second, I used experimental evolution to test the differences in adaptation to constant and fluctuating temperatures, and the efficacy of adaptation in large and small populations. Third, I used competition experiments to investigate if thermal fluctuations select for populations that are better competitors against other species. The results indicated only weak trade-offs between constant and fluctuating temperatures at genetic level, in adaptation with different population sizes, or in competitive ability of evolved populations. Based on my results, trade-offs do not seem to determine species ability to adapt to increased variation, as some individuals and populations are able to perform well across temperatures. However, the tolerance to extreme heat might be more evolutionary constrained due to little genetic variation in some species. On the other hand, high temperatures can form strong selection pressures that lead to fast adaptive responses in populations. In addition, present thermal fluctuations can affect species competitive ability, and hence should be considered when predicting species survival in future. To conclude, the results of this thesis highlight a need for reconsidering some of the hypotheses that emphasize the role of trade-offs and evolutionary constraints in adaptation to constant and differently fluctuating temperatures.

Keywords: Competition; experimental evolution; fitness trade-off; genetic architecture; population size; temperature fluctuation; thermal adaptation.

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TIIVISTELMÄ

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Ilmastonmuutos altistaa eliölajit yhä useammin kuumuudelle ja nopealle lämpötilan vaihtelulle. Vaihtelun nopeuden on oletettu vaikuttavan eliöiden lämmönsietoon, sillä tasaisiin ja vaihteleviin lämpötiloihin sopeutumisen välillä pitäisi olla kelpoisuuskustannuksia ja evolutiivisia rajoitteita. Tästä huolimatta ei ole varmuutta siitä, mitkä tekijät parantavat tai huonontavat eliölajien kykyä sopeutua muuttuviin lämpötilaoloihin. Väitöskirjassani tutkin sieni- ja bakteerilajien sopeutumista vakaisiin keski- ja äärilämpötiloihin sekä eri nopeuksilla vaihteleviin lämpötiloihin. Selvitin laskennallisen genetiikan ja geenikartoituksen avulla vaikuttavatko samat geenit lämmönsietokykyyn vakaissa ja vaihtelevissa lämpötiloissa. Kokeellisella evoluutiolla tutkin sopeutuvatko suuret ja pienet populaatiot yhtä tehokkaasti lämpötilaltaan vaihteleviin ja vakaisiin ympäristöihin. Lisäksi tein kilpailukokeita, joilla tutkittiin ovatko lämpötilaltaan vaihtelevissa ympäristöissä kehittyneet populaatiot parempia kilpailijoita muita lajeja vastaan. Tulokset osoittivat, että geneettiset erot rajoittivat lämpötilasopeutumista vain vähän, ettei populaatiokoolla ollut suurta vaikutusta sopeutumiskykyyn, eikä vaihtelevissa lämpötiloissa kehittyminen selittänyt menestystä lajien välisessä kilpailussa. Tulosteni perusteella kelpoisuuskustannukset eivät määritä eliöiden kykyä sopeutua lisääntyvään lämpötilan vaihteluun, sillä jotkin yksilöt ja populaatiot pärjäävät hyvin lämpötilasta riippumatta. Poikkeuksena vaikuttaisi olevan kuumansietokyky, jonka evoluutiota joillakin lajeilla rajoittaa vähäinen geneettisen muuntelun määrä. Toisaalta korkeat lämpötilat voivat johtaa voimakkaaseen luonnonvalintaan ja nopeaan sopeutumiseen. Lisäksi lämpötilan vaihtelu voi vaikuttaa lajien välisen kilpailun lopputulokseen, mikä tulisi huomioida ennustettaessa lajien selviytymistä tulevaisuudessa. Väitöskirjani osoittaa, että meidän tulisi uudelleenarvioida hypoteeseja, jotka korostavat kelpoisuuskustannuksien ja evolutiivisten rajoitteiden vaikutusta eliölajien sopeutuessa vakaisiin ja eri nopeuksilla vaihteleviin lämpötiloihin.

Avainsanat: Geneettinen perusta; kelpoisuuskustannus; kilpailu; kokeellinen evoluutio; lämpötilan vaihtelu; lämpötilasopeutuminen; populaatiokoko.

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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 Räsänen E., Moghadam N. N., Sidhu K., Summanen P., Littunen H-R., Ketola T. & Kronholm I. Genome-wide association study for loci influencing thermal performance curves in *Neurospora crassa*. Submitted manuscript.

II Räsänen E., Liukkonen M., Summanen P., Ketola T. & Kronholm I. Genome-wide association mapping for growth rate at fluctuating and extreme temperatures. Manuscript.

III Räsänen E., Nieminen V., Summanen P., Villalba de la Peña M., Makkonen P., Suisto K., Ketola T. & Kronholm I. The effect of population size on adaptation to fluctuating temperatures. Manuscript.

IV Räsänen E., Lindström L. & Ketola T. 2020. Environmental fluctuations drive species' competitive success in experimental invasions. *Annales Zoologici Fennici* 57: 79–87.

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ABBREVIATIONS

GWAS	Genome-wide association study
HSP	Heat shock protein
K	Population carrying capacity
PCA	Principal component analysis
r	Population intrinsic growth rate
SNP	Single nucleotide polymorphism
TPC	Thermal performance curve

1 INTRODUCTION

1.1 The evolution of thermal tolerance

1.1.1 What is thermal tolerance?

Temperature is an environmental stressor that varies in both space and time and affects all biological levels from cells to ecosystems (Wooliver *et al.* 2022, Thorogood *et al.* 2023). Stressful perturbations, such as extreme and variable temperatures, lead to reductions in organisms' fitness unless they are able to uphold physiological homeostasis by recovering their cellular structures and functions (Sørensen *et al.* 2003, Richter *et al.* 2010). The evolution of thermal tolerance is best described as a dynamic process between stress and adaptation, where organisms respond to change in environmental temperature and enhance their fitness trough phenotypic plasticity or evolutionary change at population level (Marshall *et al.* 2020). The evolutionary change in thermal tolerance requires heritable genetic variation in fitness-related traits, a selection pressure via environmental temperature, and enough evolutionary time. Hence, an organism's thermal performances and tolerance ranges are influenced by both the temperatures they experience and the thermal regimes that their ancestors encountered during population's evolutionary history (Bono *et al.* 2017).

Natural environments are usually characterized by a substantial thermal heterogeneity and the ongoing climate change is making temperatures warmer and increasingly variable (Meehl *et al.* 2000). Organisms differ in their sensitivity to thermal variation: some species are specialists for which performance depends strongly on temperature, whereas generalists have more robust performance over a wider range of temperatures, being able to adjust to fluctuations and extremes (Angilletta 2009). These strategies determine the fitness trade-offs between life history traits and might include genetic constraints that limit evolution (Ketola and Kristensen 2017). On an ecological timescale, temperature

causes disturbances that influence the abundance and geographic distribution of species (Bozinovic *et al.* 2011, Tüzün and Stoks 2018). Therefore, how species can adapt to the variation in temperature is one of the most fundamental questions in evolutionary biology (Angilletta 2009). It is important to understand what factors constrain or facilitate thermal adaptation, and how different thermal environments are selecting individuals, genotypes, and genes (Sinclair *et al.* 2016, Kristensen *et al.* 2020, Wooliver *et al.* 2022).

1.1.2 Climate change as a selection pressure

Natural selection favors organisms that maintain the highest fitness i.e. have the best reproductive success across conditions they are exposed to in their local environment. Anthropogenic climate change has raised the global mean temperature and increased the frequency and magnitude of thermal fluctuations (IPCC 2018). These changes are going to multiply the number of extreme weather events, mainly heat waves and unpredictable changes in temperature during the coming decades (Meehl *et al.* 2000). Both the mean and the variance of environmental temperature can act as agents of selection on different traits (Logan and Cox 2020). Based on species performance projections and empirical data, extreme temperatures, rather than just the rise in average temperature, are forming stronger selection pressures to evolution of thermal tolerance in ectotherms (Vasseur *et al.* 2014, Colinet *et al.* 2015, Logan and Cox 2020) and shaping species geographical distributions (Stenseth *et al.* 2002, Harley and Paine 2009, Schulte *et al.* 2011). This is alarming because most of the terrestrial organisms are ectotherms for which fitness is directly affected by the environmental temperature (Rolandi *et al.* 2018).

The increased stochasticity in temperature makes populations experience selection that varies in frequency and magnitude (Bürger and Krall 2004, Hellmann and Pineda-Krch 2007). Extreme temperatures that exceed a species' range of thermal tolerance can lead to a strong directional selection in a population, favoring phenotypes that tolerate better these conditions (Parmesan *et al.* 2000). Exposure to more extreme environments causes greater fitness reductions and so stronger selection, which is most apparent in the case of increased mortality (Buckley and Huey 2016, Buckley and Kingsolver 2021). Hence, enhancing heat tolerance is an important component of organismal fitness in nature (Lecheta *et al.* 2020). However, the adaptive potential of species upper thermal limits has been studied mostly regarding increasing or constant heat (Mitchell and Hoffmann 2010, Rolandi *et al.* 2018), and more rarely in response to temperature fluctuations (Schaum *et al.* 2018, 2022).

Studies have demonstrated that even rare extremities can influence the evolution of thermal tolerance (Buckley and Huey 2016), and that organisms' responses to thermal variability are timescale dependent (Botero *et al.* 2015, Kristensen *et al.* 2020, Kefford *et al.* 2022). Also, previous studies have suggested that the fast frequency of fluctuations might be more important to the organism's fitness than the duration or the magnitude of the exposure to extreme temperatures (Kearney *et al.* 2012, Marshall and Sinclair 2015). Temporally

fluctuating environments cause stronger selection than spatial heterogeneity because all individuals are affected, and under fast fluctuations acclimation and recovery times are shorter (Kronholm and Ketola 2018). Temporally fluctuating selection across generations can also facilitate evolution if it maintains genetic variation in populations by balancing selection (Kassen 2002, Turelli and Barton 2004). On the other hand, alternating selection might slow down the evolution by favoring different traits at different times and locations, making selection in one generation maladaptive for next generations (Kingsolver and Buckley 2017).

The thermal variability experienced in the past can make species preadapted to novel environments, depending on how much the magnitude and frequency of the disturbances differ from the historical regimes (Snell-Rood and Ehlman 2021). If environments that are now rare become more common in future, previously adaptive traits might be suboptimal, making phenotypes maladapted to novel thermal conditions and having lower fitness (Chevin and Hoffmann 2017). In this way species performances and local community compositions may change due to climatic perturbations. Disturbed areas are also creating opportunities for the appearance of well-adapted invader species (Lee and Gelembiuk 2008). Consequently, a better understanding of the rates of thermal change that species can cope with, and the mechanisms that allow physiological and adaptive responses to increased thermal variation are crucial for predicting their survival under climate change (Stenseth *et al.* 2002, Schulte *et al.* 2011).

1.1.3 Short-term plasticity and long-term adaptation

Adaptive mechanisms in thermal evolution include both short-term changes during individual's lifetime and longer-term changes across generations (Chevin *et al.* 2010, Lecheta *et al.* 2020). Specifically, as evolution occurs in units of generations, the mechanisms in response to thermal variation depend on the relationship between species generation intervals and the speed of change in the environment that creates the selection pressures (Kristensen *et al.* 2020). In phenotypic plasticity, individuals with the same genotype can produce different phenotypes by sensing cues of the fast changes in their environment (DeWitt and Scheiner 2004). Over evolutionary timescales, species respond to natural selection by thermal adaptation or evolution of phenotypic plasticity (Angilletta 2009, Hoekstra and Montooth 2013). Thermal adaptation can occur in basal tolerance preparing cells to thermal stress and preventing damage, or as dynamic plastic changes that protect cell during and after a stress (Lecheta *et al.* 2020). These mechanisms in thermal adaptation are not necessarily easy to separate and can be in work simultaneously as the preparative processes are usually fixed during development and both mechanisms can include production of stress proteins (Angilletta 2009, Lecheta *et al.* 2020).

Generally, populations and species from more variable environments are expected to exhibit more plasticity (Addo-Bediako *et al.* 2000, Snell-Rood and Ehlman 2021). Thermal plasticity adjusts an organism to a change in temperature to enhance performance and maintain fitness across a range of temperatures (Angilletta 2009). Compared to genetic evolution, plasticity can substantially

alter the phenotypic variance of populations without a genetic change. Plastic responses include thermoregulatory changes in behavior, morphology, physiology, and gene expression (Hochachka and Somero 2002, Callahan *et al.* 2008). The two often separated forms of phenotypic plasticity are developmental and dynamic plasticity (Kristensen *et al.* 2020). In developmental plasticity, phenotypic changes might remain fixed throughout the organism's life, being beneficial when conditions stay relatively constant after the early development (Meyers and Bull 2002). Thermal fluctuations that occur at the timescale of a generation might also select for genetic canalization that make phenotype robust across temperatures (Kawecki 2000). Transgenerational responses such as epigenetic changes are a form of developmental plasticity, allowing faster adaptation under intermediate and slow fluctuations spanning over many generations (Kristensen *et al.* 2020).

Fast thermal fluctuations are expected to select for increased dynamic plasticity and wider tolerance (Levins 1968, Dewitt and Langerhans 2004). In dynamic plasticity reversible mechanism enable organisms to acclimate and recover from thermal stress within a one generation (Angilletta 2009). For example, in insects, physiological heat hardening results in a reversible increase in heat tolerance after a brief heat shock (Hoffmann *et al.* 2003). These changes are fast, as the production of heat-shock proteins (HSPs) or changes in protein phosphorylation state can be activated in minutes (Schulte *et al.* 2011). HSPs are chaperones that help other proteins to maintain their structure and folding when temperature starts to break the chemical bonds, preventing the protein denaturation at high temperatures (Sørensen *et al.* 2003). There exists a variety of HSPs which are in work over different periods of time, as smaller HSPs are important at fast fluctuations and larger under more constant heat (Podrabsky and Somero 2004). At slower fluctuations, also the changes in the number of double bonds in lipids can affect the cell membranes (Hochachka and Somero 2002).

The heat shock response is conserved in cells and found across a variety of taxa (Rivera *et al.* 2021). The HSPs along with other stress resistance proteins are upregulated also in response to other abiotic and biotic stresses, making cells more resistant to these stresses by a physiological mechanism known as the general stress response (Kültz 2005). However, plasticity needs reliable cues from environmental change to allow organisms to respond effectively (Scheiner 1993). Instead, if environmental cues for predicting future conditions are unreliable in fluctuating environments, selection can favor risk spreading strategies, such as bet hedging (Meyers and Bull 2002). In diversified bet-hedging multiple phenotypes are always produced from a single genotype without need for predictive cues (McNamara *et al.* 2016). Plasticity and bet hedging can also act simultaneously, as most environments consist of both predictable and unpredictable changes (Grantham *et al.* 2016).

When organisms are exposed to thermal fluctuations, the ability to sense the environmental cues and respond through cellular signaling pathways is likely under selection (Bradshaw and Holzapfel 2006). The capacity for phenotypic plasticity is genetically defined and adaptable. For example, there is

genetic variation within populations and between species in speed of which the heat shock response is upregulated (Feder and Hofmann 1999). Thermally stressful environments might also select for longer-term evolutionary adaptations that enhance phenotypic variability and environmental robustness. For example, stress promotes regulatory variation and evolvability of gene expression, leading to persistent changes, which can occur within a short evolutionary time (López-Maury *et al.* 2008). Adaptation process can arise from standing genetic variation or de novo mutations and requires a minimum number of generations to reach fixation in a population (Ament-Velásquez 2022). Polygenic adaptation is a process in which a population adapts through small changes in allele frequencies at hundreds or thousands of loci. Polygenic adaptation can occur relatively quickly if there exists heritable genetic variation in fitness-related traits in direction of the natural selection and no constraints are met (Kristensen *et al.* 2020). Rapid evolution of increased thermal tolerance is essential in facilitating the persistence of species under fast environmental change (Schaum *et al.* 2018).

Plasticity can interact with evolution by either constraining or promoting evolutionary change (Chevin *et al.* 2010, Oostra *et al.* 2018). For example, plasticity might help populations to extend their thermal performance at extreme temperatures and so move closer to the phenotypic optimum as a steppingstone to adaptation (Schaum and Collins 2014). Hypothetically, plastic genotypes should be able to respond more effectively to selection compared with less plastic genotypes (Schaum and Collins 2014) and keep population size higher under fast thermal fluctuations (Chevin *et al.* 2010, Kristensen *et al.* 2020). There is also some experimental evidence of plasticity increasing the potential for rapid evolution of thermal tolerance in response to variable and changing environments (Collins *et al.* 2020). Conversely, plasticity can hinder evolution by limiting the exposure to stressful temperatures and so the strength of selection, for example, organism can avoid stressful conditions by moving to another place or shifting the time of dormancy (Huey *et al.* 2012, Williams *et al.* 2016). Altogether, there exist a good scientific understanding about the physiological processes that affect thermal adaptation on cellular and individual level. However, we don't know much about thermal adaptation at the genetic level in important fitness traits, such as growth, or the impacts of thermal fluctuations on the rapid evolution of thermal tolerance (Clarke 2003, Schaum *et al.* 2022).

1.1.4 Adaptation to fluctuating versus constant temperatures

Thermal fluctuations of varying frequency, amplitude, duration, and predictability are expected to form different kinds of selective pressures and affect fitness differently compared to constant temperatures (Levins 1968, DeWitt and Langerhans 2004, Botero *et al.* 2015, Melbinger and Vergassola 2015, Kronholm 2022). In fluctuating environments, organism's fitness is reduced due to repeated exposures to stressful temperatures, and extreme temperatures are likely to generate selection on different components of thermal tolerance than the phases of more benign temperatures (Gabriel and Lynch 1992, Gilchrist 1995,

Rezende *et al.* 2014, Sinclair *et al.* 2016, Logan and Cox 2020). Moreover, experimental studies have demonstrated that the evolutionary trajectories and plastic responses might differ at fluctuating and constant temperatures, and between fluctuations of varying speed (Ketola *et al.* 2004, Podrabsky and Somero 2004, Ketola and Saarinen 2015, Dey *et al.* 2016, Manenti *et al.* 2016, Schaum *et al.* 2022). Generally, organisms that experience variable environments are expected to evolve more plastic, whereas stable environments should favor more locally specialized genotypes (Kassen 2002, Condon *et al.* 2014). This means that adaptation to thermal fluctuations could be maladaptive in constant environments, and vice versa (Ketola and Kristensen 2017).

The physiological costs of thermal adaptation usually vary along an organism's body temperature (Clarke 2003). At fluctuating environments, adaptation with wider thermal tolerance and increased plasticity entails energetic costs associated e.g. with the signaling pathways, expression of stress resistance genes, replacement of denatured proteins, and restructuring cellular membranes (Somero 2002, Richter *et al.* 2010). During heat shock response, energy goes to both transcription of heat shock genes and the function of HSPs as molecular chaperones, which makes long-term upregulation not a beneficial evolutionary strategy (Angilletta 2009). Hence, selection is expected to favor generalist strategies only at highly variable environments compared to less plastic specialist with adaptation to specific temperatures (Svanbäck *et al.* 2009, Buckley and Kingsolver 2021). The speed of fluctuations defines the time available for acclimation and recovery from stress, and so strongly affects the costs of plasticity in a given environment (Angilletta 2009, Kronholm and Ketola 2018).

The costs of adaptation and plasticity are central for understanding the fitness trade-offs between life-history traits (Stearns 1989). In thermal biology, trade-offs can be defined as energetic or enzymatic compromises which are inevitable for an individual because of the associated fitness costs (Garland *et al.* 2022). Performance trade-offs occur when a trait that improves fitness at one temperature also reduces fitness at another temperature (Levins 1968, Palaima 2007). Generally, the proximate mechanisms underlying the trade-offs in thermal performance are divided in physiological and biochemical trade-offs. Physiological trade-offs are observed on the level of an individual, usually being related to acquisition and allocation of energy between traits (Van Noordwijk and De Jong 1986, Rowe and Houle 1996). Biochemical trade-offs result from thermodynamic effects that make cellular reactions faster at warmer temperatures, and affect the stability of temperature-sensitive proteins, membranes, and organelles (Huey and Kingsolver 1989, Malusare *et al.* 2023). For example, specialist-generalist trade-offs in performance may arise from the inability of organisms to optimize biochemical performances across a broad range of temperatures, or from resource allocation between efficient growth and heat shock response (Hochachka and Somero 2002, López-Maury *et al.* 2008). Thus, the ability to spend more time closer to critical thermal limits should come at the expense of individual's overall fitness (Angilletta *et al.* 2003).

The trade-offs in thermal adaptation are usually studied with thermal performance curves (TPCs) that display the range of temperatures which organisms can inhabit and the phenotypes that organisms express at specific temperatures (Huey and Stevenson 1979). In ectotherms, TPC describes the relationship between body temperature and a performance trait which is generally some temperature-dependent rate process like growth rate or metabolic activity (Angilletta 2009). TPCs are formed by measuring these fitness proxies at different constant temperatures and defining important shape parameters of the curve. The temperatures where an organisms cannot perform anymore i.e. the endpoints of the TPC, are called as the critical thermal minimum and the critical thermal maximum. The tolerance range between these minimum and maximum tells the broadness of the curve. TPCs can also be divided into areas of optimal performance and physiological stress, based on the curvature formed by the level of performance at each temperature. For ectotherms the TPCs are often left skewed, as the performance increases gradually towards the maximum performance at thermal optimum, after which there is a fast decrease at higher temperatures (Martin and Huey 2008).

TPCs are not fixed but modified by both phenotypic plasticity and adaptive evolution (Schulte *et al.* 2011, Schaum *et al.* 2022). The TPC shape parameters are used to define the population- and species-specific variation in thermal performance. These different modes of variation in TPCs, i.e. the changes in the shape and position, are expected to have a genetic basis, and express a potential for evolution when organisms adapt to contrasting thermal environments (Kingsolver *et al.* 2001, Izem and Kingsolver 2005). According to traditional hypotheses, TPCs are expected to maintain a constant area under curve during evolution, reflecting the proximate trade-offs on individual performance, and ultimately, constraining TPCs (Levins 1968, Lynch and Gabriel 1987, Gilchrist 1995, Angilletta 2009). Generally, three modes of variation are observed; the elevation in overall performance (faster-slower), horizontal shift in optimum between colder and warmer temperatures (hotter-colder), and in breadth of the TPC (Izem and Kingsolver 2005, Angilletta 2009). Two hypotheses are often suggested for thermal performance when organisms adapt to more constant vs. variable or more optimal vs. extreme temperatures. The specialist-generalist trade-off is expected to occur between adaptation to fluctuating and constant temperatures, stating that at fluctuating temperatures, selection should lead to evolution of generalists with broader TPCs but lower performance, whereas constant temperatures should select for specialists with higher but narrower TPCs (Lynch and Gabriel 1987, Gilchrist 1995, Logan and Cox 2020). The hotter-is-better trade-off is expected to evolve at increasingly warm temperatures, where individuals with higher optimal temperatures are also having better performance (Hochachka and Somero 2002, Angilletta *et al.* 2010). In nature, populations are likely to consist of individuals that form mixtures of temperature-dependent trade-offs (Kingsolver *et al.* 2001, Angilletta 2009).

As the fluctuations of varying frequency affect fitness differently, these are also expected to have characteristic changes in TPCs (Schulte *et al.* 2011, Colinet *et al.* 2015, Schaum *et al.* 2022). Under fast fluctuations, selection should favor

elevated performance curve and wider tolerance range across temperatures (Turelli and Barton 2004, Meyers *et al.* 2005). Fluctuations of longer timescale are usually experienced as constant conditions and should select for more fixed strategies, reflected as narrower TPCs, or shift in optimum (Meyers *et al.* 2005, Botero *et al.* 2015). However, critical suggestions have been made about the applicability of TPCs measured across variety of constant temperatures to predict performance at fluctuating temperatures (Schulte *et al.* 2011, Sinclair *et al.* 2016, Ketola and Kristensen 2017). Moreover, experimental studies have shown valid evidence that adaptations to thermal fluctuations are not necessarily detectable from TPCs (Ketola *et al.* 2014, Ketola and Saarinen 2015), and that distinct genetic architectures might regulate adaptation to fluctuating and constant temperatures (Sørensen *et al.* 2016, Deatherage *et al.* 2017). There is a pressing need for experiments investigating the genetic constraints on thermal adaptation, as these will ultimately limit species ability for rapid adaptation when variation in temperature increases (Angilletta 2009, Berger *et al.* 2014, Buckley and Kingsolver 2021).

1.1.5 Genetic architecture and constraints on evolution

Thermal tolerance is a polygenic trait for which genetic architecture is determined by the combined effects of many genes and interacting environmental conditions (Brakefield and Kesbeke 1997, Duun Rohde *et al.* 2016). Recent theoretical studies have suggested that the genetic architectures between constant and differently fluctuating temperatures are distinct, indicating that the evolution of thermal tolerance might be constrained when both the mean and variance of temperature increase (Botero *et al.* 2015, Melbinger and Vergassola 2015, Kronholm 2022). Genetic constraints on thermal adaptation can substantially slow down or completely prevent an organism from producing particular phenotypes, and over time, change the phenotypic distribution of a population (Hellmann and Pineda-Krch 2007, Garland *et al.* 2022). The divergence in TPCs can result from genes or alleles that hinder an organism from adapting to one temperature without decreasing performance at another temperature along the tolerance range (Angilletta 2009). More specifically, distinct genetic architectures between temperature-dependent traits, or the same trait measured under contrasting temperatures, might originate from differences in the identities of genes, amounts of genes, or the gene-gene and gene-environment interactions that influence the phenotypic expression (Fu *et al.* 2013, Duun Rohde *et al.* 2016). Additionally, the allele frequencies in a population and the distributions of allelic effects on individuals' thermal performance could constrain adaptation (Berger *et al.* 2014, Latimer *et al.* 2014, 2015).

In nature, selection is likely to simultaneously act on several traits or the direction of selection fluctuates across multiple temperatures, affecting how genotypes are mapped to phenotypes in individuals (Hellmann and Pineda-Krch 2007, Walsh and Blows 2009, Stinchcombe and Kirkpatrick 2012). The lack of heritable genetic variation in a trait to the direction of selection can constrain certain evolutionary trajectories (Roff and Fairbairn 2007), and negative genetic

correlations among traits prevent evolution towards the optimum combination of trait values (Reed *et al.* 2011). For example, high temperatures may constrain an organisms' ability to tolerate thermal variability if there is an insufficient amount of genetic variation in heat tolerance (Walters *et al.* 2012, Kefford *et al.* 2022). This shortcoming is often observed across taxa, as traits associated with heat tolerance are highly evolutionary conserved due to biochemical effects of high temperatures (Hochachka and Somero 2002, Araújo *et al.* 2013). The genetic correlations and covariances among a set of traits arise when the same genes affect thermal performance, or the effect of a gene differs between selection environments. For example, in the specialist-generalist mode of the TPC, a negative genetic correlation is expected to occur between maximal performance and thermal tolerance range (Huey and Hertz 1984). The proximate mechanisms behind genetic correlations are gene pleiotropy and linkage disequilibrium (Czesak *et al.* 2006). Antagonistically pleiotropic alleles cause negative correlations, for example, by affecting multiple biological pathways or by encoding the synthesis of different enzyme variants at different temperatures of the species tolerance range (Huey and Kingsolver 1989, Garland *et al.* 2022). In linkage disequilibrium, certain alleles of different loci often co-occur in a population e.g. due to tendency of closely located genes to be inherited together. This linkage can produce combinations of traits that are not completely beneficial to an individual fitness depending on the environment (Angilletta 2009). Also, epistatic effects between genes can influence an individual's phenotype if the expression of a one gene is covered or suppressed by other genes (Agrawal and Whitlock 2010).

Conversely, enhanced potential for phenotypic evolution is found from populations governing beneficial genetic variation and positive genetic correlations between selected traits (Agrawal and Stinchcombe 2009). When positive correlations between traits lead to individuals with high fitness, the phenotype can be canalized to be environmentally robust, i.e., always develop or being expressed under different conditions (Kawecki 2000). The expression of a specific phenotype can also be achievable to an individual only after a change in environment. For example, neutral mutations are usually accumulated and masked by organismal robustness, but this cryptic genetic variation can become beneficial and expressed under novel conditions (Kawecki 2000, Lee and Gelembiuk 2008). However, in terms of evolutionary adaptation, it should be remembered that regardless of the fitness effects, all genetic correlations constrain the capacity of traits to evolve independently (Stinchcombe and Kirkpatrick 2012), and that any amount of genetic variation is not a guarantee for an evolutionary change to occur if the trait is not related to fitness, or there is no selection in a particular environment (Kristensen *et al.* 2020).

Rapid or drastic environmental change can induce selection on the structure of the genome (Logan and Cox 2020, Berger *et al.* 2021), for example, gene pleiotropy can maintain phenotypic plasticity in a population, especially when adaptation by genetic change is constrained (Yadav *et al.* 2015). Adaptive changes are observed in TPCs when mutations cause heritable sequence polymorphisms in protein-coding regions or in regulatory areas of the genes (Lecheta *et al.* 2020,

Logan and Cox 2020). Changes in proteins usually affect the thermal optimum or the critical thermal limits, whereas regulatory mutations affect the tolerance range through variation in gene expression (Logan and Cox 2020). In fluctuating environments, mutations at transcription factor binding sites are favourable as they promote transcription, but don't cause changes in protein function (López-Maury *et al.* 2008, Lecheta *et al.* 2020). Selection could also target genes that regulate epigenetic changes such as modifications in histones or methyl groups (Furrow and Feldman 2014). At stressfully high temperatures, the adaptation can be driven also by mutations affecting multi-protein complexes that are involved in the temperature-dependent balancing of the expression of growth- and stress-related genes (López-Maury *et al.* 2008). This balancing between rapid growth and high stress resistance is a fundamental physiological challenge especially for unicellular microorganisms, as the most stress-resistant cells are non-growing (Kültz 2005, López-Maury *et al.* 2008).

Traditionally, the polygenic basis and constraints in thermal adaptation are investigated by the quantitative genetic analysis of covariation estimating statistical averages over a multitude of loci with small phenotypic effects (Fisher 1919). Genetic variance–covariance matrix (G-matrix) partitions phenotypic variance into genetic and environmental components and can be used to predict trait heritabilities and genetic correlations (Walsh and Blows 2009, Garland *et al.* 2022). In contrast to continuous TPCs, in a multivariate approach, the performances measured at each temperature can be thought as discrete and potentially genetically correlated traits (Kingsolver *et al.* 2004, Ghalambor *et al.* 2007). The existence of genetic correlations and their effects on evolutionary potential can be validated e.g. by measuring phenotypes in breeding designs or by experimental evolution resulting in correlated responses to selection (Buckley and Kingsolver 2021). When studying evolutionary potential with TPCs, the principal component analysis (PCA) of genetic variation is the common approach (Kingsolver *et al.* 2001, Izem and Kingsolver 2005). PCA partitions genetic variance across TPCs into loading vectors and estimates the percentage of phenotypic variance explained by each mode in a population (Latimer *et al.* 2015).

More recently, the advances in molecular genetics studies have made it possible to sequence whole genome data and investigate the genetic architecture of thermal tolerance on the level of individual loci (Fu *et al.* 2013). For example, genome-wide association studies (GWAS) link genotypes to phenotypes by mapping mutational markers such as single nucleotide polymorphisms (SNPs) that are assumed to be causal for the trait in question or in linkage disequilibrium with causal alleles (Mackay *et al.* 2009, Duun Rohde *et al.* 2016). Coupling quantitative genetics and next generation sequencing could give us more complete picture of the genetic variation in thermal tolerance and its evolutionary potential (Hoffmann *et al.* 2003, Cortés *et al.* 2020, Buckley and Kingsolver 2021). This is due to fact that even the best GWAS lack statistical power to account for all small-effect loci in polygenic traits, whereas quantitative genetic studies cannot tell apart loci with largest phenotypic effects or other architectural characteristics with precision (Fu *et al.* 2013).

Results from studies using full genome data have supported quantitative genetics by pointing that most traits have complex genetic architectures formed by a multitude of loci with very small effect sizes, and interactions between genes and environment (Fu *et al.* 2013, Duun Rohde *et al.* 2016). Moreover, many quantitative genetics studies have shown that TPC shape parameters in ectotherms are heritable, but their adaptation is genetically constrained and taxon or trait specific (Angilletta 2009, Kellermann *et al.* 2012, Araújo *et al.* 2013, Logan and Cox 2020). Some studies have suggested that the variation in TPCs exist mainly due to antagonistic pleiotropy underlying negative genetic correlations (Berger *et al.* 2014, Latimer *et al.* 2015). In addition, many experiments have found little variation in genomic regions important to heat stress, suggesting evolutionary constraints on heat tolerance (Addo-Bediako *et al.* 2000, Mitchell and Hoffmann 2010, Kellermann *et al.* 2012, Kristensen *et al.* 2015). However, other studies have found evidence that species can expand their heat tolerance if there is strong selection for better performance at hot temperatures, which is often observed in evolution and selection experiments using constantly high temperatures (Bennett *et al.* 1990, Holder and Bull 2001, Hangartner and Hoffmann 2016, Rolandi *et al.* 2018). Also, the studies on quantitative genetics and genome-wide sequence data have mostly focused on evaluating the effects of constant or increasing heat (Riehle *et al.* 2001, Knies *et al.* 2006, Mitchell and Hoffmann 2010, Khan *et al.* 2022).

Despite decades of research, many unknowns remain in the genetic architecture of thermal adaptation and patterns of evolutionary constraints seem largely inconsistent across taxa, underlying the need for new approaches (Angilletta 2009, Buckley and Kingsolver 2021). For example, very little is known about the genetic architecture of TPCs on the level of individual loci and the antagonistic effects of allelic variation (Berger *et al.* 2014, Latimer *et al.* 2014, 2015). To date, only few studies have quantified genetic covariation between fitness traits in the context of climate change (Hellmann and Pineda-Krch 2007), or integrated quantitative genetics with latest molecular genetics methods to investigate thermal adaptation (Gerken *et al.* 2015, Latimer *et al.* 2015, Duun Rohde *et al.* 2016, Rolandi *et al.* 2018, Lecheta *et al.* 2020). Even less studies have concerned differently fluctuating temperatures and mostly used experimental evolution and resequencing (Tobler *et al.* 2014, Deatherage *et al.* 2017, Schaum *et al.* 2018, Lambros *et al.* 2021), whereas only one study has been made on natural genetic variation (Sørensen *et al.* 2016). In order to predict species persistence under climate change, further information is needed about the genetic architectures and evolutionary constraints between populations that originate from constant and differently fluctuating thermal environments (Lecheta *et al.* 2020, Logan and Cox 2020, Buckley and Kingsolver 2021).

1.1.6 Population size and persistence under thermal fluctuations

The efficacy of thermal adaptation and the change in genomic structure strongly depend on population dynamics (Angilletta 2009, Botero *et al.* 2015). This is

because mutations create important genetic variation for natural selection and populations with more individuals have greater mutational supply with more beneficial mutations (Samani and Bell 2010). Larger populations are also more likely to gain rare beneficial mutations of large effect size that give a strong fitness advantage for individuals carrying the mutation (Bell and Collins 2008, Sniegowski and Gerrish 2010). On the other hand, smaller populations are expected to gain less new beneficial mutations and to adapt mainly through mutations of small effect size (Sniegowski and Gerrish 2010, Chavhan *et al.* 2019). This is alarming for the evolutionary potential of both small and maladapted populations, as adaptation to novel environments often involves mutations of large effect size (Reed *et al.* 2011). Fast climatic changes will also cause population bottle necks by diminishing population sizes and standing genetic variation in many species, leading to genetic drift and greater extinction risk (Frankham 2005, Willi *et al.* 2006).

In addition to population size, mutational supply is accelerated also by the rate that mutations appear in the population (Handel and Rozen 2009). Thermally stressful environments can select for increased mutational rate, for example, in clonal organisms specific mutator genes increase the rate of spontaneous mutations (Cooper *et al.* 2007). This kind of mechanisms are especially important when recombination of segregating alleles does not create new genetic variation in a population (Meyers and Bull 2002). However, more stable conditions would select for lower mutation rate as most of the new mutations are neutral or deleterious in the local environment (Kimura 1967). In fluctuating environments, new beneficial mutations are crucial for an organism to adaptively track the moving fitness optimum (Botero *et al.* 2015). An evolutionary adaptation also requires a certain number of generations for mutations to spread and fix in all individuals of the population (Desai *et al.* 2007). In very large populations, fixation rate should be slower and limiting the speed of adaptation instead of the number of new beneficial mutations (Otto and Whitlock 2013).

Recent studies have suggested that the adaptive cost and benefits in small and large populations should depend on the variability of environmental conditions they currently experience compared to the conditions during population's evolutionary history (Chavhan *et al.* 2019, 2020, 2021). In general, populations that have evolved in fluctuating environments should have less fitness costs in alternative local environments, as under fluctuations there is a stronger selection against fitness costs due to antagonistically pleiotropic alleles (Bono *et al.* 2017). Conversely, when the conditions stay constant over a longer time, antagonistic pleiotropy can evolve more freely as selection is blind to the fitness costs in alternative environments (Bono *et al.* 2017). The benefits from the lack of trade-offs in novel environments should be greater for larger populations because they have better access to beneficial mutations that don't carry fitness trade-offs between different environments (Chavhan *et al.* 2021). On the other hand, larger populations are also more likely to have mutations with trade-offs and large effect sizes, which in constant evolutionary environments, would lead to greater maladaptation when the environment changes (Chavhan *et al.* 2020).

For smaller populations, both the costs and benefits from the conditions during evolution should be minor and performances more robust across alternative environments (Chavhan *et al.* 2021).

To date, mostly theoretical simulations have been used to study the hypotheses about population size and environmental variability (Handel and Rozen 2009, Uecker and Hermisson 2011, Meyer and Shnerb 2020), and no experiments have been made to investigate how demographic constraints may apply to adaption to thermal fluctuations. Furthermore, the differences in populations' ability to persist under climate change can make some species thrive better than others (Lee and Gelembiuk 2008). These populations are likely to impose strong biotic selection pressures on the evolution of thermal tolerance in other species and on the ecology of entire communities (Angilletta 2009, Sinclair *et al.* 2016, Tüzün and Stoks 2018).

1.1.7 The evolution of traits that correlate with thermal generalism

Natural selection on a favourable trait can reinforce coadaptation in other genetically or phenotypically correlates traits, leading to correlated responses to multivariate selection (Czesak *et al.* 2006, Roff and Fairbairn 2007). This scenario requires that certain sets of genes or combinations of traits give an individual a fitness advantage over other adaptive assemblies (Sinervo and Svensson 2002, Angilletta 2009). Theoretical studies of trait coevolution have suggested that the selection for generalist strategies in fluctuating environments could also have correlated effects on species invasiveness under climate change (Lee and Gelembiuk 2008, Lande 2009, Hufbauer *et al.* 2012). This could be due to general stress resistance that indirectly leads to better competitive ability under wide range of temperatures and correlated climatic factors (Lee and Gelembiuk 2008, Zerebecki and Sorte 2011, Ketola *et al.* 2013).

In nature, thermal conditions show marked spatial and temporal variation, which can lead to adaptive divergence among local populations in thermal performance (Keller and Seehausen 2012, Richter-Boix *et al.* 2015). Frequent thermal disturbance at the populations' original habitat is assumed to preadapt them to tolerate similar conditions at introduced or colonized area (Lee and Gelembiuk 2008, Ketola *et al.* 2013, Hamilton *et al.* 2015). Such preadaptations could arise through selection for life history and demographic traits that promote rapid population growth and persistence under thermal stress (Lee and Gelembiuk 2008). Alternatively, selection could favor generalists with broad tolerance and high phenotypic plasticity, or genetically robust trait combinations that are beneficial in novel environments (Lee and Gelembiuk 2008). Studies have shown evidence that populations and species from more variable environments show higher levels of plasticity that might preadapt them to extremes (Chevin and Hoffmann 2017, Thorogood *et al.* 2023) and make them more invasive (Richards *et al.* 2006, Lardies and Bozinovic 2008, Lande 2009) or affect virulence (Ketola *et al.* 2013, Ashrafi *et al.* 2018). Thermal plasticity is known to be especially important for unicellular microorganisms to stay competitive (López-Maury *et al.* 2008). Temperature-dependent physiological traits may allow invasive species

to expand their niche and spread to thermally disturbed environments where they can competitively exclude native species and occupy the freed niche space (Lockwood *et al.* 2010). Also, shorter generation times and higher fecundity would lead to larger population sizes, which in turn would increase evolutionary potential under chronic thermal stress (Reed *et al.* 2011).

Climatic fluctuations are often associated with increased disturbance at the onset of biological invasions (Lande 2009, Parepa *et al.* 2013). The invasibility of local communities is pronounced if native species are maladapted to the thermal conditions during invasion and so weaker competitors (Shea and Chesson 2002, Melbourne *et al.* 2007). On the other hand, if the native species are also preadapted to the change in environment, the invader might not have a competitive advantage over its local competitors (Saarinen *et al.* 2019). Hence, the fitness of invasive species and the evolution of competitive strategies depends on the strategies in the native community (Nowak and Sigmund 2004). On an ecological timescale, thermal disturbances cause local extinctions that release resources for fast-growing invaders to exploit (Davis *et al.* 2000, Shea and Chesson 2002). Thus, environmental fluctuations, fluctuation-adapted invaders and fluctuation-maladapted communities are all expected to govern invasion success (Saarinen *et al.* 2019). However, little is still known about the consequences of thermal adaptation regarding species' competitive and invasive abilities. Clearly, if we want to use the theory of thermal adaptation to predict the biological consequences of climate change, it must consider the coevolution of correlated traits, and closer attention should be paid to the effects of environmental variability (Angilletta 2009, Schulte *et al.* 2011).

1.2 Aims of the thesis

The focus of my thesis are the differences in adaptation between constant and fluctuating temperatures, and fluctuations of varying speed. The aim of my studies was to reveal the factors that either constrain or facilitate the evolution of thermal tolerance. I tested experimentally adaptation to high extreme temperatures (I, II, III) and thermal fluctuations (II, III, IV). First, I studied the genetic architecture of thermal tolerance by GWAS (I, II) and quantitative genetics (II). Second, I used experimental evolution to study the effects of evolutionary environment and population size on the evolved trade-offs in thermal adaptation (III). Third, I investigated if thermal fluctuations select for generalists that have also increased competitive ability aiding experimental invasions (IV). In this project, I addressed the following summarizing questions concerning each original paper:

I. Genome-wide association study for loci influencing thermal performance curves in *Neurospora crassa*.

Are the same loci associated with growth rate at different constant temperatures? Are there trade-offs in allelic effects between temperatures?

II. Genome-wide association mapping for growth rate at fluctuating and extreme temperatures.

Are there distinct genetic architectures or genetic constraints in adaptation to constant and differently fluctuating temperatures? In which directions there are most potential for TPC evolution?

III. The effect of population size on adaptation to fluctuating temperatures.

Does population size affect the adaptation to fluctuating temperatures? How efficiently populations can adapt to constant versus differently fluctuating temperatures?

IV. Environmental fluctuations drive species' competitive success in experimental invasions.

Are temperature fluctuations selecting populations that are better competitors than populations originating from constant temperatures? Do present temperature fluctuations facilitate invasions?

2 MATERIALS AND METHODS

2.1 Study organisms

2.1.1 Filamentous fungus *Neurospora crassa* (I, II)

Neurospora crassa is a filamentous fungus in the phylum *Ascomycota*. Morphologically this species is often described as an orange mold, which produces vegetative spores (conidia) and branching filaments (hyphae) that form a mycelium. *N. crassa* has a complex life cycle with both clonal and sexual reproduction, resulting in haploid and diploid stages. In meiosis, the cells of opposite mating types (referred as *mat a* and *mat A*) cross by passage of nuclei into a common cytoplasm and produce sexual spores (ascospores). In nature, the wind-dispersed spores form a mycelium on a burned plant material after a fire (Kuo *et al.* 2014). Hence, *N. crassa* is a saprotrophic species for which the fast mycelial growth rate is an important fitness trait that allows competition with other fungi (Pringle and Taylor 2002).

The distribution range of *N. crassa* is in tropical and subtropical regions. The natural strains that we used in association mapping studies (I, II) were originally collected from south-eastern USA (Louisiana), Central America and Caribbean Basin (Ellison *et al.* 2011, Palma-Guerrero *et al.* 2013). The temperatures in the Caribbean average around 30 °C and the optimal temperature for the growth of *N. crassa* is approx. 35 °C, after which the there is a rapid decrease at higher temperatures (Moghadam *et al.* 2020). However, very little is known about the ecology and evolution of *N. crassa* in its natural habitat (Kuo *et al.* 2014). The long history as a model organism has mostly been based on the genetic attributes and easy maintenance at laboratory conditions (Lee and Dighton 2010). *N. crassa* has a fully sequenced genome of only 7 chromosomes, encoding approx. 10,000 protein-coding genes (Galagan *et al.* 2003). In a haploid stage, the recessive traits are expressed in phenotypes which allows to interpret the functions of the genes

by knockout mutants. It is also easy to make genetic crosses between strains and the clones can be divided into experimental treatments. These qualities make *N. crassa* well-suited for population genetics and evolutionary studies (Lee and Dighton 2010).

2.1.2 Fission yeast *Schizosaccharomyces pombe* (III)

Schizosaccharomyces pombe is a unicellular fungus in the phylum *Ascomycota*. Its rod-shaped cells divide by medial fission which has given it the name fission yeast. In nature, *S. pombe* lives in large distribution area and strains are usually collected from the soil or on the surface of plants or insects. Fission yeast is easy to maintain in the laboratory and it has a rapid growth rate (generation times are 2–4 h at optimal temperatures around 30 °C) which considerably slows down at extreme heat around 40 °C (Coelho *et al.* 2013). Fast reproduction makes *S. pombe* a good model species for experimental evolution studies, as it can easily reach large population sizes (Zeyl 2006). Fission yeast is also popular in studies of molecular and cellular biology as its eukaryote genome has many genes that are found also from multicellular organism (Vyas *et al.* 2021). The small genome is fully sequenced, consisting of 3 chromosomes and approx. 4,970 protein-coding genes (Galagan *et al.* 2003).

S. pombe strains are usually haploid but the life cycle has also a short diploid stage. Sexual reproduction between cells of different mating types is induced by stressful nitrogen-limited conditions (Petersen and Russell 2016). Fission yeast has 2 mating types (h^+ and h^-) and ability to reversibly switch between types by DNA-rearrangement during replication (Hanson and Wolfe 2017). In the thermal evolution experiment (III), we used haploid ancestor strains which were genetically modified to be incapable of switching their mating type. However, we wanted to have replicates of different mating types so that the evolved strains could be mated in future experiments. Altogether we had 4 ancestor lines, as there was also a genetic marker to distinguish strains in competition experiments (III). These auxotrophic mutants have 2 different alleles $ade6^{M210}$ and $ade6^{M216}$ which cause a color difference due to divergent accumulation and oxidation of adenine precursor in biosynthesis (Forsburg and Rhind 2006). When grown on selective low-adenine plates, mutants with $ade6^{M216}$ allele turn to pink and $ade6^{M210}$ get darker red color.

2.1.3 Bacterial invader *Serratia marcescens* and competitor species (IV)

Serratia marcescens is a gram-negative bacterium in the family *Yersiniaceae*. This facultatively anaerobic species has motile rod-shaped cells which form bright red colonies. Ecologically *S. marcescens* is an opportunistic pathogen that infects a variety of organisms from plants to insects, fishes, and mammals (Grimont and Grimont 1978). *S. marcescens* is assumed to have a global distribution, as it is commonly found from both aquatic and terrestrial habitats. Hence, this bacterium has also a wide thermal tolerance, and it is able to grow at temperatures ranging 5–40 °C (Grimont and Grimont 1978). The fastest growth

rates and generation times are found at optimal temperatures around 31 °C (Ketola *et al.* 2013). The superior growth ability makes *S. marcescens* also fast in utilizing the available resources in its environment.

In the invasion study (IV) *S. marcescens* was selected as an invader because it is known to be highly dominant in multispecies cultures, possibly due to resource or interference competition with other bacteria (Ketola *et al.* 2017). The 3 bacterial competitor species, *Pseudomonas putida, Pseudomonas fluorescens*, and *Novosphingobium capsulatum* were chosen based on their ability to grow well in the same medium and tolerate similar thermal range as the invader (Saarinen *et al.* 2018). All bacterial species originated from a study where clones had evolved for 79 days (corresponds to approx. 86 generations) under nearly optimal constant temperature 30 °C or fluctuating temperatures cycling 20–30–40–30–20 °C for 2 h at every temperature (Saarinen *et al.* 2018). In addition, *S. marcescens* differs from competitor species by its ability to break down DNA enzymatically, which makes it easy to distinguish the colonies with halo on DNase agar plates (Ketola *et al.* 2017).

2.2 Genome-wide association mapping (I, II)

2.2.1 Nested association mapping

To study the genetic architecture of thermal tolerance at constant and fluctuating temperatures, we used nested association mapping that combines information from historic and recent recombination (Yu *et al.* 2008). In our mapping population, we had 118 natural *N. crassa* strains (obtained from the Fungal Genetics Stock Center, USA) and some of the strains were crossed to produce families with 316 offspring strains (Moghadam *et al.* 2020). Altogether, we had 434 *N. crassa* strains in our mapping population that was used in two genome-wide association studies (I, II). First, we studied the identity of large-effect loci and possible opposite allelic effects influencing growth rate at different constant temperatures forming the TPC (I). Second, we compared whether the same or different large-effect loci were associated with growth rate at constant temperatures and fluctuations of varying speed (II). To find the statistical associations between genotypes and phenotypes, we sequenced the genomes and measured the growth rates for each of the strain in the mapping population.

2.2.2 Sequencing and genotyping

To get a dense genome-wide SNP data, we extracted genomic DNA from the natural strains and sequenced these samples with Illumina (Novogene, UK). For some of the natural strains, the sequences were obtained from previous studies (Zhao *et al.* 2015, Villalba De La Peña *et al.* 2023). For the cost-efficiency, the offspring strains were sequenced by RAD reduced representation method (Baird *et al.* 2008), which was also done with Illumina (Floragenex Inc., USA). The full

genome sequences of the offspring strains were inferred from parental genotypes by using the RAD markers to locate the recombination breakpoints in offspring genomes, and then imputing the inherited segments from the parents. After genotyping all the strains in the mapping population, the final dataset contained 1,473,869 SNPs.

2.2.3 Phenotyping by growth rate

We measured the mycelial growth rates of *N. crassa* strains by standard tube method using agar filled serological pipet tips (Ryan *et al.* 1943). The asexual spores were inoculated at the other end of the tube and sealed with a plastic cap. We tracked the growth rate by marking the position of the mycelial front to the tubes 2 times per day for 4 days. Altogether we had 7 timepoints since the data was collected only after the initial growth of the inoculum. We plotted the distance of mycelial growth against time and estimated the growth rates (mm h^{-1}) by using the slope of the regression line.

The strains were replicated 3 times, and the tubes were put in 10 thermal treatments generated in climate cabinets (MTM-313 Plant Growth Chamber, HiPoint Corp., Taiwan) (II). We had 2 fluctuating regimes, low ranging 25–35 °C and high ranging 32–42 °C, and fast and slow fluctuations with frequencies of 120 min and 480 min respectively. The fast fluctuations were estimated to span within the generation time of *N. crassa*, whereas the slow fluctuations occurred between generations (Kronholm and Ketola 2018). The growth rates were measured also at constant mean and extreme temperatures of these ranges. At higher range the growth rates were measured at constant 32 °C and 37 °C, and at 42 °C which is known to be very stressful for *N. crassa* (Mohsenzadeh *et al.* 1998). The measurements at constant lower range temperatures 25 °C, 30 °C, and 35 °C were collated from a previously published data (Moghadam *et al.* 2020). In Moghadam *et al.* (2020), the growth rates were measured at constant 20 °C, 25 °C, 30 °C, 35 °C, 37.5 °C, and 40 °C that formed the TPC (I).

2.2.4 Statistical analysis and annotations

The statistical association between SNPs and variation in growth rate at different temperatures was studied by the genome association and prediction integrated tool (GAPIT) Version 3 R-package (Wang and Zhang 2021). In GWAS, GAPIT 3 corrects for the population structure and kinship, and so controls for false positives. We used Bayesian-information and linkage-disequilibrium iteratively nested keyway method (BLINK), which is a statistically powerful multi-locus test (Huang *et al.* 2019, Wang and Zhang 2021). In BLINK, the SNP markers are removed iteratively if they are in linkage disequilibrium with the most significantly associated reference SNP, and this procedure is repeated until no more markers can be removed. We run the BLINK separately for the average growth rates at each temperature treatment and the significance threshold for the associations was set to 0.01 after a Bonferroni correction. The positive allelic effects indicated that the individuals carrying the minor allele at a specific locus

had faster growth rate compared to other individuals in the population. The candidate genes associated with growth rate were searched from the Ensemble Fungi release 57 data base and the effects of SNP variants on proteins were processed by the Variant Effect Predictor web tool. The protein annotations were further searched from the UniProt and the FungiDB databases.

2.3 Quantitative genetics (II)

2.3.1 Estimating genetic architecture

To get an overall picture of the genetic architecture of thermal tolerance in *N. crassa*, we used quantitative genetic analyses that gave statistical summaries over all loci affecting growth rate at constant and fluctuating temperatures (II). The multivariate growth rate data was used in these analyses, meaning that the growth rate measured at each temperature was modelled as a discrete and potentially genetically correlated with growth rate at other temperatures (Kingsolver *et al.* 2004). The possible trade-offs in growth rate between constant and fluctuating temperatures were investigated by forming a genetic variance–covariance matrix and principal components of the phenotypic variation.

2.3.2 The G-matrix

The growth rate data including all measurements was used in a Bayesian multilevel model where the growth at each temperature treatment was explained by the strain's genotype. From the multivariate model we obtained a G-matrix with posterior estimates for the amount of variation and the strength of covariation in growth rate at constant and fluctuating temperatures. G-matrix included genetic and environmental variances, and genetic correlations and covariances. These estimates were also used to calculate the broad-sense heritabilities, and the coefficients of variation for the genetic and environmental components.

2.3.3 Principal component analysis

The PCA was used to find the most important partitions of genetic variation underlying the variation in average growth rate at constant and fluctuating temperatures. Based on the signs of the loading vectors, the principal components were interpreted as the modes of the TPCs (Izem and Kingsolver 2005). However, in principle, PCA does not assume any mode or trade-offs (Ashrafi *et al.* 2018). The first 3 principal components explained most of phenotypic variation in the strains average growth rate. The predictive values of these principal components were also used in GWAS to investigate which SNPs and candidate genes are associated with the modes of the TPC.

2.4 Experimental evolution (III) and competition assays (III, IV)

2.4.1 Measuring evolved trade-offs

Laboratory experiments allow to directly link the thermal conditions during organisms' evolution with the resulting population-level responses (Bennett and Hughes 2009). In laboratory, the environmental conditions are well-controlled which makes it possible to test specific evolutionary hypotheses without many confounding factors that would occur in nature. Typically, the experimental evolution studies have used replicate populations of microbes and other organisms that have short life cycles and high fecundity (Zeyl 2006, Bennett and Hughes 2009). In microbes, the fast clonal reproduction through cell divisions results in large effective population sizes which makes it possible to investigate evolution in real-time over thousands of generations. Experimental evolution is also a powerful method to study the evolution and existence of trade-offs when the fitness of populations that have evolved in one environment are measured in the same and in alternative environments (Bennett and Lenski 2007).

For microbes, the variation in fitness is easy to measure by population growth rate (r) and carrying capacity (K) in monocultures, or by competition assays in bicultures (Benton and Grant 2000, Bennett and Hughes 2009). The population-level proxies r and K are common estimates of fitness that can be assessed, for example, by monitoring the optical densities with a spectrophotometer (Saarinen *et al.* 2018). In competition assays, the relative fitness is often estimated by counting the difference in the number of colonies that carry distinguishable genetic markers (Lenski *et al.* 1991). We used experimental evolution, competition assays, and other fitness measurements to investigate the trade-offs in adaptation of large and small *S. pombe* populations to constant and differently fluctuating temperatures (III). In another competition experiment, we studied how fast temperature fluctuations, either on an evolutionary or an ecological timescale, affect the competitive success of bacterial species (IV).

2.4.2 The effect of population size on thermal adaptation (III)

We studied how the population size and the timescale of thermal variation during populations evolution affect the rate and extent of adaptation to constant and fluctuating environments (III). We tested the hypotheses suggesting that larger populations adapt to their environment more efficiently than smaller populations, that adaptation to fluctuations helps to cope with alternative environments with less fitness trade-offs, and that population size affects the occurrence of trade-offs differently in constant and fluctuating environments (Bono *et al.* 2017, Chavhan *et al.* 2021). We defined the trade-offs as a concurrent adaptation to one temperature environment and a resulting maladaptation to an alternative environment. In our experiment, we allowed large and small *S. pombe* populations to evolve for approx. 500 generations at constant mean and extreme

temperatures, and at fast, intermediate, and slow frequency fluctuations. We expected the adaptations to differ between the thermal environments as different adaptive mechanisms are assumed to be in work depending on the time scale of temperature variation in relation to generation time (Botero *et al*. 2015, Kronholm 2022).

We started the evolution experiment with 4 haploid ancestor populations (obtained from Dr. Bart Nieuwenhuis) which had either h^+ or h^- mating type and *ade6*-alleles producing either pink or red colour on selective plates. We had 4 biological replicates of each of the ancestors, and small ($N_e = 10^6$) and large ($N_e = 10^7$) effective population sizes, grown in liquid culture on 96-well and 24-well plates respectively. The population sizes were selected based on a literature review in which larger populations did not show fitness costs in fluctuating environments (Chavhan *et al.* 2021). In total we had 32 populations in each of the 5 temperature treatments, giving a total number of 160 populations.

In preliminary experiments we tested the constant mean temperature 34 °C to be close to optimal, and the high extreme temperature 38 °C close to detrimental for the ancestral strains. All fluctuations cycled with a sequence of 30–38–30 °C (steps of 1 °C) and the number of generations were around 2.5 at fast, 32 at intermediate, and 100 at slow fluctuations. These frequencies were selected based on previous simulations (Kronholm 2022). The temperature treatments were generated in growth chambers (MTM-313 Plant Growth Chamber, HiPoint Corp., Taiwan) and cultures were transferred every 24 h to a fresh medium with a constant concentration. During the evolution experiment small populations had approx. 464 generations and large populations 507 generations.

The adaptation to evolutionary environment and the maladaptation to alternative environments were evaluated by competition experiments between experimental strains and their ancestors, and by measuring growth parameters in isolation. In competition assays, the samples were plated at the initial stage of the experiment (mixes were estimated to have 1:1 proportion), and again at the end of the experiment. The relative fitness of the evolved strain compared to the ancestor was counted as the change in proportions of the colonies between platings.

During competition experiments, bicultures were grown on 96-well plates in thermal cabinets (Lab Companion, ILP-12; Jeio Tech, Seoul, Korea) and transferred to fresh medium every 48 h. Competitions at constant temperatures and fast fluctuations lasted 4 days (4 cycles), and at intermediate fluctuation 13 days (1 cycle). The temperature treatments matched the conditions during evolution experiment, except that populations originating from slow fluctuations were measured at constant mean and extreme temperatures (duration of 1 cycle was 40 days). Because of the extent of conducting a full factorial experiment, we chose to compete the populations evolved at mean temperature in all treatments, and other populations in their evolution environment and at constant mean temperature to make comparisons. Each combination of competition assays was replicated 3 times, which was 1080 competition assays in total.

Evolved strains competed with an ancestor of the same mating type but a different *ade6*-allele so that they could be identified by color on low-adenine plates. We counted manually the ratio of colony-forming units for the evolved strains and ancestors and used the average of 3 replicates in statistical analyses. We used a Bayesian generalized linear mixed model in which the relative fitness was predicted by temperature treatment and its interaction with the population size. We run also separate models for small and large populations to compare posterior estimates at different treatments. Trade-offs were expected to occur when evolved strains had higher fitness relative to the ancestor in one temperature environment and lower fitness in another environment.

Based on the results of the competition experiments, we further investigated the ancestors and the evolved strains only from the large populations. First, we created a clone library by sampling 4 colonies from 80 evolved populations and the 4 ancestors. Altogether 320 clones were grown in randomized order on 100-well Bioscreen plates. The optical density measurements were made with temperature-controlled spectrophotometers (Bioscreen C®, Oy Growth Curves Ab, Ltd., Finland). The maximal growth rate r_{max} and the carrying capacity K were estimated from the growth curves measured at constant 30 °C, 34 °C, 38 °C, and at fast 30–38–30 °C fluctuations. The runs lasted 4 days and each clone plate was measured 4 times in all treatments. We used this data in a Bayesian multilevel linear model to estimate the phenotypic variation between populations, the genetic variation within populations, and the environmental variation between clones.

2.4.3 The effect of thermal preadaptation on invasion success (IV)

We studied with experimental invasions if thermal fluctuations during evolution select for increased competitive ability, and if fluctuation-maladapted community species aid invasions together with concurrent temperature fluctuations in the environment (IV). We used bacteria from a previous experimental evolution study in which strains had evolved for approx. 86 generations in thermal cabinets (Lab Companion, ILP-12; Jeio Tech, Korea) under constant 30 °C or fluctuating temperatures cycling 20–30–40–30–20 °C for 2 h at each temperature (Saarinen *et al.* 2018). In this study, we used strains from *Serratia marcescens ssp. marcescens* (ATCC® 13880™), *Pseudomonas putida* (ATCC® 12633™), *Pseudomonas fluorescens* (ATCC® 13525™) and *Novosphingobium capsulatum* (ATCC® 14666™), all originally obtained from American Type Culture Collection (ATCC®).

In experimental invasions, the bacterial competitor species *P. putida*, *P. fluorescens*, or *N. capsulatum* that had evolved at either constant or fluctuating temperature were set to compete with the dominant invader *Serratia marcescens*, which had also evolved at either constant or fluctuating temperature. We ran the competition experiments for 3 days at constant 30 °C and at fluctuating 20–30–40–30–20 °C (2 h per step) temperatures matching the conditions during evolution. Altogether we had 8 treatment combinations in the competition

experiments, and we used 1 clone per each replicate population (n = 8) of the bacterial species. In total there were 192 competition experiments.

The clones were chosen randomly to compete in bicultures in 15 ml centrifuge tubes containing sterile nutrient broth medium. To resemble the starting point of invasions where the invaders are rare, we estimated the initial invader-to-competitor ratio to be 1:24 in each assay. Both the invader and its competitor species were inoculated concurrently, hence having an equal opportunity to use the available resources. Therefore, our experiments reflected asymmetric competition rather than invasion in a strict sense, i.e. when the invader arrives later than its resident species that is already adapted to local conditions.

We plated all the samples on DNase test agar plates that allowed for separation of the invader colonies from the competitor species colonies. The colonies on each plate were counted manually and the competitive success of the invader was estimated as the proportion of *S. marcescens* colonies in the total colony count after the competition. However, there was no defined threshold value for the competitor species' displacement. We used a generalized linear mixed model to statistically separate the effects of the environment during competition, the evolution of the invader, and the evolution of the competitor species on the competitive success of *S. marcescens.*

3 MAIN RESULTS AND DISCUSSION

3.1 Genetic architecture of thermal tolerance (I, II)

To better understand the genetic architecture of thermal tolerance at constant and fluctuating temperatures, we integrated GWAS and quantitative genetics in fungus *N. crassa* (Cortés *et al.* 2020, Buckley and Kingsolver 2021). With GWAS we were able to pinpoint specific large-effect loci and study the effects of SNPs on growth rate, whereas quantitative genetics analyses gave us estimates of the genetic variation over several small-effect loci and partitioned this variation into modes of the TPC (Fisher 1919, Izem and Kingsolver 2005, Fu *et al.* 2013). We tested the hypotheses suggesting that fitness trade-offs or genetic constraints could occur in adaptation to different constant temperatures forming the TPC (Berger *et al.* 2014, Latimer *et al.* 2014, 2015) (I), or between constant temperatures and fluctuations of varying frequency (Ketola and Kristensen 2017, Kristensen *et al.* 2020) (II). Our results contradicted these hypotheses, and in general, indicated no trade-offs in adaptation to different constant and fluctuating temperatures (I, II), except that the adaptation to constant heat could be more evolutionary constrained (II).

The GWAS for loci influencing TPCs discovered unique associations at specific temperatures, as some SNPs affected growth rate of *N. crassa* only at low, intermediate, or high temperatures (I). There were also SNPs that were associated at multiple constant temperatures across the tolerance range, but their allelic effects on growth did not vary greatly in sign or magnitude depending on the temperature. There was little evidence for potential trade-offs, and only few SNPs segregated among natural populations of *N. crassa*. Moreover, most of the minor alleles slowed down growth rate, and analyses suggested negative selection in natural populations (Saltz *et al.* 2017). In line with our results, an experimental evolution study with insects found temperature-specific allelic effects on growth rate but observed no trade-offs in TPC shape (Berger *et al.* 2014).

Also, another study with fish showed evidence that genes with low pleiotropy drive thermal stress response, whereas highly pleiotropic genes have only limited importance (Papakostas *et al.* 2014).

Similar findings were made in GWAS comparing loci associated with growth rate at constant and fluctuating temperatures (II). Interestingly, many unique SNPs were found at constant temperatures or at fluctuations of both ranges and frequencies, especially at higher temperatures. This indicates that some partition of the genetic variation is specific to the type of fluctuations, and that adaptation to extreme heat could be more distinct in *N. crassa*. Similar findings were reported in an experimental evolution and resequencing study with bacteria that found 'particular signature genes' in adaptation to constant and fluctuating temperatures (Deatherage *et al.* 2017). Accordingly, another study with *Drosophila* found that an independent set of genes regulated transcriptional responses to heat stress after acclimation at fluctuating versus constant temperatures (Sørensen *et al.* 2016). The GWAS in this study (II), discovered also shared SNPs that were associated at multiple temperatures, but no trade-offs were discovered in allelic effects between temperatures, and almost all minor alleles decreased growth rate. Moreover, most of the allelic variants did not affect the protein structure and were at the regulatory area of the genes, having potential regulatory functions. However, the types of allelic variants were not associated clearly with growth at constant or fluctuating temperatures, even though regulatory area mutations are often expected to dictate the dynamic responses under thermal fluctuations (López-Maury *et al.* 2008).

When we annotated the candidate genes from both association studies (I, II), we found overlapping molecular and physiological functions between temperatures. Most of the genes encoded proteins essential for the basic functions in fungi, such as cellular growth and energy metabolism, and did not have obvious connection to plastic stress response. However, many of the physiological functions of these proteins are known to be sensitive to temperature or other environmental stresses (I, II), and probably linked to the general stress response (Verghese *et al.* 2012). In line with this assumption, it has been found that in yeast, most of the genes upregulated during heat stress are involved in changes in metabolic pathways, to ensure the energy supply and allocation during stress (Richter *et al.* 2010). For example, we found that the TORC1 multi-protein complex that works in signal transduction and temperature-dependent balancing of growth was associated at constant 42 °C (Urban *et al.* 2007, López-Maury *et al.* 2008). As an exception, a DNAJ (HSP40) chaperone, which is known to be part of the thermal stress response by preserving the structure of unfolded proteins, was associated at fast temperature fluctuations (Sørensen *et al.* 2003, Lecheta *et al.* 2020). Other GWAS and gene expression studies have also linked HSPs to variation in thermal tolerance and adaptation to climate change (Lecheta *et al.* 2020, Logan and Cox 2020). These findings are interesting as we found the slowest growth rates at high extreme temperature 42 °C and at fast 32–42 °C fluctuations.

With quantitative genetics, we estimated the TPC modes for most of the genetic variation in growth rate, that is, over many small-effect loci (II). We found

high heritability values across temperatures, meaning that there was plenty of genetic variation in growth rate among the strains. We detected also strong positive genetic correlations between the fast and slow frequency fluctuations, and between fluctuations and their mean temperatures. This indicated that most of the genetic variation in growth is shared across temperatures in *N. crassa* and predicts correlated responses to selection (Czesak *et al.* 2006). The amount of genetic variance and positive correlations together point that in *N. crassa* there should be no significant trade-offs or genetic constraints on adaptation to increased thermal variation. However, the genetic correlations and covariances with the high extreme temperature were markedly lower, pointing that the growth at 42 °C could evolve more independently from the selection at other temperatures. Similar quantitative genetic results have been found before for the growth rate of *N. crassa* at constant 40 °C (Moghadam *et al.* 2020), suggesting that different genes or amounts of gene expression might affect acclimatization at the higher thermal limit.

The high heritability values and strong positive genetic correlations between temperatures might be found because some of the strains are superior in growth rate, so called 'supergeneralists' or 'master of all temperatures' (Huey and Hertz 1984, Kristensen *et al.* 2020). We verified this suggestion by PCA and interpreted the modes of most genetic variation and evolutionary potential in TPCs (II). Most of the variation was found to be in overall performance i.e. in the faster-slower mode (83 %), whereas hot-cold shift in thermal optimum (8 %) and shape change towards better growth at high temperatures (4 %) represented a minor aspect. Thus, there should be least genetic constraints to evolution in the general growth ability regardless of the temperature, meaning that some strains grew better, and some slower across all the tested temperatures (Angilletta 2009). Thus, PCA neither supported strong genetic constraints on TPC evolution, which challenges the traditional hypothesis of specialist-generalist trade-offs between higher performance and wider tolerance (Lynch and Gabriel 1987, Gilchrist 1995). Previously, the TPC elevation has been found to predominate growth rate in *N. crassa* (Moghadam *et al.* 2020) and performance in other species (Yamahira *et al.* 2007, Shama *et al.* 2011, Ketola *et al.* 2014, Latimer *et al.* 2015, Bartheld *et al.* 2017, Schaum *et al.* 2022). In addition, TPC elevation had a nearly significant association with HSP40 protein, whereas heat tolerance was associated with TORC1.

We found little genetic variance in heat tolerance, which in *N. crassa* means most likely the variation in the induction or the magnitude of heat-shock response (Moghadam *et al.* 2020). One possible explanation to this is that the nature-derived strains have undergone selection during their thermal history and are well-adapted to heat, decreasing the amount of variation in this tolerance trait. This view was supported by the GWAS results indicating that beneficial mutations with large effects on growth were rare (I, II), and probably already fixed in natural populations. Other studies have also suggested that the ability of organisms to evolve higher heat tolerance might be limited if substantial genetic changes, such as gene duplications are required (Riehle *et al.* 2001, Hoekstra and Montooth 2013). In general, the ability to tolerate prolonged heat is known to be

a trait that is highly evolutionary conserved, and constrained, as many experiments have found little variation in critical thermal maximum across taxa (Kellermann *et al.* 2012, Araújo *et al.* 2013, Kristensen *et al.* 2015).

Altogether, there was no strong evidence for trade-offs or genetic constraints, contradicting the idea that the variation in TPCs would exist mainly due to antagonistic pleiotropy (I), or that distinct genetic architectures would underlie adaptation to constant and fluctuating temperatures (II). Small-effect loci indicated a shared polygenic basis of thermal tolerance in *N. crassa* across temperatures, evolving mainly via individual's general growth ability i.e. elevated TPCs. Large-effect loci supported this view as many associated genes had overlapping functions of general importance, which could be due to cells general stress response. Previous studies with *Drosophila* have made similar conclusions, as most of the QTL effects in standing genetic variation and accumulated mutational variation corresponded to the elevation in TPCs (Latimer *et al.* 2014, 2015). In our study, the increasing variation in temperature should not set bounds on the evolutionary potential of *N. crassa*, but our results suggest that the adaptation to extreme heat is probably more constrained (II). In next experiments, measuring the growth rates of deletion mutants for the genes associated in GWAS (I, II) at fluctuating and constant temperatures would clarify their causality for thermal tolerance in *N. crassa*.

3.2 Selection at constant and fluctuating temperatures (III)

When organisms adapt genetically to the local environment, they are expected to lose fitness in alternative environments due to antagonistically pleiotropic mutations (Cooper and Lenski 2000, Bennett and Lenski 2007). We investigated the genetic trade-offs in *S. pombe* populations after they were allowed to evolve for approx. 500 generations at constant temperatures or under fast, intermediate, or slow fluctuations (III). We tested the hypothesis suggesting that antagonistic pleiotropy should evolve more readily in constant environments due to relaxed selection than in variable environments where different selection pressures alternate (Bono *et al.* 2017). We also tested the hypothesis that evolution in constant and fluctuating environments could diverge from each other and lead to specific adaptations (Botero *et al.* 2015, Kristensen *et al.* 2020). To demonstrate the adaptations to evolutionary environment and trade-offs in alternative environments, we measured the fitness of all evolved strains by competition experiments against their ancestors. Then we further investigated the possible mechanisms of adaptation and populations evolutionary potential by measuring the r_{max} and K separately for each ancestor strain and for the strains evolved in large populations.

We found that the populations adapted to constant heat had the greatest fitness increase relative to the ancestors, as they were stronger competitors at extreme temperature. This indicates that during the evolution, populations had gained some beneficial mutations to tolerate stressfully high temperatures.

Furthermore, specialists are often assumed to adapt faster to their constant environment by fixing beneficial mutations due to increased efficacy of selection (Snell-Rood and Ehlman 2021). Also, an experimental evolution study with bacteria have demonstrated that the adaptive responses are fastest and most extensive at constant extreme temperatures (Bennett *et al.* 1992). In clonal organisms, the thermally stressful environments can select for mutator genes and elevated baseline production of heat shock proteins, which are unbeneficial at more constant conditions (Kimura 1967, Cooper *et al.* 2007, Hoekstra and Montooth 2013, Lecheta *et al.* 2020). However, we did not find a reciprocal maladaptation when populations adapted to extreme heat were competed at constant mean temperature. Also, populations evolved at mean temperature did not show significant fitness improvements compared to the ancestors at constant mean temperature, or at fast or intermediate fluctuations. This could mean that the ancestors were already close to their thermal optimum and well-adapted to the experimental mean temperature, but also having high levels of phenotypic plasticity that makes them tolerate fluctuations. However, populations evolved at mean temperature clearly lacked some aspects of heat tolerance, as they had decrease in relative fitness when competing at extreme temperature. The extensive adaptation and maladaptation observed in populations that had evolved at constant temperatures indicates that these conditions can act as stronger selective pressures than temperature variation (Bennett *et al.* 1992, Kingsolver *et al.* 2009, Snell-Rood and Ehlman 2021).

In general, when populations evolved in thermally variable environments, no clear adaptations were detected to fast, intermediate, or slow fluctuations. This supports the idea that the ancestor populations have attributes that make them readily tolerate fluctuations, for example, yeast populations might optimize their fitness by tuning the rate of switching between individual phenotypes depending on the frequency of environmental fluctuations (Acar *et al.* 2008). On the other hand, in fluctuating environments the direction and intensity of selection varies, which could make adaptation to require more generations to occur, as fluctuations might slow down fixation and purge mutations from population (Snell-Rood *et al.* 2010, Kingsolver and Buckley 2017). The evolution of thermal generalists has been observed in evolution experiments made with bacteria for thousands of generations (Bennett *et al.* 1992, Kassen 2002, Ketola *et al.* 2013). However, these studies have often found that the fitness evolves fast during the first thousand generations, which is closer to the length of our study. In competition experiments, the only significant maladaptation to mean temperature was found for populations that had evolved under fast fluctuations. This is in line with studies suggesting that tolerance to fast fluctuations requires allocation to dynamic stress response, which is costly at nonstressful conditions (Sørensen *et al.* 2003, Kristensen *et al.* 2020). It was also interesting that, for the populations evolved at slow fluctuations, there was no comparable maladaptation to extreme temperature as with populations that had evolved at mean temperature. Slow fluctuations had an estimated cycle of 100 generations which should be increasingly experienced as constant conditions. Hence, genetic adaptation should be favored as an adaptive mechanism over plasticity and lead,

for example, to the evolution of two distinct specialist lines (Botero *et al.* 2015, Snell-Rood and Ehlman 2021, Kronholm 2022).

The average values of r_{max} and K estimated from the growth curves were plotted as reaction norms to see the within population variation at range mean 34 °C, at extremes 30 °C and 38 °C, and at fast fluctuations 30–38–30 °C. From these reaction norms we detected the fitness improvements and decrements in evolved strains compared their own ancestor. We also quantified the phenotypic variance between populations from the same evolutionary treatment, between clones indicating the amount of genetic variation within populations, and environmental variation that depicts the sensitivity of phenotypes to evolutionary environments. On average, we found low phenotypic variance between populations for both r_{max} and K, pointing that populations with the same thermal history tended to evolve similar phenotypes due to uniformity of selection pressures (Ketola and Kronholm 2023). Overall, most of the variation for both growth parameters was attributed to environmental variation between clones. This suggests that, in general, the high thermal sensitivity of growth parameters to the evolutionary treatment could stem from bet-hedging or phenotypic plasticity (Tonsor *et al.* 2013, Ketola and Kronholm 2023). By the randomization of the clone libraries and controlled conditions during measurements, we can rule out that this observation would originate from a systematic error caused by external factors such as differences in handling the samples. In general, the populations from fluctuations had an equal variance with ancestors, whereas populations from constant temperatures had evolved lower environmental variance. This contradicts the idea that alternating temperatures in fluctuating environments would lead to higher environmental variance, affecting the accessibility of genetic variation to natural selection (Ketola and Kronholm 2023). However, populations from constant temperatures showed genetic canalization that prevents evolutionary change by hiding genetic variation from selection, and thus makes phenotypes less sensitive to changes in temperature (Kawecki 2000). It has been suggested that ancestral plasticity is more likely to lead to genetic canalization in constant environments where plasticity is costly (Scheiner and Levis 2021). In yeasts, one possible mechanism for genetic canalization is the loss of stop codons and the consequent preservation of the reading frame in protein synthesis (Giacomelli *et al.* 2007).

The genetic variation within populations did not differ much between evolutionary treatments for either r_{max} or K. However, there was a slight indication of more genetic variance within populations that had evolved at constant temperatures. The lower genetic variation in fluctuating environments could be due to positive selection fixing the traits related to generalism or plasticity (Botero *et al.* 2015, Kronholm 2022). This is opposite to the common expectation that fluctuating environments would maintain higher amount of genetic variation, though the evidence also from other experiments has been mixed (Bürger and Gimelfarb 2002, Kassen 2002). Interestingly, there were high levels of genetic variance in the ability to grow at extreme temperature within populations from different evolution environments. This supports the extensive evolutionary response to extreme temperature that was found in competition

experiments. From the reaction norms we were also able to detect that the extreme-evolved populations tolerated heat better than their ancestors, as they had on average higher r_{max} and K. In competition experiments, the populations evolved at mean temperature had drastic maladaptation to extreme heat, but the reaction norms showed that still a fraction of the clones grew better than their ancestor in extreme environment. Similar results were found in an experimental evolution experiment showing that most, but not all bacterial lines adapted to low temperature were maladapted to high temperature (Bennett and Lenski 2007).

In our experiment, the populations originating from fast, intermediate, and slow fluctuations seemed to do on average worse than their ancestors at 38 °C. It has been shown in a meta-analysis, that if organisms evolve to be more plastic, there will be a trade-off with fixing higher upper thermal limits (Barley *et al.* 2021). However, the evolution under thermal fluctuations of different frequency improved populations r_{max} at fast fluctuations and at more optimal temperatures 30 °C and 34 °C. This indicates that thermal fluctuations could select for better ability to grow fast during the times of more benign conditions, for example, by reversible plasticity (Kristensen *et al.* 2020). The same was not detected for the K, as fluctuation-adapted populations did generally worse than their ancestors in all treatments. This could mean that, due to cost associated with a fast growth rate in short-term, the population density could be lower in long-term (Bideault *et al.* 2023).

In a summary, the competition experiments supported the idea that extreme heat forms a strong selection pressure, leading to prevalent adaptive responses over populations. On the other hand, if populations are maladapted to cope with high temperatures, there will be drastic fitness costs. Along with this, other studies have suggested that high extreme temperatures can select for larger shifts in TPCs than the changing mean temperatures (Angilletta *et al.* 2010, Buckley and Huey 2016). However, we did not find strong evidence for the trade-offs between competition environments or the expected differences in adaptation to constant and fluctuating temperatures. One plausible explanation is that the ancestors were already near the fitness optimum at experimental mean temperature and at fluctuations spanning over mostly favorable temperatures. This was supported by the high levels of environmental variance in r_{max} and K which could mean that ancestors had growth attributes related to generalism or plasticity (Tonsor *et al.* 2013, Ketola and Kronholm 2023). However, there was no evidence that the evolution under fluctuations would have maintained more genetic variation in populations or led to higher environmental variance. Rather, we found high levels of genetic variation in the ability to grow at extreme temperature, and an indication for the genetic canalization in populations that had originated from constant temperatures.

3.3 The effect of population size (III)

We investigated the evolution of genetic trade-offs in large and small *S. pombe* populations and how efficiently they can adapt to constant temperatures and fluctuations of different frequency (III). We tested the hypotheses concerning the effects of population size, and the interaction of the population size and the evolution environment (Chavhan *et al.* 2021). After evolution in thermal treatments for approx. 500 generations, the strains were competed against ancestors to estimate relative fitness. We found no clear evidence that larger populations would have adapted to temperature more efficiently than smaller populations, and that population sizes had a similar amount of fitness costs regardless of their evolution environment.

Larger populations are expected to have faster evolution and higher fitness due to larger number of beneficial mutations and better ability to recover from population bottlenecks compared to smaller populations (Botero *et al.* 2015). Contradicting this assumption, in our experiment, there was no significant difference in relative fitness between small and large populations when tested over all temperature treatments. However, when we tested the differences between population sizes separately for each treatment, we found two cases where large populations had significantly higher relative fitness than small populations. This increase was detected when populations that had evolved at extreme temperature competed in a matching environment, indicating that the larger populations adapted more efficiently to extreme heat. A previous experimental evolution study with yeast has also found that populations with larger effective population size have more extensive adaptive responses to stressful conditions (Samani and Bell 2010). In addition, we found that the larger populations that had evolved at constant mean temperature had significantly higher relative fitness at fast fluctuations, even though there was no clear adaptation compared to ancestral fitness when observed over all populations.

One reason why we did not detect a significant population size effect in general, could be that the effective population sizes chosen for the evolution experiment were too similar. In a previous literature review of bacterial experimental evolution, it was suggested that in fluctuating environments, populations with $N_e \approx 10^8$ are less likely to show fitness costs than smaller populations with $N_e \leq 10^7$ (Chavhan *et al.* 2021). In our experiment, the difference between population sizes was smaller due to technical limitations, but the same order of magnitude (small $N_e = 10^6$ and large $N_e = 10^7$). However, when Chavhan *et al.* (2021) tested the hypotheses concerning populations size and environmental variability in an evolution experiment, the difference between population sizes they had was even larger. Another possibility regarding our experiment is that population size can have both positive and negative effects on fitness, which could cancel out each other. For example, even though higher mutational supply is beneficial in large populations, simultaneously occurring beneficial mutations can compete for fixation, slowing down evolution by clonal interference (Gerrish

and Lenski 1998). Conversely, small populations are less likely to get beneficial mutations, but the evolution could be accelerated due to faster fixation rates (Handel and Rozen 2009).

In addition, we did not find that population size or its interaction with evolution environment would have defined the relative fitness of an evolved population. With pairwise comparisons between competition environments, we found that both large and small populations that had evolved at high temperature were better competitors in a matching competition environment than in an alternative mean competition environment. Similar finding was made for large and small populations that had evolved at constant mean temperature and were better competitors in a matching competition environment than in an alternative extreme competition environment. Previous studies have suggested that large populations should be especially vulnerable to sudden environmental changes due to rapid adaptation to local environment (Chavhan *et al.* 2020), and therefore the optimal adaptation would be a balance between the speed of adaptation and the exposure to environmental change (Chavhan *et al.* 2019).

The only significant difference in the number of trade-offs was found when large populations that had evolved at intermediate fluctuations were slightly better competitors in a matching environment than at alternative mean temperature, an interaction that was not found for small populations. This finding is opposite to Chavhan *et al*. (2021) who demonstrated that large bacterial populations could avoid fitness costs in alternative environments when they have evolved at fluctuating resource environments, and that the mechanism of cost avoidance was the enrichment of beneficial mutations in the same generalist line. In their literature review, Chavhan *et al*. (2021) also pointed a bunch of studies that showed evidence for an indirect link between population size and environmental variability affecting trade-offs (e.g. Bennett and Lenski 1999, Buckling *et al.* 2007, Ketola and Saarinen 2015). Yet, our study is the first one attempting to directly test the effects of population size on adaptation to constant and fluctuating temperatures.

3.4 **The evolution of correlated traits (IV)**

A predictive understanding of how temperature fluctuations affect interspecific competition and diversity in bacterial communities is currently lacking (Lax *et al.* 2020). It has been hypothesized that stressful perturbations during biological invasions, preadapted invader species, and maladapted community species could increase the rate of competitive exclusion (Lee and Gelembiuk 2008, Saarinen *et al.* 2019). To investigate if thermal fluctuations select for traits that correlate with increased invasiveness, we used bacterial species that had evolved either at constant or rapidly fluctuating temperatures (Saarinen *et al.* 2018) and implemented interspecific competition experiments in similarly constant or fluctuating temperatures (IV). The results showed that temperature fluctuations during competition, i.e. on an ecological timescale, made the invader

S. marcescens more successful. However, there was no evidence that the invaders evolution under temperature fluctuations, or the competitor species evolution at constant temperature would have affected the outcome of the competition.

Climatic fluctuations are often associated with increased disturbance (Lande 2009, Parepa *et al.* 2013), and several field studies have found disturbed environments to be more prone to invasions than non-disturbed (Davis *et al.* 2000, Melbourne *et al.* 2007). In accordance with our hypothesis, the fast temperature fluctuations during competition clearly promoted invaders competitive success, emphasizing the importance of ecological timescale in invasions (Shea and Chesson 2002). Fluctuations can, for example, cause local extinctions that release resources for fast-growing invaders to exploit and allow to take over the freed niche space (Lee and Gelembiuk 2008, Lockwood *et al.* 2010). Previous laboratory studies with microbes have mainly focused on the fluctuations in resource availability (Li and Stevens 2012, Liu *et al.* 2012), whereas our study is among the first ones to investigate the direct effect of thermal fluctuations on microbial competition (Descamps-Julien and Gonzalez 2005, Saarinen *et al.* 2019, Lax *et al.* 2020). On the other hand, there is also some evidence that thermal variation could facilitate species coexistence and maximize the diversity in local communities if disturbances happen on an intermediate intensity and timescale (Descamps-Julien and Gonzalez 2005, Jiang and Morin 2007).

Invasive species are often suggested to originate from disturbed and heterogenous areas, which could preadapt them to novel environments (Lee and Gelembiuk 2008, Hufbauer *et al.* 2012). For example, fast thermal fluctuations might select for generalist strategies and life history traits, such as fast growth rate, shorter generation times and higher fecundity that make species more successful invaders (Colinet *et al.* 2015, Chevin and Hoffmann 2017, Lax *et al.* 2020). On the contrary to these hypotheses, we did not find that rapid thermal fluctuations would have preadapted *S. marcescens* to be more competitive than other bacterial species in fluctuating environment. Another study has found that the matching timescale between phenotypic plasticity and environmental fluctuations during competition can make cyanobacteria having advantage over other species (Stomp *et al.* 2008).

Invasions could also be pronounced if the native species are maladapted to the prevailing thermal conditions and so weaker competitors (Blackburn and Duncan 2001, Shea and Chesson 2002, Duncan *et al.* 2011). In our experiment, the maladaptation of competitor species to fluctuations did not affect the competitive success of *S. marcescens*. Along with this, none of the interactions between studied factors were found to affect the outcome of the competition, pointing that these factors were not dependent on each other. Some studies have found stronger evidence for interactions where the environmental conditions during competition, and the traits of competing species acted together (Kreyling *et al.* 2008, Mächler and Altermatt 2012, Saarinen *et al.* 2019). For example, a previous study has shown that the similarity in traits between competing species could lead to competitive exclusion in temporally fluctuating environments, if different species are not having the competitive advantage at different times (Stomp *et al.* 2008).

Altogether, our results demonstrated that the temperature fluctuations did not significantly affect the evolutionary processes that were assumed to explain invasion success. There are few possible reasons that arise from the design of the evolution experiment from which the populations originated (Saarinen *et al.* 2018) and the competition assays we implemented in this study (IV). First, the competitive success of *S. marcescens* was high when measured after 3 days of competition, as it had almost outcompeted its competitor species. This dominant growth ability could have confounded us from observing the effects of temperature treatments. However, our additional analyses indicated that the competitive success of *S. marcescens* was not explained by its growth characteristics nor the ability to compete with other species by resource or interference competition. In previous competition experiments, *S. marcescens* has been set against multiple species in the same culture and competitor species were supplemented to maintain the community, leading to less pronounced invasions (Ketola *et al.* 2017, Saarinen *et al.* 2019). However, in Saarinen *et al*. (2019) the nonsignificant effect of the invader's evolution environment after 3 days of competition was comparable to ours, and the significant effect was found only at the subsequent stages of invasion.

In the evolution experiment preceding our invasion study, the growth rates and yields were estimated for the evolved bacterial populations at constant and rapidly fluctuating environments (Saarinen *et al.* 2018). The results showed increased growth ability for some of the competitor species but not for the invader *S. marcescens*. This lack of adaptation might well explain why the evidence also for the thermal preadaptation and increased invasiveness was missing in competition experiments. Another possibility could be that during the experimental evolution there was not enough time for a thermal generalism to evolve when starting from a homogeneous gene pool. However, the evolutionary time in an experimental setup closely similar to ours has been sufficient for adaptations to occur in response to selection (Ketola *et al*. 2013). This suggests that within the given time, the selection for improved competitive ability was not very strong.

Overall, our study highlights the importance of the present environmental fluctuations in promoting species' competitive success and potentially facilitating biological invasions. This points that conserving relatively undisturbed habitats could be the best way to prevent large-scale competitive exclusions in nature (Hobbs and Huenneke 1992). Surprisingly, the relative effect of thermal fluctuations on the evolutionary timescale seemed minor, which might be explained by species-specific traits or some technical limitations in study design. Further studies on the multifactorial nature of invasions are needed to forecast the general patterns of how temperature alters microbial communities (Lax *et al.* 2020). The information emerging from microbial studies is also important since climate change will increase the spread of pathogens into new habitats and hosts (Bennett and Hughes 2009, Ricciardi *et al*. 2017).

3.5 Absence of trade-offs in thermal adaptation (I, II, III, IV)

In this thesis I integrated methods from molecular and quantitative genetics and used experimental evolution to study the evolution of thermal tolerance. The results from the studies (I, II, III, IV) did not find strong support for the hypothesized fitness trade-offs or evolutionary constraints between adaptation to constant and fluctuating temperatures. To summarize, there were no prominent trade-offs in thermal tolerance at the levels of: individual loci and allelic effects (I, II), most of the genetic variation and genetic correlations (II), adaptation with larger or smaller population size (III), or evolutionary responses at constant and fluctuating temperatures (III, IV). However, the tolerance to extreme heat seemed to be a special case with specific large-effect loci, little genetic variation, and more independent evolution in some species (I, II). On the other hand, high temperatures might form a strong selection pressure that facilitates fast evolution (III). Next, I will discuss previously presented biological explanations that help to outline my conclusions about the absence of trade-offs in thermal adaptation.

The costs of adaptation have long been one of the fundamental assumptions in evolutionary biology (Darwin 1859, Levins 1968, Stearns 1989). Today, TPC trade-offs are part of the models that are used to predict species survival under climate change (Schulte *et al.* 2011, Sinclair *et al.* 2016, Buckley and Kingsolver 2021). Paradoxically, the evidence for the existence of trade-offs in thermal adaptation has been elusive, as fitness costs and genetic constraints are not always demonstrated in experiments, or they are weaker than expected (Bennett *et al.* 1992, Bennett and Lenski 1993, 2007, Angilletta 2009, Bideault *et al.* 2023). This lack of evidence has led to a debate about the existence of genotypes that are superior in both thermal tolerance and performance (Huey and Hertz 1984, Scheiner 1993, Conover *et al.* 2009, Kristensen *et al.* 2020). According to the TPC optimality models and life-history biology, the evolution of superior genotypes should be prevented by specialist-generalist trade-offs at multiple biological levels (Levins 1968, Stearns 1989, Angilletta 2009). However, empirical evidence shows that supergeneralists, that are not hindered by the cost of plasticity, exist across taxa (Kassen 2002, Conover *et al.* 2009, Latimer *et al.* 2015, Manenti *et al.* 2015, Murren *et al.* 2015, Schaum *et al.* 2022), and that they can outcompete specialists due to lack of trade-offs in alternative thermal environments (Kassen 2002, Callahan *et al.* 2008, Duncan *et al.* 2011, Ketola *et al.* 2013). Likewise, many TPC studies have found most of the genetic variance to be in overall performance across temperatures, i.e. in the faster-lower mode (Yamahira *et al.* 2007, Shama *et al.* 2011, Latimer *et al.* 2015, Moghadam *et al.* 2020), which indicates that the evolution of positively correlated performance traits between extreme and benign temperatures are possible. Especially, the TPCs measured for growth have been found to exhibit the faster-slower mode (Angilletta 2009).

To overcome the costs caused by trade-offs, superior generalists must acquire additional energy to perform well across temperatures, or alternatively,

reallocate their energy among different traits (Angilletta 2009). Thus, the life history explanation why faster-slower mode predominates TPCs comes from the acquisition and allocation trade-offs between different traits (Van Noordwijk and De Jong 1986, Houle 1991). According to a hypothesis of condition-dependent traits, the lack of trade-offs on individual level can be explained by the larger amount of genetic variation in individual's overall fitness traits compared to stress tolerance (Rowe and Houle 1996, Kristensen *et al.* 2020). For example, if individuals differ genetically in their ability to acquire resources, some of them will have higher general condition which is reflected to their ability to perform well across temperatures (Van Noordwijk and De Jong 1986). In this way, the condition-dependent traits can capture the high levels of the genetic variance and resources in condition, and correlations between traits remain positive (Rowe and Houle 1996, Berger *et al*. 2014). Indeed, it has been found that the traits associated with individual's overall fitness are usually those related to cells resource uptake and energy efficiency (Kristensen *et al.* 2005, Pedersen *et al.* 2008), and that the evolution of faster growth rate often involves changes in resource acquisition and metabolic assimilation, accompanied with evidence for superior generalist that grow better across temperatures (Billerbeck *et al.* 2000, Van Doorslaer and Stoks 2005).

Evolution can reduce fitness costs over time through genetic mechanisms and strong selection against trade-offs (Murren *et al.* 2015). For example, mutations that compensate the effects of antagonistically pleiotropic mutations can accumulate in genes that regulate the basic cellular pathways and individual's overall fitness (Yadav *et al.* 2015). Genetic resolutions to costs might also explain why trade-offs are often observed in the laboratory experiments or between species, but rarely in natural populations or within species (Glazier 1999, Leroi *et al.* 2005, Yadav *et al.* 2015, Agrawal 2020). Furthermore, the polygenic nature of thermal tolerance found in many experiments of both quantitative and molecular genetics indicates that there is not a single 'magic bullet' i.e. one major gene for thermal adaptation (Cortés *et al.* 2020, Lecheta *et al.* 2020). So, it is well recognized that the genetic basis of thermal tolerance is complex, and that antagonistic pleiotropy between adaptation to different temperatures does not necessarily constrain evolution (Hochachka and Somero 2002, Kassen 2002, Buckling *et al.* 2007). It is also possible that there are no real costs altogether if mutations are not antagonistically, but synergistically pleiotropic, meaning that some genotypes just adapt better to multiple environments (Sackman and Rokyta 2019, Chavhan *et al.* 2021). This could be the case if adaptation to temperature happens mainly by mutations of positive or neutral pleiotropy.

Selection may generate associations among traits when correlated environmental conditions produce correlated selection pressures (Roff and Fairbairn 2007, Agrawal *et al*. 2010). Nearby temperatures are likely to create very similar selection pressures for organisms, and accordingly, the genetic correlations between performances at contiguous temperatures are usually strong and positive (Moghadam *et al*. 2020). Conversely, performances at extreme temperatures have often lower and even negative genetic correlations with

performances at other temperatures, indicating pronounced trade-offs (Travisano and Lenski 1996, Kingsolver *et al.* 2004, Ørsted *et al.* 2019). In general, trade-offs between different temperatures are probably less likely because temperature forms more continuous selection gradient compared to discrete factors like uptake of different nutritional resources (Chavhan *et al*. 2021). In other words, because the same pathways regulate thermal tolerance at cellular level, it is less likely to find separate adaptations for tolerating different temperatures than for acquiring different nutrients. For example, the basal production of HSPs at benign temperatures is beneficial also when thermal conditions become suddenly more extreme (Chevin and Hoffmann 2017).

In addition, the detection of trade-offs can be obscured by the patterns of past selection. For example, detecting the costs of phenotypic plasticity is affected by the amount of variation in plasticity between individuals, such as, whether thermal specialists and generalists are present in a population, or alternatively, if the physiological trade-offs are genetically fixed among most of the individuals (Stearns 1989, Snell-Roog and Ehlman 2021). Fluctuating selection pressures can also purge deleterious mutations from a population, reducing the expression of costly traits and trade-offs (Snell-Rood *et al.* 2010, Kristensen *et al.* 2020). If deleterious mutations are removed by natural selection, most of the mutations in a population could be selectively neutral and under drift. It is important to remember that, in addition to adaptive explanations, neutral evolutionary processes can explain the observed patterns of variation and trade-offs within and between populations (Angilletta 2009, Snell-Roog and Ehlman 2021).

The results of this thesis and the literature discussed highlight the need for reconsidering some of hypotheses that emphasize the role of antagonistic pleiotropy and trade-offs in thermal adaptation (Lynch and Gabriel 1987, Gilchrist 1995). There are several, but not mutually exclusive biological explanations why trade-offs are often absent in experiments studying the cost of plasticity in fluctuating environments or the variation in TPCs (Angilletta 2009, Snell-Roog and Ehlman 2021). The diversity of possible mechanisms could itself be one explanation for the mixed evidence of trade-offs if the mechanisms act simultaneously and are important to varying degrees (Kassen 2002, Snell-Roog and Ehlman 2021). When modeling TPCs, the acquisition and allocation tradeoffs should be incorporated to make realistic predictions about the overall fitness of an individual and the populations' evolutionary potential (Angilletta 2009, Agrawal *et al*. 2010, Berger *et al*. 2014). Furthermore, it is important for future studies to investigate trade-offs at multiple levels of biological organization, from alleles to interspecific interactions affecting ecosystems, and preferably combine different study methods to get comprehensive results.

4 CONCLUSIONS AND FUTURE DIRECTIONS

Considering my results in the context of climate change, some individuals in a population should be able to adapt when variance in temperature increases, because despite some unique large-effect loci, thermal tolerance has a shared polygenic basis (I, II), and most of the genetic variance is in individuals' overall fitness (II). Further, if we want to predict species survival and distribution in future, then present thermal fluctuations (IV), and the magnitude and duration of extreme heat exposure seems critical (II, III), most likely to very small populations (III). Moreover, it is important to think how the theory of thermal adaptation and the experimental methods could be improved based on the results of this thesis and the studies referred within.

The fundamental conclusion that can be made about the multitude of hypotheses and mixed evidence is that there is no universally applicable theory of thermal evolution. Other studies have come to similar conclusions, emphasizing that thermal tolerance is a property that emerges from complex interactions between biochemical and genetic constraints and various selection pressures (Angilletta 2009, Buckley and Huey 2016, Buckley and Kingsolver 2021). Hence, variation in thermal tolerance might be too idiosyncratic to be explained by any generalizable hypothesis that would provide predictions about specific biological systems (Angilletta 2009, Gunderson and Stillman 2015). This stems from the fact that nature is multivariate, and consequently, the relationships between temperature, ecology and evolution are too, making theoretical models only caricatures and often too simplistic to predict multivariate fitness costs (Clarke 2003, Walsh and Blows 2009, Garland *et al.* 2022). So, opposite to making generalizations, natural complexity should be added to the models when predicting thermal evolution in response to climate change (Buckley and Huey 2016, Buckley and Kingsolver 2021). In nature, a multitude of environmental factors covary with thermal variability, as well as multiple biological traits correlate with thermal tolerance, both that affect fitness and the shape of the TPC (Somero 2010, Todgham and Stillman 2013, Sinclair *et al.* 2016). Also, populations are likely to cope with thermal stress by using

simultaneously different adaptive mechanisms that occur within and between generations (Kristensen *et al*. 2020).

Trade-offs in thermal adaptation arise from local adaptation, and several studies have underlined that TPCs are context dependent (Angilletta *et al.* 2002, Kristensen *et al.* 2020, Pallarés *et al.* 2021, Verspagen *et al.* 2023). This dependency includes specificity to the trait measured, the environmental conditions under which measurements are made, and the population and species in question (Gunderson and Stillman 2015, Manenti *et al.* 2016, Ketola and Kristensen 2017, Kellermann *et al.* 2019, Barley *et al.* 2021). An important implication from this is that the TPCs measured for a one trait might not be generalizable to the whole individual fitness because overall fitness constitutes of many fitness related traits (Angilletta *et al.* 2003, Kellermann *et al.* 2019). Similarly, the physiological allocation trade-offs on the individual level do not always translate into evolutionary trade-offs in TPCs, as populations usually consist of individuals that represent trade-offs to varying degrees (Kingsolver *et al.* 2004, Wooliver *et al.* 2022). The applicability of TPCs depends also on the fitness relevance of the trait in the selective environment, for example, the performances measured under constant conditions might not be a reliable proxy for predicting performances under thermal fluctuations and adaptation to climate change (Chevin and Hoffmann 2017, Ketola and Kristensen 2017, Kristensen *et al.* 2020).

Based on the multiple reasons listed above, it should be worthwhile to expand the framework of studying thermal tolerance by incorporating additional factors to experiments and models. These factors could be e.g., several fitness components, multiple environmental stressors, natural timescales and predictability of thermal variability, processes at ecological and evolutionary timescales, alternative genetic architectures, population- or individual-level variation, and interactions among species (Reed *et al.* 2010, Todgham and Stillman 2013, Kellermann *et al.* 2019, Capblancq *et al.* 2020, Kristensen *et al.* 2020, Barley *et al.* 2021). In addition, to accurately quantify the evolutionary constraints on thermal adaptation, it would be useful to integrate methods that use information from the selective environments, phenotypes, and the genetic architectures underlying TPCs (Angilletta 2009). One approach to do this is by experimental evolution and whole-genome resequencing that allows following the genetic change as well as the coincidental change in fitness across temperatures (Tobler *et al.* 2014, Schaum *et al.* 2018). Another interesting new avenue is to use nature-derived environmental data to predict how organisms' genomes response to changes in climatic selection pressures. In genotype-environment association studies (GEAs), the genomic data is statistically associated with climate variables measured at sampling site or with historical data (López-Hernández and Cortés 2019, Capblancq *et al.* 2020, Cortés *et al.* 2020). GEAs can be combined with GWAS and quantitative genetics to track phenotypic variation in climate adaptative traits and identify specific loci under selection (Forester *et al.* 2016, Hoban *et al.* 2016, López-Hernández and Cortés 2019).

A novel way forward is also by using study species that have previously gained little attention. Most studies on thermal tolerance have been made with bacteria, animals, and plants, whereas other groups like fungi have stayed

relatively understudied (Fisher and Lang 2016, Abu Bakar *et al.* 2020). Microbial experiments have been used to apprehend the evolutionary responses to extreme and fluctuating temperatures and to some extent, these results might be applicable to multicellular and sexual organisms (Kassen 2002, Chavhan *et al.* 2021). Moreover, the symbiotic or pathogenic bacteria and fungi can alter the TPCs of higher-level organisms and their geographical distributions (Abu Bakar *et al.* 2020, Wooliver *et al.* 2022). Studying the fungal responses to thermal stress is important also because they have a key role in ecosystems as decomposers fuelling the carbon cycle in a soil (Romero-Olivares *et al.* 2015). In general, more experimental work is needed on the adaptive potential of TPCs across organisms, and studying natural variation in contrast to experimentally evolved populations would help us to make better generalizations (Childress and Letcher 2017, Ketola and Kristensen 2017, Malusare *et al.* 2023). Applying genome data to species distribution models could also benefit nature conservation if species tolerance ranges are driven by genetic constraints (Kellermann *et al.* 2009, Capblancq *et al.* 2020).

To summarize, the future challenges in studying thermal adaptation are best tackled by adding natural complexity to the models, integrating multiple methods, and using genomic and environmental data collected from nature (Cortés *et al.* 2020, Buckley and Kingsolver 2021). Further work examining the genetic architecture of thermal tolerance at fluctuating environments could be accompanied by laboratory experiments that reflect natural timescales of thermal variability (Barley *et al.* 2021). However, one should be cautious not to make too strong generalizations about thermal tolerance and species responses to climate change based on single studies, as TPCs are context dependent (Kellermann *et al.* 2019). The ability of an ecosystem to recover its original function after a thermal disturbance is also likely to depend on complex interactions between species (Kristensen *et al.* 2020). To this end, I suggest more studies to verify the effects of fluctuating and extreme temperatures on the existence of fitness trade-offs and evolutionary constraints in thermal adaptation.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Sopeutuminen lämpötilan vaihteluun ja äärilämpötiloihin

Lämpötila on ympäristötekijä, jonka vaihtelu vaikuttaa kaikkeen elolliseen soluista ekosysteemeihin. Ihmisen aiheuttama ilmastonmuutos on paitsi nostanut maapallon keskilämpötilaa, myös lisännyt lämpötilassa tapahtuvaa vaihtelua. Korkeat äärilämpötilat ja nopeat lämpötilan muutokset aiheuttavat fysiologista stressiä, jota paremmin sietääkseen eliöt joutuvat sopeutumaan. Lämpötilasopeutuminen voi tapahtua joko nopeina biokemiallisina reaktioina yksilön kehossa, tai kehittyä useita satoja sukupolvia vaativan geneettisen evoluution tuloksena. Sopeutumismekanismien onkin oletettu riippuvan siitä, kuinka nopeaa lämpötilan vaihtelu on suhteessa eliölajin elinkaaren pituuteen. Lämpötilaolojen pysyessä pitkään vakaina luonnonvalinnan tulisi suosia yksilöitä, jotka ovat sopeutuneet muita paremmin kyseisiin oloihin. Vaihtelevissa lämpötiloissa luonnonvalinnan tulisi sen sijaan suosia yksilöitä, jotka eivät ole erikoistuneita tiettyyn lämpötilaan, vaan pärjäävät kohtalaisesti lämpötilasta riippumatta. Tämä tarkoittaa, että eliöyksilöiden ei pitäisi voida sopeutua samanaikaisesti sekä vakaisiin että vaihteleviin lämpötilaolosuhteisiin. Sopeutumista voivat rajoittaa energeettiset kustannukset, joita elintoimintojen ylläpitäminen tietynlaisessa lämpötilassa vaatii, tai geneettiset erot, jotka vaikeuttavat nopeaa sopeutumista uudenlaiseen lämpötilaympäristöön. On myös oletettu, että yksilömäärältään pienemmät populaatiot olisivat hitaampia sopeutumaan, koska niillä on vähemmän hyödyllistä geneettistä muuntelua. Lisäksi lämpötilan vaihteluun sopeutumisen on ehdotettu johtavan yleisesti hyvin menestyvien vieraslajien kehittymiseen, jotka voivat kilpailullaan syrjäyttää muita eliölajeja niiden omissa elinympäristöissä.

Lämmönsietokyvyn evoluutiota rajoittavien ja nopeuttavien tekijöiden tarkempi tutkiminen on tärkeää, jotta voitaisiin paremmin ennustaa eliölajien mahdollisuuksia selvitä ilmastonmuutoksesta. Tällä hetkellä ei esimerkiksi tiedetä, mitkä geenit eliöiden perimässä määrittävät lämpötilasopeutumista erilaisiin oloihin, tai millaisia sopeumia eliöt kehittävät lämpötilaltaan vakaissa ja eri nopeuksilla vaihtelevissa ympäristöissä. Väitöskirjassani tutkin edellä mainittuja kysymyksiä geneettisten menetelmien ja kokeellisen evoluution avulla. Selvitin laskennallisella genetiikalla ja geenikartoituksilla vaikuttavatko samat geenit lämmönsietokykyyn vakaissa keski- ja äärilämpötiloissa sekä hitaasti ja nopeasti vaihtelevissa lämpötiloissa (I, II). Kokeellisessa evoluutiossa kasvatin suuria ja pieniä populaatiota satoja sukupolvia lämpötilaltaan vakaissa keski- ja äärilämpötiloissa sekä eri nopeuksilla vaihtelevissa lämpötiloissa nähdäkseni sopeutuvatko populaatiot yhtä tehokkaasti erilaisiin oloihin (III). Lisäksi tein kilpailukokeita, joissa tutkittiin ovatko vaihtelevissa lämpötiloissa kehittyneet lajit parempia kilpailijoita vakaissa lämpötiloissa kehittyneitä lajeja vastaan, sekä sitä, miten kilpailun aikana tapahtuva lämpötilan vaihtelu vaikuttaa lajien menestykseen (IV). Tutkimuseliöinäni käytin sieni- ja

bakteerilajeja, joiden kasvua ja määrää mittaamalla tehtiin päätelmiä sopeutumisesta kokeellisiin olosuhteisiin.

Väitöskirjani tulokset eivät antaneet tukea aiemmille oletuksille siitä, että vakaisiin ja vaihteleviin lämpötiloihin sopeutumisen välillä olisi voimakkaita energeettisiä tai evolutiivisia rajoitteita. Geneettiset menetelmät osoittivat, että suurin osa lämmönsietokykyyn vaikuttavista geeneistä ovat samoja riippumatta lämpötilasta, mutta geenikartoitus löysi myös yksittäisiä geenejä, jotka olivat tyypillisiä vain tiettyihin vakaisiin tai vaihteleviin lämpötiloihin sopeutumiselle (I, II). Lisäksi jotkin populaatiot vaikuttivat pärjäävän hyvin lämpötilasta riippumatta. Poikkeuksena oli kuumansietokyky, jonka geneettinen perusta erosi enemmän muista lämpötilakäsittelyistä ja jonka evoluutiota voi rajoittaa vähäinen geneettisen muuntelun määrä (II). Toisaalta evoluutiokokeessa havaittiin, että geneettisen muuntelun määrä ei rajoita kaikkia lajeja, ja että korkeat äärilämpötilat voivat johtaa voimakkaaseen luonnonvalintaan nopeuttaen evoluutiota (III). Evoluutiokokeessa kuumansietokykyä rajoitti eniten vakaassa keskilämpötilassa kasvaminen, jonka aikana populaatiot todennäköisesti menettivät energeettisesti kalliita solujen suojamekanismeja. Yleisesti ottaen populaatiokoolla ei havaittu olevan vaikutusta siihen, miten nopeasti ja tehokkaasti populaatiot sopeutuivat vakaisiin tai vaihteleviin lämpötiloihin (III). Ainoastaan korkeassa äärilämpötilassa suuret populaatiot sopeutuivat pieniä paremmin, mikä voi johtua siitä, että merkittävää sopeutumista ei ylipäänsä havaittu keskilämpötilassa tai vaihtelevissa lämpötiloissa. Populaatioiden evolutiivisella taustalla ei ollut merkitystä myöskään kilpailukokeissa, joissa vaihtelevissa lämpötiloissa kasvaneet lajit eivät olleet vahvempia kilpailijoita muita lajeja vastaan (IV). Sen sijaan kilpailun aikana tapahtuvat lämpötilan vaihtelut vaikuttivat merkittävästi lajien välisen kilpailun lopputulokseen, mikä voi johtua vaihtelun aiheuttamista häiriöistä ympäristöoloissa.

Tutkimustulosteni perusteella voidaan päätellä, että lisääntyvä lämpötilan vaihtelu ei välttämättä estä eliölajeja sopeutumasta tulevaisuuden oloihin. Tulokseni myös haastavat aiempia oletuksia, joiden mukaan eliöyksilöt ja populaatiot eivät voi olla samanaikaisesti hyvin sopeutuneita sekä vakaisiin että vaihteleviin lämpötiloihin. Tuloksilleni löytyy tukea teorioista, joiden mukaan lämpötilasta riippumatta yleisesti hyvin menestyvien lajien olemassaolo on mahdollista, jos suurin osa lämmönsietokykyä säätelevistä geeneistä ovat samoja. Syynä voi olla myös yleinen stressireaktio, joka parantaa yksilön sietokykyä yhtäaikaisesti monien ympäristötekijöiden vaihtelulle. Yhteenvetona voidaan todeta, että lämmönsietokyky on monimutkainen ominaisuus, johon vaikuttavat useat geenit ja ympäristötekijät. Lämmönsietokyvyn evoluutiota tutkiessa tulisi ottaa huomioon tämä monimutkaisuus, mutta myös mahdollisuus, että sopeutuminen vakaisiin ja vaihteleviin ympäristöihin on ainakin osittain eri geneettisen perustan säätelemää. Vaikein rajoite sopeutumiselle ovat todennäköisesti korkeat äärilämpötilat, joihin suuremmat populaatiot voivat kyetä sopeutumaan pieniä tehokkaammin. Lisäksi lämpötilan vaihtelu voi parantaa joidenkin eliölajien kilpailukykyä ja auttaa niitä levittäytymään uusiin elinympäristöihin.

REFERENCES

Abu Bakar N., Karsani S.A. & Alias S.A. 2020. Fungal survival under temperature stress: a proteomic perspective. *PeerJ* 8, e10423, doi:10.7717/peerj.10423.

Acar M., Mettetal J.T. & Van Oudenaarden A. 2008. Stochastic switching as a survival strategy in fluctuating environments. *Nat. Genet.* 40: 471–475.

Addo-Bediako A., Chown S.L. & Gaston K.J. 2000. Thermal tolerance, climatic variability and latitude. *P. Roy. Soc. Lond. B Bio.* 267: 739–745.

Agrawal A.A. 2020. A scale-dependent framework for trade-offs, syndromes, and specialization in organismal biology. *Ecology* 101, e02924, doi:10.1002/ecy.2924.

Agrawal A.A., Conner J.K. & Rasmann S. 2010. Tradeoffs and negative correlations in evolutionary ecology. In: Bell M.A., Futuyma D.J., Eanes W.F. & Levinton J.S. (eds.), *Evolution since Darwin: The First 150 Years*, Sinauer Associates, Sunderland, Massachusetts, pp. 243–268

Agrawal A.F. & Stinchcombe J.R. 2009. How much do genetic covariances alter the rate of adaptation? *P. Roy. Soc. B-Biol. Sci.* 276: 1183–1191.

Agrawal A.F. & Whitlock M.C. 2010. Environmental duress and epistasis: how does stress affect the strength of selection on new mutations? *Trends Ecol. Evol.* 25: 450–458.

Ament-Velásquez S.L. 2022. The Dynamics of Adaptation to Stress from Standing Genetic Variation and de novo Mutations. *Mol. Biol. Evol.* 39, msac242, doi:0.1093/molbev/msac242.

Angilletta M.J. 2009. *Thermal adaptation: a theoretical and empirical synthesis*. Oxford University Press, Oxford, U.K.

Angilletta M.J., Huey R.B. & Frazier M.R. 2010. Thermodynamic Effects on Organismal Performance: Is Hotter Better? *Physiol. Biochem. Zool.* 83: 197–206.

Angilletta M.J., Niewiarowski P.H. & Navas C.A. 2002. The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* 27: 249–268.

Angilletta M.J., Wilson R.S., Navas C.A. & James R.S. 2003. Tradeoffs and the evolution of thermal reaction norms. *Trends Ecol. Evol.* 18: 234–240.

Araújo M.B., Ferri-Yáñez F., Bozinovic F., Marquet P.A., Valladares F. & Chown S.L. 2013. Heat freezes niche evolution. *Ecol. Lett.* 16: 1206–1219.

Ashrafi R., Bruneaux M., Sundberg L.-R., Pulkkinen K., Valkonen J. & Ketola T. 2018. Broad thermal tolerance is negatively correlated with virulence in an opportunistic bacterial pathogen. *Evol. Appl.* 11: 1700–1714.

Baird N.A., Etter P.D., Atwood T.S., Currey M.C., Shiver A.L., Lewis Z.A., Selker E.U., Cresko W.A. & Johnson E.A. 2008. Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers. *PLoS ONE* 3, e3376, doi: 10.1371/journal.pone.0003376.

Barley J.M., Cheng B.S., Sasaki M., Gignoux-Wolfsohn S., Hays C.G., Putnam A.B., Sheth S., Villeneuve A.R. & Kelly M. 2021. Limited plasticity in

thermally tolerant ectotherm populations: evidence for a trade-off. *P. Roy. Soc. B-Biol. Sci*. 288, 20210765, doi:10.1098/rspb.2021.0765.

Bartheld J.L., Artacho P. & Bacigalupe L. 2017. Thermal performance curves under daily thermal fluctuation: A study in helmeted water toad tadpoles. *J. Therm. Biol.* 70: 80–85.

Bell G. & Collins S. 2008. Adaptation, extinction and global change. *Evol. Appl.* 1: 3–16.

Bennett A.F. & Hughes B.S. 2009. Microbial experimental evolution. *Am. J. Physiol-Reg. I.* 297: R17–R25.

Bennett A.F. & Lenski R.E. 1993. Evolutionary adaptation to temperature ii. Thermal niches of experimental lines of *Escherichia coli*. *Evolution* 47: 1–12.

Bennett A.F. & Lenski R.E. 1999. Experimental Evolution and Its Role in Evolutionary Physiology. *Am. Zool.* 39: 346–362.

Bennett A.F. & Lenski R.E. 2007. An experimental test of evolutionary trade-offs during temperature adaptation. *Proc. Natl. Acad. Sci. U.S.A.* 104: 8649–8654.

Bennett A.F., Dao K.M. & Lenski R.E. 1990. Rapid evolution in response to high-temperature selection. *Nature* 346: 79–81.

Bennett A.F., Lenski R.E. & Mittler J.E. 1992. Evolutionary adaptation to temperature. I. Fitness responses of E*scherichia coli* to changes in its thermal environment. *Evolution* 46: 16–30.

Benton T.G. & Grant A. 2000. Evolutionary fitness in ecology: Comparing measures of fitness in stochastic, density-dependent environments. *Evol. Ecol. Res. 2*: 769–789.

Berger D., Stångberg J., Baur J. & Walters R. 2021. Elevated temperature increases genome-wide selection on de novo mutations. *P. Roy. Soc. B-Biol. Sci*. 288, 20203094, doi:10.1098/rspb.2020.3094.

Berger D., Walters R.J. & Blanckenhorn W.U. 2014. Experimental evolution for generalists and specialists reveals multivariate genetic constraints on thermal reaction norms. *J. Evolution. Biol.* 27: 1975–1989.

Bideault A., Préfontaine S., Barbier M., Loreau M. & Gravel D. 2023. The robustness of thermal performance curves limits adaptation in growth rate of wild bacterial strains. *TechRxiv*. doi:10.22541/au.167330464.48160133/v1.

Billerbeck J.M., Schultz E.T. & Conover D.O. 2000. Adaptive variation in energy acquisition and allocation among latitudinal populations of the Atlantic silverside. *Oecologia* 122: 210–219.

Blackburn T.M. & Duncan R.P. 2001. Determinants of establishment success in introduced birds. *Nature* 414: 195–197.

Bono L.M., Smith L.B., Pfennig D.W. & Burch C.L. 2017. The emergence of performance trade-offs during local adaptation: insights from experimental evolution. *Mol. Ecol.* 26: 1720–1733.

Botero C.A., Weissing F.J., Wright J. & Rubenstein D.R. 2015. Evolutionary tipping points in the capacity to adapt to environmental change. *Proc. Natl. Acad. Sci. U.S.A.* 112: 184–189.

Bozinovic F., Bastías D.A., Boher F., Clavijo-Baquet S., Estay S.A. & Angilletta M.J. 2011. The Mean and Variance of Environmental Temperature Interact

to Determine Physiological Tolerance and Fitness. *Physiol. Biochem. Zool.* 84: 543–552.

Bradshaw W.E. & Holzapfel C.M. 2006. Evolutionary Response to Rapid Climate Change. *Science* 312: 1477–1478.

Brakefield P.M. & Kesbeke F. 1997. Genotype–environment interactions for insect growth in constant and fluctuating temperature regimes. *P. Roy. Soc. Lond. B Bio.* 264: 717–723.

Buckley L.B. & Huey R.B. 2016. How Extreme Temperatures Impact Organisms and the Evolution of their Thermal Tolerance. *Integr. Comp. Biol.* 56: 98–109.

Buckley L.B. & Kingsolver J.G. 2021. Evolution of Thermal Sensitivity in Changing and Variable Climates. *Annu. Rev. Ecol. Evol. Syst.* 52: 563–586.

Buckling A., Brockhurst M.A., Travisano M. & Rainey P.B. 2007. Experimental adaptation to high and low quality environments under different scales of temporal variation. *J. Evolution. Biol.* 20: 296–300.

Bürger R. & Gimelfarb A. 2002. Fluctuating environments and the role of mutation in maintaining quantitative genetic variation. *Genet. Res.* 80: 31–46.

Bürger R. & Krall C. 2004. Quantitative-Genetic Models and Changing Environments. In: Ferrière R., Dieckmann U. & Couvet D. (eds.), *Evolutionary Conservation Biology*, Cambridge University Press, pp. 171–187.

Callahan H.S., Maughan H. & Steiner U.K. 2008. Phenotypic Plasticity, Costs of Phenotypes, and Costs of Plasticity: Toward an Integrative View. *Ann. N.Y. Acad. Sci.* 133: 44–66.

Capblancq T., Fitzpatrick M.C., Bay R.A., Exposito-Alonso M. & Keller S.R. 2020. Genomic Prediction of (Mal)Adaptation Across Current and Future Climatic Landscapes. *Annu. Rev. Ecol. Evol. Syst.* 51: 245–269.

Chavhan Y., Karve S. & Dey S. 2019. Adapting in larger numbers can increase the vulnerability of *Escherichia coli* populations to environmental changes. *Evolution* 73: 836–846.

Chavhan Y., Malusare S. & Dey S. 2020. Larger bacterial populations evolve heavier fitness trade-offs and undergo greater ecological specialization. *Heredity* 124: 726–736.

Chavhan Y., Malusare S. & Dey S. 2021. Interplay of population size and environmental fluctuations: A new explanation for fitness cost rarity in asexuals. *Ecol. Lett.* 24: 1943–1954.

Chevin L.-M. & Hoffmann A.A. 2017. Evolution of phenotypic plasticity in extreme environments. *Philos. T. Roy. Soc. B.* 372, 20160138, doi:10.1098/rstb.2016.0138.

Chevin L.-M., Lande R. & Mace G.M. 2010. Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory. *PLoS Biol.* 8, e1000357, doi:10.1371/journal.pbio.1000357.

Childress E.S. & Letcher B.H. 2017. Estimating thermal performance curves from repeated field observations. *Ecology* 98: 1377–1387.

Clarke A. 2003. Costs and consequences of evolutionary temperature adaptation. *Trends Ecol. Evol.* 18: 573–581.

Coelho M., Dereli A., Haese A., Kühn S., Malinovska L., DeSantis M.E., Shorter J., Alberti S., Gross T. & Tolić-Nørrelykke I.M. 2013. Fission Yeast Does Not Age under Favorable Conditions, but Does So after Stress. *Curr. Biol.* 23: 1844–1852.

Colinet H., Sinclair B.J., Vernon P. & Renault D. 2015. Insects in Fluctuating Thermal Environments. *Annu. Rev. Entomol.* 60: 123–140.

Collins S., Boyd P.W. & Doblin M.A. 2020. Evolution, Microbes, and Changing Ocean Conditions. *Annu. Rev. Mar. Sci.* 12: 181–208.

Condon C., Cooper B.S., Yeaman S. & Angilletta Jr. M.J. 2014. Temporal Variation Favors the Evolution of Generalists in Experimental Populations of *Drosophila Melanogaster*. *Evolution* 68: 720–728.

Conover D.O., Duffy T.A. & Hice L.A. 2009. The Covariance between Genetic and Environmental Influences across Ecological Gradients: Reassessing the Evolutionary Significance of Countergradient and Cogradient Variation. *Ann. N.Y. Acad. Sci.* 1168: 100–129.

Cooper V.S. & Lenski R.E. 2000. The population genetics of ecological specialization in evolving *Escherichia coli* populations. *Nature* 407: 736–739.

Cooper V.S., Bennett A.F. & Lenski R.E. 2007. Evolution of thermal dependence of growth rate of *Escherichia coli* populations during 20,000 generations in a constant environment. *Evolution* 55: 889–896.

Cortés A.J., López-Hernández F. & Osorio-Rodriguez D. 2020. Predicting Thermal Adaptation by Looking Into Populations' Genomic Past. *Front. Genet.* 11, 564515, doi:10.3389/fgene.2020.564515.

Czesak M.E., Fox C.W. & Wolf J.B. 2006. Experimental Evolution of Phenotypic Plasticity: How Predictive Are Cross-Environment Genetic Correlations? *The Am. Nat*. 168: 323–335.

Darwin C. 1859. *On the Origin of Species by Means of Natural Selection.* Murray, London.

Davis M.A., Grime J.P. & Thompson K. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *J. Ecol.* 88: 528–534.

Deatherage D.E., Kepner J.L., Bennett A.F., Lenski R.E. & Barrick J.E. 2017. Specificity of genome evolution in experimental populations of *Escherichia coli* evolved at different temperatures. *Proc. Natl. Acad. Sci. U.S.A.* 114: E1904-E1912.

Desai M.M., Fisher D.S. & Murray A.W. 2007. The Speed of Evolution and Maintenance of Variation in Asexual Populations. *Curr. Biol.* 17: 385–394.

Descamps-Julien B. & Gonzalez A. 2005. Stable coexistence in a fluctuating environment: an experimental demonstration. *Ecology* 86: 2815–2824.

DeWitt T.J. & Langerhans R.B. 2004. Integrated solutions to environmental heterogeneity: theory of multimoment reaction norms. In: DeWitt T.J. & Scheiner S.M. (eds.), *Phenotypic Plasticity: Functional and Conceptual Approaches*, Oxford, Oxford University Press, pp. 98–111.

DeWitt T.J. & Scheiner S.M. 2004. Phenotypic variation from single genotypes: a primer. In: DeWitt T.J. & Scheiner S.M. (eds.), *Phenotypic plasticity: functional and conceptual approaches*, Oxford, Oxford University Press, pp. 1–9.

Dey S., Proulx S.R. & Teotónio H. 2016. Adaptation to Temporally Fluctuating Environments by the Evolution of Maternal Effects. *PLOS Biol.* 14, e1002388, doi:10.1371/journal.pbio.1002388.

Duncan A.B., Fellous S., Quillery E. & Kaltz O. 2011. Adaptation of *Paramecium caudatum* to variable conditions of temperature stress. *Res. Microbiol.* 162: 939–944.

Duun Rohde P., Krag K., Loeschcke V., Overgaard J., Sørensen P. & Nygaard Kristensen T. 2016. A Quantitative Genomic Approach for Analysis of Fitness and Stress Related Traits in a *Drosophila melanogaster* Model Population. *Int. J. Genomics* 2016: 1–11.

Ellison C.E., Hall C., Kowbel D., Welch J., Brem R.B., Glass N.L. & Taylor J.W. 2011. Population genomics and local adaptation in wild isolates of a model microbial eukaryote. *Proc. Natl. Acad. Sci. U.S.A.* 108: 2831–2836.

Feder M.E. & Hofmann G.E. 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and Ecological Physiology. *Annu. Rev. Physiol.* 61: 243–282.

Fisher K.J. & Lang G.I. 2016. Experimental evolution in fungi: An untapped resource. *Fungal Genet. Biol.* 94: 88–94.

Fisher R.A. 1919. XV.—The correlation between relatives on the supposition of Mendelian inheritance. *Earth Env. Sci. T. R. So.* 52: 399–433.

Forester B.R., Jones M.R., Joost S., Landguth E.L. & Lasky J.R. 2016. Detecting spatial genetic signatures of local adaptation in heterogeneous landscapes. *Mol. Ecol.* 25: 104–120.

Forsburg S.L. & Rhind N. 2006. Basic methods for fission yeast. *Yeast* 23: 173–183.

Frankham R. 2005. Genetics and extinction. *Biol. Conserv.* 126: 131–140.

Fu W., O'Connor T.D. & Akey J.M. 2013. Genetic architecture of quantitative traits and complex diseases. *Curr. Opin. Genet. Dev.* 23: 678–683.

Furrow R.E. & Feldman M.W. 2014. Genetic variation and the evolution of epigenetic regulation: special section. *Evolution* 68: 673–683.

Gabriel W. & Lynch M. 1992. The selective advantage of reaction norms for environmental tolerance. *J. Evolution. Biol.* 5: 41–59.

Galagan J.E., Calvo S.E., Borkovich K.A., Selker E.U., Read N.D., Jaffe D., FitzHugh W., Ma L.-J., Smirnov S., Purcell S., Rehman B., Elkins T., Engels R., Wang S., Nielsen C.B., Butler J., Endrizzi M., Qui D., Ianakiev P., Bell-Pedersen D., Nelson M.A., Werner-Washburne M., Selitrennikoff C.P., Kinsey J.A., Braun E.L., Zelter A., Schulte U., Kothe G.O., Jedd G., Mewes W., Staben C., Marcotte E., Greenberg D., Roy A., Foley K., Naylor J., Stange-Thomann N., Barrett R., Gnerre S., Kamal M., Kamvysselis M., Mauceli E., Bielke C., Rudd S., Frishman D., Krystofova S., Rasmussen C., Metzenberg R.L., Perkins D.D., Kroken S., Cogoni C., Macino G., Catcheside D., Li W., Pratt R.J., Osmani S.A., DeSouza C.P.C., Glass L., Orbach M.J., Berglund J.A., Voelker R., Yarden O., Plamann M., Seiler S., Dunlap J., Radford A., Aramayo R., Natvig D.O., Alex L.A., Mannhaupt G., Ebbole D.J., Freitag M., Paulsen I., Sachs M.S., Lander E.S., Nusbaum C. & Birren B. 2003. The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature* 422: 859–868.

Garland T., Downs C.J. & Ives A.R. 2022. Trade-Offs (and Constraints) in Organismal Biology. *Physiol. Biochem. Zool.* 95: 82–112.

Gerken A.R., Eller O.C., Hahn D.A. & Morgan T.J. 2015. Constraints, independence, and evolution of thermal plasticity: Probing genetic architecture of long- and short-term thermal acclimation. *Proc. Natl. Acad. Sci. U.S.A.* 112: 4399–4404.

Gerrish P.J. & Lenski R.E. 1998. The fate of competing beneficial mutations in an asexual population. *Genetica* 102: 127-144.

Ghalambor C.K., McKay J.K., Carroll S.P. & Reznick D.N. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* 21: 394–407.

Giacomelli M.G., Hancock A.S. & Masel J. 2007. The Conversion of 3′ UTRs into Coding Regions. *Mol. Biol. Evol.* 24: 457–464.

Gilchrist G.W. 1995. Specialists and Generalists in Changing Environments. I. Fitness Landscapes of Thermal Sensitivity. *Am. Nat.* 146: 252–270.

Glazier D.S. 1999. Trade-offs between reproductive and somatic (storage) investments in animals: a comparative test of the Van Noordwijk and De Jong model. *Evol. Ecol.* 13: 539–555.

Grantham M.E., Antonio C.J., O'Neil B.R., Zhan Y.X. & Brisson J.A. 2016. A case for a joint strategy of diversified bet hedging and plasticity in the pea aphid wing polyphenism. *Biol. Lett.* 12, 20160654, doi:10.1098/rsbl.2016.0654.

Grimont P.A.D. & Grimont F. 1978. The genus *Serratia*. *Ann. Rev. Microb.* 32: 221–248.

Gunderson A.R. & Stillman J.H. 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. R. Soc. B Biol. Sci.* 282, 20150401, doi:10.1098/rspb.2015.0401.

Hamilton J.A., Okada M., Korves T. & Schmitt J. 2015: The role of climate adaptation in colonization success in *Arabidopsis thaliana*. *Mol. Ecol.* 24: 2253–2263.

Handel A. & Rozen D.E. 2009. The impact of population size on the evolution of asexual microbes on smooth versus rugged fitness landscapes. *BMC Evol. Biol.* 9: 1-10.

Hangartner S. & Hoffmann A.A. 2016. Evolutionary potential of multiple measures of upper thermal tolerance in *Drosophila melanogaster*. *Funct. Ecol.* 30: 442–452.

Hanson S.J. & Wolfe K.H. 2017. An Evolutionary Perspective on Yeast Mating-Type Switching. *Genetics* 206: 9–32.

Harley C.D.G. & Paine R.T. 2009. Contingencies and compounded rare perturbations dictate sudden distributional shifts during periods of gradual climate change. *Proc. Natl. Acad. Sci. U.S.A.* 106: 11172–11176.

Hellmann J.J. & Pineda-Krch M. 2007. Constraints and reinforcement on adaptation under climate change: Selection of genetically correlated traits. *Biol. Conserv.* 137: 599–609.

Hoban S., Kelley J.L., Lotterhos K.E., Antolin M.F., Bradburd G., Lowry D.B., Poss M.L., Reed L.K., Storfer A. & Whitlock M.C. 2016. Finding the Genomic

Basis of Local Adaptation: Pitfalls, Practical Solutions, and Future Directions. *Am. Nat.* 188: 379–397.

Hobbs R.J. & Huenneke L.F. 1992. Disturbance, Diversity, and Invasion: Implications for Conservation. *Conserv. Biol.* 6: 324–337.

Hochachka P.W. & Somero G.N. 2002. *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford University Press, New York.

Hoekstra L.A. & Montooth K.L. 2013. Inducing extra copies of the Hsp70 gene in *Drosophila melanogaster* increases energetic demand. *BMC Evol. Biol.* 13: 1–11.

Hoffmann A.A., Sørensen J.G. & Loeschcke V. 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J. Therm. Biol.* 28: 175–216.

Holder K.K. & Bull J.J. 2001. Profiles of Adaptation in Two Similar Viruses. *Genetics* 159: 1393–1404.

Houle D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. *Evolution* 45: 630–648.

Huang M., Liu X., Zhou Y., Summers R.M. & Zhang Z. 2019. BLINK: a package for the next level of genome-wide association studies with both individuals and markers in the millions. *GigaScience* 8, giy154, doi:10.1093/gigascience/giy154.

Huey R.B. & Hertz P.E. 1984. Is a jack-of-all-temperatures a master of none? *Evolution* 38: 441–444.

Huey R.B. & Kingsolver J.G. 1989. Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol*. 4: 131–135.

Huey R.B. & Stevenson R.D. 1979. Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches. *Am. Zool.* 19: 357–366.

Huey R.B., Kearney M.R., Krockenberger A., Holtum J.A.M., Jess M. & Williams S.E. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philos. T. Roy. Soc. B* 367: 1665–1679.

Hufbauer R.A., Facon B., Ravigné V., Turgeon J., Foucaud J., Lee C.E., Rey O. & Estoup A. 2012. Anthropogenically induced adaptation to invade (AIAI): contemporary adaptation to human-altered habitats within the native range can promote invasions. *Evol. Appl.* 5: 89–101.

IPCC 2018: Summary for policymakers. — An IPCC Special Report on the impacts of global warming of 1.5 °C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty, World Meteorological Organization, Geneva, Switzerland.

Izem R. & Kingsolver J.G. 2005. Variation in Continuous Reaction Norms: Quantifying Directions of Biological Interest. *Am. Nat.* 166: 277–289.

Jiang L. & Morin P.J. 2007. Temperature fluctuation facilitates coexistence of competing species in experimental microbial communities. *J. Anim. Ecol.* 76: 660–668.

Kassen R. 2002. The experimental evolution of specialists, generalists, and the maintenance of diversity. *J. Evolution. Biol.* 15: 173–190.

Kawecki T.J. 2000. The Evolution of Genetic Canalization Under Fluctuating Selection. *Evolution* 54: 1–12.

Kearney M.R., Matzelle A. & Helmuth B. 2012. Biomechanics meets the ecological niche: the importance of temporal data resolution. *J. Exp. Biol.* 215: 1422–1424.

Kefford B.J., Ghalambor C.K., Dewenter B., Poff N.L., Hughes J., Reich J. & Thompson R. 2022. Acute, diel, and annual temperature variability and the thermal biology of ectotherms. *Glob. Change Biol.* 28: 6872–6888.

Keller I. & Seehausen O. 2012. Thermal adaptation and ecological speciation. *Mol. Ecol.* 21: 782–799.

Kellermann V., Van Heerwaarden B., Sgrò C.M. & Hoffmann A.A. 2009. Fundamental Evolutionary Limits in Ecological Traits Drive *Drosophila* Species Distributions. *Science* 325: 1244–1246.

Kellermann V., Overgaard J., Hoffmann A.A., Fløjgaard C., Svenning J.-C. & Loeschcke V. 2012. Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. *Proc. Natl. Acad. Sci. U.S.A.* 109: 16228–16233.

Kellermann V., Chown S.L., Schou M.F., Aitkenhead I., Janion-Scheepers C., Clemson A., Scott M.T. & Sgrò C.M. 2019. Comparing thermal performance curves across traits: how consistent are they? *J. Exp. Biol.* 222, jeb.193433, doi: 10.1242/jeb.193433.

Ketola T. & Kristensen T.N. 2017. Experimental Approaches for Testing if Tolerance Curves Are Useful for Predicting Fitness in Fluctuating Environments. *Front. Ecol. Evol.* 5, 129, doi:10.3389/fevo.2017.00129.

Ketola T. & Kronholm I. 2023. Experimental evolution of evolutionary potential in fluctuating environments. *J. Evolution. Biol.* 36: 945–949.

Ketola T. & Saarinen K. 2015. Experimental evolution in fluctuating environments: tolerance measurements at constant temperatures incorrectly predict the ability to tolerate fluctuating temperatures. *J. Evolution. Biol.* 28: 800–806.

Ketola T., Saarinen K. & Lindström L. 2017. Propagule pressure increase and phylogenetic diversity decrease community's susceptibility to invasion. *BMC Ecol.* 17: 15.

Ketola T., Laakso J., Kaitala V. & Airaksinen S. 2004. Evolution of hsp90 expression in *Tetrahymena thermophila* (protozoa, ciliata) populations exposed to thermally variable environments. *Evolution* 58: 741–748.

Ketola T., Kellermann V.M., Loeschcke V., López-Sepulcre A. & Kristensen T.N. 2014. Does environmental robustness play a role in fluctuating environments?. *Evolution* 68: 587–594.

Ketola T., Mikonranta L., Zhang J., Saarinen K., Örmälä A.-M., Friman V.-P., Mappes J. & Laakso J. 2013. Fluctuating temperature leads to evolution of thermal generalism and preadaptation to novel environments. *Evolution* 67: 2936-2944.

Khan M.I., Kainat Z., Maqbool S., Mehwish A., Ahmad S., Suleman H.M., Mahmood Z., Ali M., Aziz A., Rasheed A. & Li H. 2022. Genome-wide

association for heat tolerance at seedling stage in historical spring wheat cultivars. *Front. Plant Sci.* 13, 972481, doi:10.3389/fpls.2022.972481.

Kimura M. 1967. On the evolutionary adjustment of spontaneous mutation rates. *Genet. Res.* 9: 23–34.

Kingsolver J.G. & Buckley L.B. 2017. Quantifying thermal extremes and biological variation to predict evolutionary responses to changing climate. *Philos. T. Roy. Soc. B.* 372, 20160147, 10.1098/rstb.2016.0147.

Kingsolver J.G., Gomulkiewicz R. & Carter P.A. 2001. Variation, selection and evolution of function-valued traits. In: Hendry A.P. & Kinnison M.T. (eds.), *Microevolution Rate, Pattern, Process*, Springer Netherlands, Dordrecht, pp. 87–104.

Kingsolver J.G., Ragland G.J. & Diamond S.E. 2009. Evolution in a constant environment: thermal fluctuations and thermal sensitivity of laboratory and field populations of *Manduca sexta*. *Evolution* 63: 537–541.

Kingsolver J.G., Ragland G.J. & Shlichta J.G. 2004. Quantitative genetics of continuous reaction norms: thermal sensitivity of caterpillar growth rates. *Evolution* 58: 1521–1529.

Knies J.L., Izem R., Supler K.L., Kingsolver J.G. & Burch C.L. 2006. The Genetic Basis of Thermal Reaction Norm Evolution in Lab and Natural Phage Populations. *PLoS Biol.* 4, e201, doi:10.1371/journal.pbio.0040201.

Kreyling J., Beierkuhnlein C., Ellis L. & Jentsch A. 2008. Invasibility of grassland and heath communities exposed to extreme weather events – additive effects of diversity resistance and fluctuating physical environment. *Oikos* 117: 1542–1554.

Kristensen T.N., Ketola T. & Kronholm I. 2020. Adaptation to environmental stress at different timescales. *Ann. N.Y. Acad. Sci.* 1476: 5–22.

Kristensen T.N., Overgaard J., Lassen J., Hoffmann A.A. & Sgrò C. 2015. Low evolutionary potential for egg-to-adult viability in *Drosophila melanogaster* at high temperatures. *Evolution* 69: 803–814.

Kristensen T.N., Sørensen P., Kruhøffer M., Pedersen K.S. & Loeschcke V. 2005. Genome-Wide Analysis on Inbreeding Effects on Gene Expression in *Drosophila melanogaster*. *Genetics* 171: 157–167.

Kronholm I. 2022. Evolution of anticipatory effects mediated by epigenetic changes. *Environ. Epigenet.* 8, dvac007, doi:10.1093/eep/dvac007.

Kronholm I. & Ketola T. 2018. Effects of acclimation time and epigenetic mechanisms on growth of *Neurospora* in fluctuating environments. *Heredity* 121: 327–341.

Kültz D. 2005. Molecular and evolutionary basis of the cellular stress response. *Annu. Rev. Physiol.* 67: 225–257.

Kuo H.-C., Hui S., Choi J., Asiegbu F.O., Valkonen J.P.T. & Lee Y.-H. 2014. Secret lifestyles of *Neurospora crassa*. *Sci. Rep.* 4, 5135, doi:10.1038/srep05135.

Lambros M., Pechuan-Jorge X., Biro D., Ye K. & Bergman A. 2021. Emerging Adaptive Strategies Under Temperature Fluctuations in a Laboratory Evolution Experiment of *Escherichia Coli*. *Front. Microbiol.* 12, 724982, doi:10.3389/fmicb.2021.724982.

Lande R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *J. Evolution. Biol.* 22: 1435–1446.

Lardies M.A. & Bozinovic F. 2008. Genetic variation for plasticity in physiological and life-history traits among populations of an invasive species, the terrestrial isopod *Porcellio laevis*. *Evol. Ecol. Res.* 10: 747–762.

Latimer C.A.L., Foley B.R. & Chenoweth S.F. 2015. Connecting thermal performance curve variation to the genotype: a multivariate QTL approach. *J. Evolution. Biol.* 28: 155–168.

Latimer C.A.L., McGuigan K., Wilson R.S., Blows M.W. & Chenoweth S.F. 2014. The contribution of spontaneous mutations to thermal sensitivity curve variation in *Drosophila serrata*. *Evolution* 68: 1824–1837.

Lax S., Abreu C.I. & Gore J. 2020. Higher temperatures generically favour slower-growing bacterial species in multispecies communities. *Nat. Ecol. Evol.* 4: 560–567.

Lecheta M.C., Awde D.N., O'Leary T.S., Unfried L.N., Jacobs N.A., Whitlock M.H., McCabe E., Powers B., Bora K., Waters J.S., Axen H.J., Frietze S., Lockwood B.L., Teets N.M. & Cahan S.H. 2020. Integrating GWAS and Transcriptomics to Identify the Molecular Underpinnings of Thermal Stress Responses in *Drosophila melanogaster*. *Front. Genet.* 11, 658, doi:10.3389/fgene.2020.00658.

Lee C.E. & Gelembiuk G.W. 2008. Evolutionary origins of invasive populations. *Evol. Appl.* 1: 427–448.

Lee K. & Dighton J. 2010. *Neurospora*, a potential fungal organism for experimental and evolutionary ecology. *Fungal Biol. Rev.* 24: 85–89.

Lenski R.E., Rose M.R., Simpson S.C. & Tadler S.C. 1991. Long-Term Experimental Evolution in *Escherichia coli*. I. Adaptation and Divergence During 2,000 Generations. *Am. Nat.* 138: 1315–1341.

Leroi A.M., Bartke A., Benedictis G.D., Franceschi C., Gartner A., Gonos E., Feder M.E., Kivisild T., Lee S., Kartal-Özer N., Schumacher M., Sikora E., Slagboom E., Tatar M., Yashin A.I., Vijg J. & Zwaan B. 2005. What evidence is there for the existence of individual genes with antagonistic pleiotropic effects? *Mech. Ageing Dev.* 126: 421–429.

Levins R. 1968. *Evolution in Changing Environments.* Princeton University Press, Princeton.

Li W. & Stevens M.H.H. 2012. Fluctuating resource availability increases invasibility in microbial microcosms. *Oikos* 121: 435–441.

Liu M., Bjørnlund L., Rønn R., Christensen S. & Ekelund F. 2012. Disturbance Promotes Non-Indigenous Bacterial Invasion in Soil Microcosms: Analysis of the Roles of Resource Availability and Community Structure. *PLoS ONE* 7, e45306, doi:10.1371/journal.pone.0045306.

Lockwood B.L., Sanders J.G. & Somero G.N. 2010. Transcriptomic responses to heat stress in invasive and native blue mussels (genus *Mytilus*): molecular correlates of invasive success. *J. Exp. Biol.* 213: 3548–3558.

Logan M.L. & Cox C.L. 2020. Genetic Constraints, Transcriptome Plasticity, and the Evolutionary Response to Climate Change. *Front. Genet.* 11, 538226, doi:10.3389/fgene.2020.538226.

López-Hernández F. & Cortés A.J. 2019. Last-Generation Genome–Environment Associations Reveal the Genetic Basis of Heat Tolerance in Common Bean (*Phaseolus vulgaris L.*). *Front. Genet.* 10, 954, doi:10.3389/fgene.2019.00954.

López-Maury L., Marguerat S. & Bähler J. 2008. Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. *Nat. Rev. Genet.* 9: 583–593.

Lynch M. & Gabriel W. 1987. Environmental tolerance. *Am. Nat.* 129: 283–303.

Mächler E. & Altermatt F. 2012. Interaction of Species Traits and Environmental Disturbance Predicts Invasion Success of Aquatic Microorganisms. *PLoS ONE* 7, e45400, doi:10.1371/journal.pone.0045400.

Mackay T.F.C., Stone E.A. & Ayroles J.F. 2009. The genetics of quantitative traits: challenges and prospects. *Nat. Rev. Genet.* 10: 565–577.

Malusare S.P., Zilio G. & Fronhofer E.A. 2023. Evolution of thermal performance curves: A meta-analysis of selection experiments. *J. Evolution. Biol.* 36: 15–28.

Manenti T., Loeschcke V., Moghadam N.N. & Sørensen J.G. 2015. Phenotypic plasticity is not affected by experimental evolution in constant, predictable or unpredictable fluctuating thermal environments. *J. Evolution. Biol.* 28: 2078–2087.

Manenti T., Sørensen J.G., Moghadam N.N. & Loeschcke V. 2016. Few genetic and environmental correlations between life history and stress resistance traits affect adaptation to fluctuating thermal regimes. *Heredity* 117: 149–154.

Marshall K.E. & Sinclair B.J. 2015. The relative importance of number, duration and intensity of cold stress events in determining survival and energetics of an overwintering insect. *Funct. Ecol.* 29: 357–366.

Marshall K.E., Gotthard K. & Williams C.M. 2020. Evolutionary impacts of winter climate change on insects. *Curr. Opin. Insect Sci.* 41: 54–62.

Martin T.L. & Huey R.B. 2008. Why "Suboptimal" Is Optimal: Jensen's Inequality and Ectotherm Thermal Preferences. *Am. Nat.* 171: E102–E118.

McNamara J.M., Dall S.R.X., Hammerstein P. & Leimar O. 2016. Detection vs. selection: integration of genetic, epigenetic and environmental cues in fluctuating environments. *Ecol. Lett.* 19: 1267–1276.

Meehl G.A., Karl T., Easterling D.R., Changnon S., Pielke R., Changnon D., Evans J., Groisman P.Y., Knutson T.R., Kunkel K.E., Mearns L.O., Parmesan C., Pulwarty R., Root T., Sylves R.T., Whetton P. & Zwiers F. 2000. An Introduction to Trends in Extreme Weather and Climate Events: Observations, Socioeconomic Impacts, Terrestrial Ecological Impacts, and Model Projections. *B. Am. Meteorol. Soc.* 81: 413–416.

Melbinger A. & Vergassola M. 2015. The Impact of Environmental Fluctuations on Evolutionary Fitness Functions. *Sci. Rep.* 5, 15211, doi:10.1038/srep15211.

Melbourne B.A., Cornell H.V., Davies K.F., Dugaw C.J., Elmendorf S., Freestone A.L., Hall R.J., Harrison S., Hastings A., Holland M., Holyoak M., Lambrinos J., Moore K. & Yokomizo H. 2007. Invasion in a heterogeneous world: resistance, coexistence or hostile takeover? *Ecol. Lett.* 10: 77–94.

Meyer I. & Shnerb N.M. 2020. Evolutionary dynamics in fluctuating environment. *Phys. Rev. Res.* 2, 023308, doi:10.1103/PhysRevResearch.2.023308.

Meyers L.A. & Bull J.J. 2002. Fighting change with change: adaptive variation in an uncertain world. *Trends Ecol. Evol.* 17: 551–557.

Meyers L.A., Ancel F.D. & Lachmann M. 2005. Evolution of Genetic Potential. *PLoS Comp. Biol.* 1, e32, doi:10.1371/journal.pcbi.0010032.

Mitchell K.A. & Hoffmann A.A. 2010. Thermal ramping rate influences evolutionary potential and species differences for upper thermal limits in *Drosophila*. *Funct. Ecol.* 24: 694–700.

Moghadam N.N., Sidhu K., Summanen P.A.M., Ketola T. & Kronholm I. 2020. Quantitative genetics of temperature performance curves of *Neurospora crassa*. *Evolution* 74: 1772–1787.

Mohsenzadeh S., Saupe-Thies W., Steier G., Schroeder T., Fracella F., Ruoff P. & Rensing L. 1998. Temperature Adaptation of House Keeping and Heat Shock Gene Expression in *Neurospora crassa*. *Fungal Genet. Biol.* 25: 31–43.

Murren C.J., Auld J.R., Callahan H., Ghalambor C.K., Handelsman C.A., Heskel M.A., Kingsolver J.G., Maclean H.J., Masel J., Maughan H., Pfennig D.W., Relyea R.A., Seiter S., Snell-Rood E., Steiner U.K. & Schlichting C.D. 2015. Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. *Heredity* 115: 293–301.

Nowak M.A. & Sigmund K. 2004. Evolutionary Dynamics of Biological Games. *Science* 303: 793–799.

Oostra V., Saastamoinen M., Zwaan B.J. & Wheat C.W. 2018. Strong phenotypic plasticity limits potential for evolutionary responses to climate change. *Nat. Commun.* 9, 1005, doi:10.1038/s41467-018-03384-9.

Ørsted M., Hoffmann A.A., Rohde P.D., Sørensen P. & Kristensen T.N. 2019. Strong impact of thermal environment on the quantitative genetic basis of a key stress tolerance trait. *Heredity* 122: 315–325.

Otto S.P. & Whitlock M.C. 2013. Fixation Probabilities and Times. *eLS*, doi:10.1002/9780470015902.a0005464.pub3.

Palaima A. 2007. The fitness cost of generalization: present limitations and future possible solutions: the fitness cost of generalization. *Biol. J. Linn. Soc.* 90: 583–590.

Pallarés S., Verberk W.C.E.P. & Bilton D.T. 2021. Plasticity of thermal performance curves in a narrow range endemic water beetle. *J. Therm. Biol.* 102, 103113, doi:10.1016/j.jtherbio.2021.103113.

Palma-Guerrero J., Hall C.R., Kowbel D., Welch J., Taylor J.W., Brem R.B. & Glass N.L. 2013. Genome Wide Association Identifies Novel Loci Involved in Fungal Communication. *PLoS Genet.* 9, e1003669, doi:10.1371/journal.pgen.1003669.

Papakostas S., Vøllestad L.A., Bruneaux M., Aykanat T., Vanoverbeke J., Ning M., Primmer C.R. & Leder E.H. 2014. Gene pleiotropy constrains gene expression changes in fish adapted to different thermal conditions. *Nat. Commun.* 5, 4071, doi:10.1038/ncomms5071.

Parepa M., Fischer M. & Bossdorf O. 2013. Environmental variability promotes plant invasion. *Nat. Commun.* 4, 1604, doi:10.1038/ncomms2632.

Parmesan C., Root T.L. & Willig M.R. 2000. Impacts of Extreme Weather and Climate on Terrestrial Biota. *B. Am. Meteorol. Soc.* 81: 443–450.

Pedersen K.S., Kristensen T.N., Loeschcke V., Petersen B.O., Duus J.Ø., Nielsen N.C. & Malmendal A. 2008. Metabolomic Signatures of Inbreeding at Benign and Stressful Temperatures in *Drosophila melanogaster*. *Genetics* 180: 1233–1243.

Petersen J. & Russell P. 2016. Growth and the Environment of *Schizosaccharomyces pombe*. *Cold Spring Hab. Protoc.* 2016, pdb.top079764, doi:10.1101/pdb.top079764.

Podrabsky J.E. & Somero G.N. 2004. Changes in gene expression associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish *Austrofundulus limnaeus*. *J. Exp. Biol.* 207: 2237–2254.

Pringle A. & Taylor J.W. 2002. The fitness of filamentous fungi. *Trends Microbiol.* 10: 474–481.

Reed T.E., Schindler D.E. & Waples R.S. 2011. Interacting Effects of Phenotypic Plasticity and Evolution on Population Persistence in a Changing Climate. *Conserv. Biol.* 25: 56–63.

Reed T.E., Waples R.S., Schindler D.E., Hard J.J. & Kinnison M.T. 2010. Phenotypic plasticity and population viability: the importance of environmental predictability. *P. Roy. Soc. B-Biol. Sci.* 277: 3391–3400.

Rezende E.L., Castañeda L.E. & Santos M. 2014. Tolerance landscapes in thermal ecology. *Funct. Ecol.* 28: 799–809.

Ricciardi A., Blackburn T. M., Carlton J. T., Dick J. T., Hulme P. E., Iacarella J. C., Jeschke, J. M., Liebhold, A. M., Lockwood, J. L., MacIsaac, H. J., Pyšek, P., Richardson, D. M., Ruiz, G. M., Simberloff, D., J. Sutherland, W. J., Wardle, D. A. & Aldridge, D. C. 2017. Invasion science: a horizon scan of emerging challenges and opportunities. *Trends Ecol. Evol.* 32: 464–474.

Richards C.L., Bossdorf O., Muth N.Z., Gurevitch J. & Pigliucci M. 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol. Lett.* 9: 981–993.

Richter K., Haslbeck M. & Buchner J. 2010. The Heat Shock Response: Life on the Verge of Death. *Mol. Cell* 40: 253–266.

Richter-Boix A., Katzenberger M., Duarte H., Quintela M., Tejedo M. & Laurila A. 2015. Local divergence of thermal reaction norms among amphibian populations is affected by pond temperature variation. *Evolution* 69: 2210–2226.

Riehle M.M., Bennett A.F. & Long A.D. 2001. Genetic architecture of thermal adaptation in *Escherichia coli*. *Proc. Natl. Acad. Sci.* 98: 525–530.

Rivera H.E., Aichelman H.E., Fifer J.E., Kriefall N.G., Wuitchik D.M., Smith S.J. & Davies S.W. 2021. A framework for understanding gene expression plasticity and its influence on stress tolerance. *Mol. Ecol.* 30: 1381–1397.

Roff D.A. & Fairbairn D.J. 2007. The evolution of trade-offs: where are we? *J. Evolution. Biol.* 20: 433–447.

Rolandi C., Lighton J.R.B., De La Vega G.J., Schilman P.E. & Mensch J. 2018. Genetic variation for tolerance to high temperatures in a population of *Drosophila melanogaster*. *Ecol. Evol.* 8: 10374–10383.

Romero-Olivares A.L., Taylor J.W. & Treseder K.K. 2015. *Neurospora discreta* as a model to assess adaptation of soil fungi to warming. *BMC Evol. Biol.* 15: 1–8.

Rowe L. & D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. B Biol. Sci*. 263: 1415–1421.

Ryan F.J., Beadle G.W. & Tatum E.L. 1943. The Tube Method of Measuring the Growth Rate of *Neurospora*. *Am. J. Bot.* 30: 784–799.

Saarinen K., Lindström L. & Ketola T. 2019. Invasion triple trouble: environmental fluctuations, fluctuation-adapted invaders and fluctuation-mal-adapted communities all govern invasion success. *BMC Evol. Biol.* 19: 1-8.

Saarinen K., Laakso J., Lindström L. & Ketola T. 2018. Adaptation to fluctuations in temperature by nine species of bacteria. *Ecol. Evol.* 8: 2901–2910.

Sackman A.M. & Rokyta D.R. 2019. No Cost of Complexity in Bacteriophages Adapting to a Complex Environment. *Genetics* 212: 267–276.

Saltz J.B., Hessel F.C. & Kelly M.W. 2017. Trait Correlations in the Genomics Era. *Trends Ecol. Evol.* 32: 279–290.

Samani P. & Bell G. 2010. Adaptation of experimental yeast populations to stressful conditions in relation to population size. *J. Evol. Biol.* 23: 791–796.

Schaum C.-E. & Collins S. 2014. Plasticity predicts evolution in a marine alga. *Proc. R. Soc. B Biol. Sci*. 281, 20141486, doi:10.1098/rspb.2014.1486.

Schaum C.-E., Buckling A., Smirnoff N. & Yvon-Durocher G. 2022. Evolution of thermal tolerance and phenotypic plasticity under rapid and slow temperature fluctuations. *Proc. R. Soc. B Biol. Sci*. 289, 20220834, doi:10.1098/rspb.2022.0834.

Schaum C.-E., Buckling A., Smirnoff N., Studholme D.J. & Yvon-Durocher G. 2018. Environmental fluctuations accelerate molecular evolution of thermal tolerance in a marine diatom. *Nat. Commun.* 9, 1719, doi:10.1038/s41467-018-03906-5.

Scheiner S.M. 1993. Genetics and Evolution of Phenotypic Plasticity. *Annu. rev. ecol. syst.* 24: 35–68.

Scheiner S.M. & Levis N.A. 2021. The loss of phenotypic plasticity via natural selection: Genetic assimilation. In: Pfennig D.W. (ed.), *Phenotypic Plasticity and Evolution: Causes, Consequences, Controversies*, CRC Press, Boca Raton, Florida, pp. 161–181.

Schulte P.M., Healy T.M. & Fangue N.A. 2011. Thermal Performance Curves, Phenotypic Plasticity, and the Time Scales of Temperature Exposure. *Integr. Comp. Biol.* 51: 691–702.

Shama L.N.S., Campero-Paz M., Wegner K.M., De Block M. & Stoks R. 2011. Latitudinal and voltinism compensation shape thermal reaction norms for growth rate. *Mol. Ecol.* 20: 2929–2941.

Shea K. & Chesson P. 2002. Community ecology theory as a framework for biological invasions. *Trends Ecol. Evol.* 17: 170–176.

Sinclair B.J., Marshall K.E., Sewell M.A., Levesque D.L., Willett C.S., Slotsbo S., Dong Y., Harley C.D.G., Marshall D.J., Helmuth B.S. & Huey R.B. 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol. Lett.* 19: 1372–1385.

Sinervo B. & Svensson E. 2002. Correlational selection and the evolution of genomic architecture. *Heredity* 89: 329–338.

Snell-Rood E.C. & Ehlman S.M. 2021. Ecology and Evolution of Plasticity. In: Pfennig D.W. (ed.), *Phenotypic Plasticity and Evolution: Causes, Consequences, Controversies*, CRC Press, Boca Raton, Florida, pp. 139–160.

Snell-Rood E.C., Van Dyken J.D., Cruickshank T., Wade M.J. & Moczek A.P. 2010. Toward a population genetic framework of developmental evolution: the costs, limits, and consequences of phenotypic plasticity. *BioEssays* 32: 71–81.

Sniegowski P.D. & Gerrish P.J. 2010. Beneficial mutations and the dynamics of adaptation in asexual populations. *Philos. T. Roy. Soc. B* 365: 1255–1263.

Somero G.N. 2002. Thermal Physiology and Vertical Zonation of Intertidal Animals: Optima, Limits, and Costs of Living. *Integr. Comp. Biol.* 42: 780–789.

Somero G.N. 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* 213: 912–920.

Sørensen J.G., Kristensen T.N. & Loeschcke V. 2003. The evolutionary and ecological role of heat shock proteins. *Ecol. Lett.* 6: 1025–1037.

Sørensen J.G., Schou M.F., Kristensen T.N. & Loeschcke V. 2016. Thermal fluctuations affect the transcriptome through mechanisms independent of average temperature. *Sci. Rep.* 6, 30975, doi:10.1038/srep30975.

Stearns S.C. 1989. Trade-Offs in Life-History Evolution. *Funct. Ecol.* 3: 259–268.

Stenseth N.C., Mysterud A., Ottersen G., Hurrell J.W., Chan K.-S. & Lima M. 2002. Ecological Effects of Climate Fluctuations. *Science* 297: 1292–1296.

Stinchcombe J.R. & Kirkpatrick M. 2012. Genetics and evolution of function-valued traits: understanding environmentally responsive phenotypes. *Trends Ecol. Evol.* 27: 637–647.

Stomp M., Van Dijk M.A., Van Overzee H.M.J., Wortel M.T., Sigon C.A.M., Egas M., Hoogveld H., Gons H.J. & Huisman J. 2008. The Timescale of Phenotypic Plasticity and Its Impact on Competition in Fluctuating Environments. *Am. Nat.* 172: E169–E185.

Svanbäck R., Pineda-Krch M. & Doebeli M. 2009. Fluctuating Population Dynamics Promotes the Evolution of Phenotypic Plasticity. *Am. Nat.* 174: 176–189.

Thorogood R., Mustonen V., Aleixo A., Aphalo P.J., Asiegbu F.O., Cabeza M., Cairns J., Candolin U., Cardoso P., Eronen J.T., Hällfors M., Hovatta I., Juslén A., Kovalchuk A., Kulmuni J., Kuula L., Mäkipää R., Ovaskainen O.,

Pesonen A.-K., Primmer C.R., Saastamoinen M., Schulman A.H., Schulman L., Strona G. & Vanhatalo J. 2023. Understanding and applying biological resilience, from genes to ecosystems. *npj Biodivers.* 2, 16, doi:10.1038/s44185-023-00022-6.

Tobler R., Franssen S.U., Kofler R., Orozco-terWengel P., Nolte V., Hermisson J. & Schlötterer C. 2014. Massive Habitat-Specific Genomic Response in *D. melanogaster* Populations during Experimental Evolution in Hot and Cold Environments. *Mol. Biol. Evol.* 31: 364–375.

Todgham A.E. & Stillman J.H. 2013. Physiological Responses to Shifts in Multiple Environmental Stressors: Relevance in a Changing World. *Integr. Comp. Biol.* 53: 539–544.

Tonsor S.J., Elnaccash T.W. & Scheiner S.M. 2013. Developmental instability is genetically correlated with phenotypic plasticity, constraining heritability, and fitness. *Evolution* 67: 2923–2935.

Travisano M. & Lenski R.E. 1996. Long-Term Experimental Evolution in *Escherichia coli.* IV. Targets of Selection and the Specificity of Adaptation. *Genetics* 143: 15–26.

Turelli M. & Barton N.H. 2004. Polygenic Variation Maintained by Balancing Selection: Pleiotropy, Sex-Dependent Allelic Effects and G x E Interactions. *Genetics* 166: 1053–1079.

Tüzün N. & Stoks R. 2018. Evolution of geographic variation in thermal performance curves in the face of climate change and implications for biotic interactions. *Curr. Opin. Insect Sci.* 29: 78–84.

Uecker H. & Hermisson J. 2011. On the Fixation Process of a Beneficial Mutation in a Variable Environment. *Genetics* 188: 915–930.

Urban J., Soulard A., Huber A., Lippman S., Mukhopadhyay D., Deloche O., Wanke V., Anrather D., Ammerer G., Riezman H., Broach J.R., De Virgilio C., Hall M.N. & Loewith R. 2007. Sch9 Is a Major Target of TORC1 in *Saccharomyces cerevisiae*. *Mol. Cell* 26: 663–674.

Van Doorslaer W. & Stoks R. 2005. Growth rate plasticity to temperature in two damselfly species differing in latitude: contributions of behaviour and physiology. *Oikos* 111: 599–605.

Van Noordwijk A.J. & De Jong G. 1986. Acquisition and Allocation of Resources: Their Influence on Variation in Life History Tactics. *Am. Nat.* 128: 137–142.

Vasseur D.A., DeLong J.P., Gilbert B., Greig H.S., Harley C.D.G., McCann K.S., Savage V., Tunney T.D. & O'Connor M.I. 2014. Increased temperature variation poses a greater risk to species than climate warming. *P. Roy. Soc. B-Biol. Sci.* 281, 20132612, doi:10.1098/rspb.2013.2612.

Verghese J., Abrams J., Wang Y. & Morano K.A. 2012. Biology of the Heat Shock Response and Protein Chaperones: Budding Yeast (*Saccharomyces cerevisiae*) as a Model System. *Microbiol. Mol. Biol. R.* 76: 115–158.

Verspagen N., Ikonen S., Maes D., Stefanescu C., DiLeo M.F. & Saastamoinen M. 2023. Thermal plasticity in development and diapause strategy in a temperate butterfly across a latitudinal gradient. *Funct. Ecol.* 37: 3111–3123.

Villalba De La Peña M., Summanen P.A.M., Liukkonen M. & Kronholm I. 2023. Chromatin structure influences rate and spectrum of spontaneous mutations in *Neurospora crassa*. *Genome Res.* 33: 599–611.

Vyas A., Freitas A.V., Ralston Z.A. & Tang Z. 2021. Fission Yeast *Schizosaccharomyces pombe*: A Unicellular "Micromammal" Model Organism. *Curr. Protoc.* 1, e151, doi:10.1002/cpz1.151.

Walsh B. & Blows M.W. 2009. Abundant Genetic Variation + Strong Selection = Multivariate Genetic Constraints: A Geometric View of Adaptation. *Annu. Rev. Ecol. Evol. Syst.* 40: 41–59.

Walters R.J., Blanckenhorn W.U. & Berger D. 2012. Forecasting extinction risk of ectotherms under climate warming: an evolutionary perspective. *Funct. Ecol.* 26: 1324–1338.

Wang J. & Zhang Z. 2021. GAPIT Version 3: Boosting Power and Accuracy for Genomic Association and Prediction. *Genom. Proteom. Bioinf.* 19: 629–640.

Willi Y., Van Buskirk J. & Hoffmann A.A. 2006. Limits to the Adaptive Potential of Small Populations. *Annu. Rev. Ecol. Evol. Syst.* 37: 433–458.

Williams C.M., Buckley L.B., Sheldon K.S., Vickers M., Pörtner H.-O., Dowd W.W., Gunderson A.R., Marshall K.E. & Stillman J.H. 2016. Biological Impacts of Thermal Extremes: Mechanisms and Costs of Functional Responses Matter. *Integr. Comp. Biol.* 56: 73–84.

Wooliver R., Vtipilthorpe E.E., Wiegmann A.M. & Sheth S.N. 2022. A viewpoint on ecological and evolutionary study of plant thermal performance curves in a warming world. *AoB PLANTS* 14, plac016, doi:10.1093/aobpla/plac016.

Yadav A., Radhakrishnan A., Bhanot G. & Sinha H. 2015. Differential Regulation of Antagonistic Pleiotropy in Synthetic and Natural Populations Suggests Its Role in Adaptation. *G3-Genes Genom. Genet.* 5: 699–709.

Yamahira K., Kawajiri M., Takeshi K. & Irie T. 2007. Inter- and intrapopulation variation in thermal reaction norms for growth rate: evolution of latitudinal compensation in ectotherms with a genetic constraint. *Evolution* 61: 1577–1589.

Yu J., Holland J.B., McMullen M.D. & Buckler E.S. 2008. Genetic Design and Statistical Power of Nested Association Mapping in Maize. *Genetics* 178: 539–551.

Zerebecki R.A. & Sorte C.J.B. 2011. Temperature Tolerance and Stress Proteins as Mechanisms of Invasive Species Success. *PLoS ONE* 6, e14806, doi:10.1371/journal.pone.0014806.

Zeyl C. 2006. Experimental evolution with yeast. *FEMS Yeast Res* 6: 685–691.

Zhao J., Gladieux P., Hutchison E., Bueche J., Hall C., Perraudeau F. & Glass N.L. 2015. Identification of Allorecognition Loci in *Neurospora crassa* by Genomics and Evolutionary Approaches. *Mol. Biol. Evol.* 32: 2417–2432.

ORIGINAL PAPERS

I

GENOME-WIDE ASSOCIATION STUDY FOR LOCI INFLUENCING THERMAL PERFORMANCE CURVES IN *NEUROSPORA CRASSA*

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IV

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Environmental fluctuations drive species' competitive success in experimental invasions

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Climate change is presumed to increase both the number and frequency of fluctuations in environmental conditions. Fluctuations can affect the ecological and evolutionary processes that make species more successful competitors. For example, fluctuating conditions can create selection pressures for traits that are profitable in adaptation to fast climate change. On an ecological timescale, environmental fluctuations can facilitate species competitive success by reducing other species' population sizes. Climate change could then enhance species invasions into new areas if fluctuation-adapted invaders displace their native competitors in chancing environments. We tested experimentally whether fast environmental fluctuations, either past (on an evolutionary timescale) or present (on an ecological timescale) affect species competitive success. Bacteria that evolved in either constant or fluctuating temperature were set to compete with the dominant invader *Serratia marcescens*, which had also evolved in either constant or fluctuating temperature. Moreover, the competition experiments were conducted in environments with similarly constant or fluctuating thermal conditions. The results showed that temperature fluctuations during competition, i.e. on an ecological timescale, made the invader more successful. Surprisingly, we found that the invaders' or its competitor species' evolution in fluctuating environments did not affect the outcome of the competition. Our study highlights the importance of the present environmental fluctuations in promoting species' competitive success and potentially facilitating biological invasions.

Introduction

It is predicted that as a result of climate change temperatures and also the variability in environmental conditions will increase (IPCC 2018). Global warming has already enhanced the spread of many invasive species (Dukes & Mooney 1999, Clements & Ditommaso 2011), but it is also possible that species' evolution under fluctuating conditions will contribute to its ability to invade new areas (Lee & Gelembiuk 2008, Saarinen *et al.* 2019). This is because fluctuating conditions create selection pressures for traits helping to adapt to fast climate change (Levins 1968, Botero *et al.* 2015). In addition to increased species' invasiveness, fluctuations in environmental conditions could accelerate global biodiversity loss by making native com-

munities and their environments more vulnerable to invasions (Parepa *et al.* 2013, Saarinen *et al.* 2019). Invasive species are known to be a problem in many ecosystems, and they can, for example, competitively displace native species (Mooney & Cleland 2001). This calls for studies to predict the success of invasive species under future changing environmental conditions (Ricciardi *et al.* 2017).

Climatic fluctuations are often associated with increased disturbance in natural environments (Parepa *et al.* 2013). On an ecological timescale, environmental fluctuations can facilitate species invasions by increasing variation in native species' population sizes, thus reducing competition and releasing resources for invaders to exploit (Davis 2009). Several field and laboratory studies have found disturbed environments to be more prone to invasions than non-disturbed (Burke & Grime 1996, Davis *et al.* 2000, Melbourne *et al.* 2007, Li & Stevens 2012, Liu *et al.* 2012). In addition, invasive species have been suggested to originate from areas that are heterogeneous and disposed to disturbances (Baker 1974, Lee & Gelembiuk 2008, Foucaud *et al.* 2010, Hufbauer *et al.* 2012). For example, if the species has evolved in a disturbed environment, it might have pre-adaptations which increase its invasion success in the new environment with similar conditions (Bock 1959, Lee & Gelembiuk 2008, Hamilton *et al.* 2015). Human-altered environments are becoming universal, and if species can adapt to this type and intensity of disturbances, they could become successful invaders worldwide. This scenario is known as the anthropogenically induced adaptation to invade hypothesis (AIAI; Hufbauer *et al.* 2012).

Species evolution under environmental fluctuations, which are fast in relation to species' generation time, might select for characteristics, such as generalism and phenotypic plasticity that make them subsequently successful as invaders (Levins 1968, Meyers *et al.* 2005, Lee & Gelembiuk 2008, Ketola *et al.* 2013, Kristensen *et al.* 2018). These qualities can increase their ability to tolerate a wide range of conditions; for example, the adaptation to fluctuating temperature by thermal generalism would allow species to prosper in various environments under climate change (Zerebecki & Sorte 2011). Moreover, the adaptation to fluctuating conditions can make the invader more competitive than the native species (Lee & Gelembiuk 2008, Duncan *et al.* 2011). This is true especially if the native species have not adapted to the prevailing fluctuating conditions (Shea & Chesson 2002). On the other hand, if the native species are also pre-adapted to tolerate fluctuations, the invader might not have a competitive advantage over its local competitors (Saarinen *et al.* 2019). However, there is little information if the lack of adaptation to fluctuations in native species could make communities less resistant against invasions and increase the risk of extinctions due to competition with the invader (Marvier *et al.* 2004, Melbourne *et al.* 2007).

The experimental evolution studies on adaptation to fluctuating environments are numerous (Kassen 2002), yet very few studies exist on the possible effects of fluctuations promoting the biological invasions (Lee & Gelembiuk 2008). Thus, our aim was to test how rapid temperature fluctuations in the environment during competition (i.e. on an ecological timescale), and during the evolution of both the invader and its competitor species (i.e. on an evolutionary timescale), affect the competitive success of the invader. To investigate the multifactorial nature of invasions, we used several bacterial species that had evolved either in constant or fluctuating temperature and implemented inter-specific competition experiments in similarly constant or fluctuating thermal conditions (Saarinen *et al.* 2018). With this experimental evolution setup and high levels of replication, we were able to tease apart the effects that are co-occurring in nature.

As an invader we used a strongly competitive species *Serratia marcescens*, competing with other bacterial species in bicultures. In our competition experiments, the invader started as rare compared with its competitor species. Both the invader and its competitor species were inoculated concurrently, hence having an equal opportunity to use the available resources. Therefore, our experiments reflect asymmetric competition rather than invasion in a strict sense, i.e. when the invader arrives later than its resident species. The level of competitive success of the invader was defined by continuous metrics as the proportion of invader clones in the total number of bacterial clones under specific environmental conditions.

We hypothesized that: (1) disturbed, thermally fluctuating, environments promote species' competitive success, (2) evolution under fluctuating temperature increases species' competitive success, and (3) species evolved under constant temperature are less resistant to competition in thermally fluctuating environments.

Material and methods

Study species

In our study, we used the following four species, all originally obtained from ATCC® (American Type Culture Collection): *Serratia marcescens* ssp. *marcescens* (ATCC® 13880™), *Pseudomonas putida* (ATCC® 12633™), *Pseudomonas fluorescens* (ATCC® 13525™) and *Novosphingobium capsulatum* (ATCC® 14666™). Bacterial species were chosen based on their abilities to grow well in the same medium and to tolerate rapidly fluctuating temperatures of the experiment. Before the experiments, the clones had evolved for 79 days in thermal cabinets (Lab Companion, ILP-12; Jeio Tech, Seoul, Korea) in two temperature regimes: constant temperature of 30 °C for the whole period, and fluctuating temperature cycling as follows: 20–30–40–30–20 °C each temperature kept for 2 h (for details *see* Saarinen *et al.* 2018). This corresponded to ca. 86 generations in all species and treatments. The constant temperature was near the optimal temperature for all the bacterial species, when the maximum growth rate and yield were measured (Saarinen *et al.* 2018). *Serratia marcescens* was chosen as an invader because it is known to dominate the other study species, i.e. reflecting typical invasive species in this respect, and is also easy to distinguish from the other species when using DNase agar plates (Smith *et al.* 1969, Ketola *et al.* 2017).

Competition experiments

Our study design allowed us to separate the effects of the environment during competition, the evolution of the invader and the evolution of the competitor species (constant *vs*. fluctuating temperature in all cases) on the competitive success of *S. marcescens*. In this experiment, the competitive success was calculated as the proportion of the *S. marcescens* colonies in the total colony count including also the competitor species colonies. This means that there was no defined threshold value for the competitor species' displacement. The invader clone that had evolved in either constant or fluctuating temperature competed with the competitor species' clone that had also evolved in either constant or fluctuating temperature. We implemented competition experiments in two environments which matched the conditions during bacterial evolution, one with constant (30 °C) and the other with fluctuating (20–30–40 °C, at two-hour intervals) temperature. Altogether we had eight different treatment combinations.

In the competition experiment, we used one clone per replicate population (n = 8) for each bacterial species. After the evolution (*see* "Study species"), clones were isolated from each of the populations and frozen at –80 °C (1:1 in 80% glycerol). At first, we propagated all study species separately from frozen samples for three days at 30 °C and measured clones' inoculum sizes as optical densities (OD) in temperature-controlled spectrophotometers (Bioscreen C®, Oy Growth Curves Ab, Ltd., Helsinki, Finland). After that, one clone from each *S. marcescens* population was chosen randomly to compete with one clone from each population of its competitor species in biculture. The initial invader-to-competitor ratio was 1:24. This experiment was repeated with all three competitor species (*P. putida*, *P. fluorescens*, *N. capsulatum*). In total there were 192 competition experiments.

The experimental microcosms were 15 ml centrifuge tubes (Sarstedt, Numbrecht, Germany) containing 3 ml of sterile nutrient broth medium (10 g of nutrient broth powder [Difco, Becton, Dickinson & Company, Sparks, MD] and 1.25 g of yeast extract (Difco) in 1 l of sterile ddH_2O). We initiated the competition experiments by pipetting 2 µl of *S. marcescens* and 48 µl of one of its competitor species into all of the 192 tubes. Species were inoculated concurrently so that there was an equal opportunity for consuming the resources. Half of the tubes were kept in constant (30 °C) and half in fluctuating

(*see* "Study species") temperatures. The tube caps were kept loose to ensure the gas exchange. We allowed the species to compete for three days (72 h), after which we sampled 500 µl of bacterial suspension from each tube into cryotubes containing 500 µl of 80% glycerol and stored them at –80 °C for later analysis.

Determination of competitive success

To determine the competitive success of *S. marcescens*, we counted the invader colonies in each sample after three days of competition. We plated all the 192 frozen samples in a random order. We used a standard dilution-series technique, i.e., we pipetted 100 µl of thawed bacterial suspension into 900 µl of sterile ddH_2O and repeated the tenfold dilution six times to achieve 10^{-5}-fold and 10^{-6}-fold dilutions of the original samples. These dilutions allowed us to count separate colonies on agar plates. The discrimination of species, *S. marcescens* or other, was conducted on DNase test agar plates (Becton, Dickinson and Company, Sparks, MD; premade at Tammertutkan maljat, Tampere, Finland). DNase plates allow for separation of the invader colonies from the competitor species colonies because only *S. marcescens* can break down DNA enzymatically, which is seen as a clear halo around the colonies (Smith *et al.* 1969, Ketola *et al.* 2017). After two to three days of propagation at room temperature, we counted the *S. marcescens* colonies and all bacterial colonies on each plate to estimate the competitive success (μ) of the invader expressed as the proportion of *S. marcescens* colonies in the total colony count.

Data analysis

We tested the effect of the environment temperature during competition, as well as that of the evolution temperature of the invader and competitor species, on the competitive success of *S. marcescens*. We modeled the odds of encountering *S. marcescens* colonies in all bacterial colonies on a DNase agar plate. As we had a non-normal proportion data and the analysis included random effects, we analyzed the results with generalized linear mixed model (GLMMs; Bolker *et al.* 2009). We used a binomial error distribution and a logit link, and set the total number of colonies on a plate as a denominator to control for the total number of events in a trial. All analyses were run in SPSS ver. 24.0 (IBM-SPSS, Chicago, IL).

The three fixed factors were the environment during competition, the evolution of the invader and the evolution of the competitor species, which all had two levels, constant and fluctuating temperatures. We fitted these three fixed factors, all their two-way interactions and the three-way interaction as explanatory variables. The identity of the *S. marcescens* clone, regardless of its evolution regime, and the identity of the competitor species were fitted as random factors. This was done to control for the non-independency of the observations, arising from the fact that the competitive success of the same invader clones was measured in two environments and against several competitor species. In addition, we also performed backward model selection for the full factorial model to find the most reduced model by removing effects for which $p > 0.1$. This procedure did not change the biological interpretation of the results.

To test the sensitivity of the main results, we re-ran our model including also the fixed effect of competitor species' identity, and all possible two- to four-way interactions with other fitted factors. This allows test the responses of competitor species to the environment temperature during competition and the evolution temperature of the invader and its competitor species. In addition, we tested a model in which the inoculum sizes of both the competitor species' clones and the invaders' clones were added as covariates to control for the differences in starting cell densities. Moreover, using the data from Saarinen *et al*. (2018), we tested whether growth characteristics of the invader clones at nearly optimal thermal conditions (30 °C), extreme thermal conditions (20 °C and 40 °C) or during thermal fluctuations (*see* "Study species") affected the competitive success of the invader by including them in the analysis as fixed covariates. As all additional analyses confirmed our findings from the full factorial model, in the following we present the results from the full factorial and

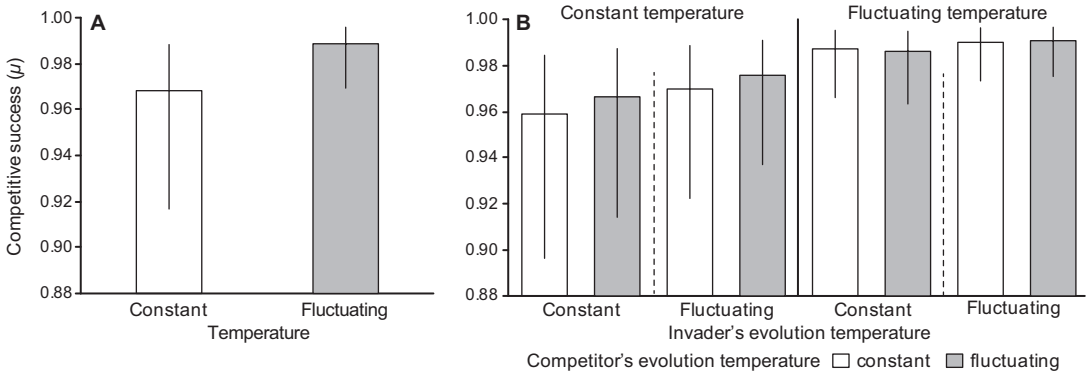

Fig. 1. The effects of (**A**) the environment temperature during competition, and (**B**) the three-way interaction of the environment temperature during competition, the invader's evolution temperature and the competitor's evolution temperature. Competitive success (μ) is the proportion of *Serratia marcescens* colonies in the total colony count after three days (72 h) of competition. Shown are the estimated marginal means with 95% confidence limits (CL).

the reduced models only. All count data, clones' inoculum sizes and additional data of maximum growth rates and yields are available at https://doi.org/10.17011/jyx/dataset/68316.

Results

The full factorial model indicated high competitive success of *S. marcescens* after three days of competition (all-data mean $\mu = 0.981$, 95%CL = 0.949–0.993). In all the studied species' pairs, there was clearly one factor, the environment temperature during competition, which had an effect on the competitive success. *Serratia marcescens* was more successful when the environment temperature during competition was fluctuating ($\mu = 0.989$, 95%CL = 0.969–0.996) rather than constant ($\mu = 0.968$, 95%CL = 0.917–0.988, $p = 0.001$) (Fig. 1A and Table 1). The invader's evolution temperature, the competitor's evolution temperature, as well as all the studied interactions had no effect on the competitive success of *S. marcescens* (Fig. 1B and Table 1). The random effect of the invader clones' identity was significant ($\sigma^2_{\text{logit}} = 0.503$, SE = 0.205, $Z = 2.447$, $p = 0.014$), but the effect of the competitor species identity was non-significant ($\sigma^2_{\text{logit}} = 0.271$, SE = 0.275, $Z = 0.985$, $p = 0.325$).

The reduced model produced the same results. The only difference was that competitive

Table 1. The results of the generalized linear mixed model testing the effects of the environment temperature during competition, as well as the evolution temperature of the invader and the competitor species on the competitive success (μ) of *Serratia marcescens* after three days (72 h) of competition. Full model and the most reduced model after backward model selection with removal criterion of $p > 0.1$; df1 was 1 for all factors and factor interactions.

	Full model			Reduced model		
	F	df2	*p*	*F*	df2	*p*
Environment during competition	141.679	182	0.001	155.031	186	0.001
Invader's evolution	0.792	14	0.389	0.815	14	0.382
Competitor's evolution	1.533	182	0.217	3.184	186	0.076
Environment during competition × invader's evolution	0.001	182	0.981			
Environment during competition × competitor's evolution	1.624	182	0.204			
Invader's evolution × competitor's evolution	0.256	182	0.614			
Environment during competition × invader's evolution × competitor's evolution	0.165	182	0.685			
	AIC = 1153.181			AIC = 1139.420		

success of the invader seemed insignificantly higher when the competitor species had evolved in fluctuating (μ = 0.982, 95%CL = 0.952–0.994) rather than in constant temperature (μ = 0.980, 95%CL = 0.945–0.993, p = 0.076; *see* Table 1). The AIC value for the reduced model was slightly better than that for the full model (*see* Table 1).

Discussion

Ongoing climate change is increasing fluctuations in global temperature (IPCC 2018), and therefore can potentially increase chances of successful species invasions now and in future (Stachowicz *et al.* 2002, Parepa *et al.* 2013). Despite that, very few studies have tested this prediction (Kreyling *et al.* 2008, Lee & Gelembiuk 2008, Ketola *et al.* 2013, Saarinen *et al.* 2019). Experimental evaluation of the relative importance of ecological and evolutionary processes, which affect species' invasion success at different timescales is also lacking (Lee 2002, Facon *et al.* 2006). In our manipulative laboratory experiment, we quantified the effect of temperatures fluctuating on the ecological timescale and during the evolution of the invader and its competitor on the competitive success of the invader. We found that fast temperature fluctuations in the environment during competition clearly facilitated competitive success (Fig. 1A and Table 1). However, we did not detect any evidence for the effect of fluctuating temperatures during invaders' or its competitor's evolution, or the interactions between the studied factors on the outcome of the competition (Fig. 1B and Table 1).

In accordance with our hypothesis, *S. marcescens* was more successful, when the temperature during competition was fluctuating, rather than when it was constant (Fig. 1A and Table 1). This result indicates that disturbed environments are more prone to invasions than less disturbed, and is consistent with the results of previous studies carried out on plants and bacteria (Burke & Grime 1996, Davis *et al.* 2000, Li & Stevens 2012, Saarinen *et al.* 2019). Our study is among the first ones to show experimental evidence that fluctuations in temperature, not just in resource availability, could also affect species' competitive success, and potentially its ability to invade (Davis *et al.* 2000, Melbourne *et al.* 2007, Liu *et al.* 2012, Saarinen *et al.* 2019). Experiments providing data on fluctuations in other environmental factors and their interactions could offer interesting insights (Kreyling *et al.* 2008, Parepa *et al.* 2013). However, it should be remembered that disturbed and fluctuating environments may differ, and besides the anthropogenic pressure, there is also natural variation in environmental conditions which could affect species' invasion success (Ricciardi & MacIsaac 2000, Winkler *et al.* 2008). Contrary to competitive exclusion, environmental fluctuations can also act as a factor promoting species' coexistence and maintaining diversity (Chesson 2000).

Unexpectedly, we found that evolution of *S. marcescens* in the fluctuating temperature had no effect on its competitive success (Fig. 1B and Table 1). This contradicts the notion that adaptation to fluctuating environments improves species' invasiveness (Lee & Gelembiuk 2008). Some earlier studies found evidence for the higher invasion success of species and populations that evolved in disturbed habitats (Baker 1974, Winkler *et al.* 2008, Foucaud *et al.* 2010, Ketola *et al.* 2013, Saarinen *et al.* 2019). Also, permissively fluctuating temperatures can select for enhanced performance traits such as faster growth rate (Colinet *et al.* 2015). In our study, all bacterial cultures were initiated from clones of replicate populations which had evolved in specific environments. Even when starting from a homogeneous gene pool, the evolutionary time in an experimental setup closely similar to ours, is sufficient for adaptations to occur in response to selection (Ketola *et al.* 2013). This suggests that within a given time the selection for improved competitive ability was not very strong. Alternatively the lack of results could be due to clone-specific evolution of growth traits that could mask treatment effects. However, our additional analyses indicated that the competitive success of *S. marcescens* was not explained by its growth characteristics nor the ability to compete with other species (resource or interference competition).

Like invaders, competitor species could also benefit from the evolution in fluctuating environments (Saarinen *et al*. 2019). Contrary to our

hypothesis, the results did not show higher competitive success of *S. marcescens* when the competitor species had evolved in a constant-temperature environment (Fig. 1B and Table 1). In the reduced model, the effect of the competitor's evolution temperature was insignificant (p = 0.076; *see* Table 1) but pointing to better competitive success of the invader when its competitor had evolved in a fluctuating-temperature environment. Whether the species' evolution in a constant thermal environment could affect their adaptation to thermally fluctuating environments is unclear. For example, in the long-term study of tobacco hornworm (*Manduca sexta*) (Kingsolver *et al.* (2009) did not find divergence in the responses of fluctuation- and constant-environment adapted populations to fast temperature fluctuations. In another experiment, populations of the pitcher plant mosquito (*Wyeomyia smithii*) from different geographic locations showed no genetic differences in their response to diurnal fluctuations (Ragland & Kingsolver 2008). In both studies, the between-population differences in life-history traits were dependent on the mean temperature rather than the variation in temperature (Ragland & Kingsolver 2008, Kingsolver *et al.* 2009). On the other hand, bacterial communities that had adapted to constant conditions were found to be more vulnerable to invasion, especially during the early stages of the experiment (Saarinen *et al.* 2019). More studies are needed to assess whether climate change can facilitate biological invasions by favoring invasive species over their native competitors (Stachowicz *et al.* 2002).

In addition, we found no effect of the interaction between the environment temperature during competition and that during the invader's evolution (Fig. 1B and Table 1). This contradicts the anthropogenically induced adaptation to invade hypothesis and the previous findings on invasive species, which show evidence that pre-adaptation of organisms to matching environmental conditions makes them more successful in invading new areas (Ricciardi & MacIsaac 2000, Bossdorf *et al.* 2008, Winkler *et al.* 2008, Foucaud *et al.* 2010, Hufbauer *et al.* 2012, Hamilton *et al.* 2015). Along with this, none of the interactions were found to affect the competitive success of *S. marcescens*, pointing that the studied factors were not dependent on each other (*see* Fig. 1B and Table 1). Some studies found stronger evidence for these interactions, when the environmental conditions during invasion, the traits of the invader and the attributes of its native competitors acted together (Kreyling *et al.* 2008, Litchman 2010, Mächler & Altermatt 2012, Saarinen *et al*. 2019).

In our study, the detection of the effects of temperature fluctuations on competition might have been confounded by the high overall competitive success of *S. marcescens*. Although we cannot separate the effect of the overall competitive success, it was clear that the temperature fluctuations during competition improved the success of the rare species. Previous studies carried out with the same bacterial strains, but setting *S. marcescens* against multiple species in the same culture and adding the competitor species frequently, did not find as pronounced invasions (Ketola *et al.* 2017, Saarinen *et al.* 2019). In addition, it could be argued that our artificial community was too simple as we used only one competitor species as "the community". Indeed, this is a simplification of nature, where also biodiversity and composition of native communities are assumed to affect their resistance to invasions (Davis 2009, Ketola *et al.* 2017). Recent studies highlighted, for example, the importance of multiple invaders and the abundance of the invader relative to that of the resident community as the primary drivers of invasion (Kinnunen *et al.* 2018, Rivett *et al.* 2018). However, the aim of our study was not to mimic the complexity of natural communities, but to efficiently test the theories of invasion biology (Naughton *et al.* 2015). The information emerging from microbial studies is also important since climate change is going to increase the spread of pathogens into new habitats and hosts (Bennett & Hughes 2009, Litchman 2010, Ricciardi *et al.* 2017).

To conclude, we found that rapid temperature fluctuations during competition improve the competitive success of *S. marcescens*, pointing that the ecological context could be extremely important also in invasions (Shea & Chesson 2002). Furthermore, we found no evidence that evolution under fluctuating conditions or competitors' lack of adaptation to tolerate temperature fluctuations makes the invader more successful. Our findings show that the current

environmental variation resulting from climate change (IPCC 2018) could be the most prominent factor in promoting species' competitive success in fluctuating environments. However, further studies aiming at distinguishing the traits of the invader, the attributes of its native competitors and the environmental conditions during invasion, should be undertaken (Facon *et al.* 2006, Lee & Gelembiuk 2008). Considering these factors together would allow us to make more accurate predictions of the species' ability to invade under novel, fluctuating climatic conditions (Kreyling *et al.* 2008, Litchman 2010, Mächler & Altermatt 2012, Saarinen *et al.* 2019).

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References

Baker, H. G. 1974: The evolution of weeds. — *Annu. Rev. Ecol. Syst.* 5: 1–24.

Bennett, A. F. & Hughes, B. S. 2009: Microbial experimental evolution. — *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297: R17–R25, https://doi.org/10.1152/ajpregu.90562.2008.

Bock, W. J. 1959: Preadaptation and multiple evolutionary pathways. — *Evolution* 13: 194–211.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H. & White, J. S. 2009: Generalized linear mixed models: a practical guide for ecology and evolution. — *Trends Ecol. Evol.* 24: 127–135.

Bossdorf, O., Lipowsky, A. & Prati, D. 2008: Selection of preadapted populations allowed *Senecio inaequidens* to invade Central Europe. — *Divers. Distrib.* 14: 676–685.

Botero, C. A., Weissing, F. J., Wright, J. & Rubenstein, D. R. 2015: Evolutionary tipping points in the capacity to adapt to environmental change. — *Proc. Natl. Acad. Sci. USA* 112: 184–189.

Burke, M. J. & Grime, J. 1996: An experimental study of plant community invasibility. — *Ecology* 77: 776–790.

Chesson, P. 2000: Mechanisms of maintenance of species diversity. — *Annu. Rev. Ecol. Syst.* 31: 343–366.

Clements, D. & Ditommaso, A. 2011: Climate change and weed adaptation: can evolution of invasive plants lead to greater range expansion than forecasted? — *Weed Res.* 51: 227–240.

Colinet, H., Sinclair, B. J., Vernon, P. & Renault, D. 2015: Insects in fluctuating thermal environments. — *Annu. Rev. Entomol.* 60: 123–140.

Davis, M. A. 2009: *Invasion biology*. — Oxford University Press, Oxford, UK.

Davis, M. A., Grime, J. P. & Thompson, K. 2000: Fluctuating resources in plant communities: a general theory of invasibility. — *J. Ecol.* 88: 528–534.

Dukes, J. S. & Mooney, H. A. 1999: Does global change increase the success of biological invaders? — *Trends Ecol. Evol.* 14: 135–139.

Duncan, A. B., Fellous, S., Quillery, E. & Kaltz, O. 2011: Adaptation of *Paramecium caudatum* to variable conditions of temperature stress. — *Res. Microbiol.* 162: 939–944.

Facon, B., Genton, B. J., Shykoff, J., Jarne, P., Estoup, A. & David, P. 2006: A general eco-evolutionary framework for understanding bioinvasions. — *Trends Ecol. Evol.* 21: 130–135.

Foucaud, J., Orivel, J., Loiseau, A., Delabie, J.H., Jourdan, H., Konghouleux, D., Vonshak, M., Tindo, M., Mercier, J. & Fresneau, D. 2010: Worldwide invasion by the little fire ant: routes of introduction and eco-evolutionary pathways. — *Evol. Appl.* 3: 363–374.

Hamilton, J. A., Okada, M., Korves, T. & Schmitt, J. 2015: The role of climate adaptation in colonization success in *Arabidopsis thaliana*. — *Mol. Ecol.* 24: 2253–2263.

Hufbauer, R. A., Facon, B., Ravigné, V., Turgeon, J., Foucaud, J., Lee, C. E., Rey, O. & Estoup, A. 2012: Anthropogenically induced adaptation to invade (AIAI): contemporary adaptation to human-altered habitats within the native range can promote invasions. — *Evol. Appl.* 5: 89–101.

IPCC 2018: *Summary for policymakers*. — An IPCC Special Report on the impacts of global warming of 1.5 °C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty, World Meteorological Organization, Geneva, Switzerland.

Kassen, R. 2002: The experimental evolution of specialists, generalists, and the maintenance of diversity. — *J. Evolution Biol.* 15: 173–190.

Ketola, T., Saarinen, K. & Lindström, L. 2017: Propagule pressure increase and phylogenetic diversity decrease community's susceptibility to invasion. — *BMC Ecol.* 17: 15, https://doi.org/10.1186/s12898-017-0126-z.

Ketola, T., Mikonranta, L., Zhang, J., Saarinen, K., Örmälä, A., Friman, V., Mappes, J. & Laakso, J. 2013: Fluctuating temperature leads to evolution of thermal generalism and preadaptation to novel environments. — *Evolution* 67: 2936–2944.

Kingsolver, J. G., Ragland, G. J. & Diamond, S. E. 2009: Evolution in a constant environment: thermal fluctuations and thermal sensitivity of laboratory and field populations of *Manduca sexta*. — *Evolution* 63: 537–541.

Kinnunen, M., Dechesne, A., Albrechtsen, H. J. & Smets, B. F. 2018. Stochastic processes govern invasion success in microbial communities when the invader is phylogenetically close to resident bacteria. — *ISME J.* 12: 2748–2756.

Kreyling, J., Beierkuhnlein, C., Ellis, L. & Jentsch, A. 2008: Invasibility of grassland and heath communities exposed to extreme weather events – additive effects of diversity resistance and fluctuating physical environment. — *Oikos* 117: 1542–1554.

Kristensen, T. N., Ketola, T. & Kronholm, I. 2018: Adaptation to environmental stress at different timescales. — *Ann. New York Acad. Sci.*, https://doi.org/10.1111/nyas.13974.

Lee, C. E. 2002: Evolutionary genetics of invasive species. — *Trends. Ecol. Evol.* 17: 386–391.

Lee, C. E. & Gelembiuk, G. W. 2008: Evolutionary origins of invasive populations. — *Evol. Appl.* 1: 427–448

Levins, R. 1968: *Evolution in changing environments: some theoretical explorations.* — Princeton University Press, Princeton, NJ.

Li, W. & Stevens, M. H. H. 2012: Fluctuating resource availability increases invasibility in microbial microcosms. — *Oikos* 121: 435–441.

Litchman, E. 2010: Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. — *Ecol. Lett.* 13: 1560–1572.

Liu, M., Bjørnlund, L., Rønn, R., Christensen, S. & Ekelund, F. 2012: Disturbance promotes non-indigenous bacterial invasion in soil microcosms: analysis of the roles of resource availability and community structure. — *PLoS ONE* 7(10): e45306, https://doi.org/10.1371/journal.pone.0045306.

Marvier, M., Kareiva, P. & Neubert, M. G. 2004: Habitat destruction, fragmentation, and disturbance promote invasion by habitat generalists in a multispecies metapopulation. — *Risk Anal.* 24: 869–878.

Melbourne, B. A., Cornell, H. V., Davies, K. F., Dugaw, C. J., Elmendorf, S., Freestone, A. L., Hall, R. J., Harrison, S., Hastings, A. & Holland, M. 2007: Invasion in a heterogeneous world: resistance, coexistence or hostile takeover? — *Ecol. Lett.* 10: 77–94.

Meyers, L. A., Ancel, F. D. & Lachmann, M. 2005: Evolution of genetic potential. — *PLoS Comput Biol* 1(3): e32, https://doi.org/10.1371/journal.pcbi.0010032.

Mooney, H. A. & Cleland, E. E. 2001: The evolutionary impact of invasive species. — *Proc. Natl. Acad. Sci. USA* 98: 5446–5451.

Mächler, E. & Altermatt, F. 2012: Interaction of species traits and environmental disturbance predicts invasion success of aquatic microorganisms. — *PLoS ONE* 7(9): e45400, https://doi.org/10.1371/journal.pone.0045400.

Naughton, H., Alexandrou, M., Oakley, T. & Cardinale, B. 2015: Phylogenetic distance does not predict competition in green algal communities. — *Ecosphere* 6(7): 116, http://dx.doi.org/10.1890/ES14-00502.1.

Parepa, M., Fischer, M. & Bossdorf, O. 2013: Environmental variability promotes plant invasion. — *Nat. Commun.* 4, 1604, https://doi.org/10.1038/ncomms2632.

Ragland, G. J. & Kingsolver, J. G. 2008: The effect of fluctuating temperatures on ectotherm life-history traits: comparisons among geographic populations of *Wyeomyia smithii*. — *Evol. Ecol. Res.* 10: 29–44.

Ricciardi, A. & MacIsaac, H. J. 2000: Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. — *Trends Ecol. Evol.* 15: 62–65.

Ricciardi, A., Blackburn, T. M., Carlton, J. T., Dick, J. T., Hulme, P. E., Iacarella, J. C., Jeschke, J. M., Liebhold, A. M., Lockwood, J. L., MacIsaac, H. J., Pyšek, P., Richardson, D. M., Ruiz, G. M., Simberloff, D., J. Sutherland, W. J., Wardle, D. A. & Aldridge, D. C. 2017. Invasion science: a horizon scan of emerging challenges and opportunities. — *Trends Ecol. Evol.* 32: 464–474.

Rivett, D. W., Jones, M. L., Ramoneda, J., Mombrikotb, S. B., Ransome, E. & Bell, T. 2018. Elevated success of multispecies bacterial invasions impacts community composition during ecological succession. — *Ecol. Lett.* 21: 516–524.

Saarinen, K., Lindström, L. & Ketola, T. 2019: Invasion triple trouble: Environmental fluctuations, fluctuation-adapted invaders and fluctuation-mal-adapted communities all govern invasion success. — *BMC Evol. Biol.* 19: 42, https://doi.org/10.1186/s12862-019-1348-9.

Saarinen, K., Laakso, J., Lindström, L. & Ketola, T. 2018: Adaptation to fluctuations in temperature by nine species of bacteria. — *Ecol. Evol.* 8: 2901–2910.

Shea, K. & Chesson, P. 2002: Community ecology theory as a framework for biological invasions. — *Trends Ecol. Evol.* 17: 170–176.

Smith, P. B., Hancock, G. A. & Rhoden, D. L. 1969: Improved medium for detecting deoxyribonuclease-producing bacteria. — *Appl. Microbiol.* 18: 991–993.

Stachowicz, J. J., Terwin, J. R., Whitlatch, R. B. & Osman, R. W. 2002: Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. — *Proc. Natl. Acad. Sci. USA* 99: 15497–15500.

Winkler, G., Dodson, J. J. & Lee, C. E. 2008: Heterogeneity within the native range: population genetic analyses of sympatric invasive and noninvasive clades of the freshwater invading copepod *Eurytemora affinis*. — *Mol. Ecol.* 17: 415–430.

Zerebecki, R. A. & Sorte, C. J. 2011: Temperature tolerance and stress proteins as mechanisms of invasive species success. — *PLoS ONE* 6(4): e14806, https://doi.org/10.1371/journal.pone.0014806.