Master's Thesis

The effect of population size on adaptation to fluctuating temperatures

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Climate change increases variation in environmental conditions and reduces the population sizes of many species, thus making evolutionary adaptation more difficult. According to the general theory, evolutionary adaptation to one environment weakens the adaptation to alternative environments due to antagonistically pleiotropic alleles. However, antagonistic pleiotropy is rarely observed in experiments. A new theory has been put forward to explain this paradox, stating that the interplay of environmental variation and population size can affect the detection of fitness trade-offs. Based on this, larger populations may adapt faster to fluctuating environments than smaller populations and are able to avoid fitness costs between environments. We wanted to evaluate whether large populations adapt more efficiently to fluctuating temperatures than small populations. We did an evolution experiment with fission yeast (*Schizosaccharomyces pombe)*, where strains evolved for 500 generations at constant and fluctuating temperatures. Evolved strains competed against ancestral strains in temperatures that matched the conditions during evolution to detect possible adaptations and in alternative environments to detect trade-offs. We did not find evidence for the interaction of environmental variation and population size. Population size did not affect adaptation to temperature fluctuations, and no trade-offs were observed. One possible explanation for the results is that temperature adaptation requires a lot of time and genetic evolution.

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Ilmastonmuutos lisää elinolosuhteissa tapahtuvaa vaihtelua ja pienentää monien eliölajien populaatiokokoja, mikä vaikeuttaa evolutiivista sopeutumista. Yleisen teorian mukaan evolutiivinen sopeutuminen yhdenlaiseen ympäristöön heikentää antagonistisen pleiotropian vaikutuksesta sopeutumista toisenlaiseen ympäristöön. Antagonistista pleiotropiaa ei kuitenkaan usein havaita tutkimuksissa. Paradoksia selittämään on esitetty uusi teoria, jonka mukaan kelpoisuuserojen havaitsemiseen voivat vaikuttaa ympäristön olosuhteiden vaihtelu ja populaation koko. Suurempien populaatioiden voidaan olettaa sopeutuvan pienempiä populaatioita nopeammin vaihteleviin ympäristöihin ja voivan välttää kelpoisuuskulut ympäristöjen välillä. Halusimme selvittää, sopeutuvatko suuret populaatiot tehokkaammin lämpötilan vaihteluun, kuin pienet populaatiot, sekä havaitaanko suurten ja pienten populaatioiden välillä eroja kelpoisuusristiriesassa vaihtelevan ja vakaiden lämpötilojen kesken. Toteutimme evoluutiokokeen halkihiivalla (*Schizosaccharomyces pombe*), jossa kannat kehittyivät 500 sukupolven ajan vakaissa ja vaihtelevissa lämpötiloissa. Kehittyneitä kantoja kilpailutettiin kantapopulaatioita vastaan evoluutiokoetta vastaavissa ympäristöissä mahdollisten sopeutumien havaitsemiseksi. Tuloksissamme emme havainneet ympäristön vaihtelun ja populaation koon vuorovaikutuksia. Populaatiokoolla ei myöskään ollut vaikutusta lämpötilan vaihteluun sopeutumisessa eikä ristiriesoja havaittu. Yksi mahdollinen selitys tuloksille on, että lämpötilaan sopeutuminen vaatii paljon aikaa ja geneettistä kehitystä.

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1 INTRODUCTION

Climate change increases the average temperature and frequency of extreme weather conditions (Gunderson and Stillman 2015). Extreme conditions mean higher maximum temperatures and increased frequency and duration (Field *et al.* 2012). Yet, we do not know how organisms are able to adapt to these changing conditions (Botero *et al.* 2015). According to earlier research, the rise in climate fluctuation and the frequency of extreme events have more significant biological impact on species than average warming (Thompson *et al.* 2013). As fluctuations increase, organisms have to spend more time in extreme temperatures. Organisms are adapted to the same environmental conditions in which their ancestors lived, and environmental changes outside of these conditions are likely to weaken fitness and decrease population sizes (Jiang *et al.* 2010; O'Dea *et al.* 2016). It is also known that population size affects the rate of adaptation, the extent of adaptation, and the effectiveness of natural selection (Chavhan et al. 2020).

1.1 Population size effects

The population size is a significant factor affecting the adaptation rate (Chavhan *et al.* 2020). It is generally assumed that large populations adapt faster than small populations through rare beneficial mutations with significant effects (Handel and Rozen 2009; Chavhan *et al.* 2019). In large populations, more beneficial mutations occur in each generation, shortening the waiting time for new beneficial mutations. They have an increased probability of achieving mutations that have significant benefits in adaptation (Chavhan *et al.* 2019). Also, in a large population with many beneficial mutations, all possible mutations may be present in each generation and the following fixed mutation is likely to increase fitness (Hall *et al.* 2010).

More mutations are not always beneficial. Simultaneously occurring beneficial mutations compete for fixation, slowing down the process known as clonal interference. Small populations can escape clonal interference and adapt faster since they have fewer mutations every generation (Vahdati and Wagner 2018). In addition, small populations fix beneficial mutations more than large populations, which increases variation in adaptation pathways between populations (Handel and Rozen 2009). It has been noted that under long-term selection, small populations show a reduced response. Small populations contain lower variation, they are more sensitive during the selection process, and new mutations occur less frequently. In addition, the adaptive capacity of small populations can be affected by their reduced individual fitness. Decreasing fitness is directly proportional to the population growth rate, which affects the rate at which a population can respond to selection without becoming extinct (Willi *et al.* 2006).

1.2 Environmental effects

Temperature can be considered the most stressful abiotic factor that organisms face. It affects the biochemistry and physiology of organisms, which is why organisms have to react to temperature changes (Brown *et al.* 2017; Perez and Aron 2020). These temperature changes can appear in the habitats of organisms as a long-term climate change or as a sudden environmental change (Brown *et al.* 2017). For example, organisms can react to sudden changes, by migrating to a more favorable habitat or physiological adaptation. Evolutionary adaptation is required when responding to long-term change (Bürger and Lynch 1995).

Organisms have ideal temperature ranges in which they can perform optimally. Temperatures outside this range usually cause fitness costs (Yampolsky *et al.* 2014). Due to natural selection, organisms can function optimally at the temperatures to which they are adapted, minimizing fitness costs at those temperatures (Porcelli *et al.* 2017). In environments that organisms commonly face, selection removes deleterious mutations more effectively than in environments that are rarely faced. Thus, those harmful mutations in the new environment cause lower adaptation. All these processes produce a population well adapted to the current conditions but likely poorly adapted to new conditions (Hoffmann and Hercus 2000).

If the adaptation time is limited, at least one beneficial mutation in the population must be fixed before conditions become lethal. This depends on the mutation rate and effective population size (Samani and Bell 2010). In contrast, increased fitness in unfavorable environments is associated with decreased fitness in favorable environments (Hoffmann and Hercus 2000).

1.3 Interaction of population size and environment

If growth conditions remain constant long enough, every population will reach the same level of adaptation provided mutations that have independent effects on fitness (Samani and Bell 2010). As environmental conditions remain the same for a long time, large populations can specialize better than small populations. Due to higher genetic variance, larger populations are expected to evolve faster, emphasizing the effects of natural selection, as selection favors beneficial mutations and eliminates harmful ones. Large populations can be vulnerable to sudden environmental changes due to rapid adaptation to their current environment. The best adaptation would, therefore, be a trade-off between adaptation speed and exposure to environmental changes (Chavhan *et al.* 2019).

In antagonistic pleiotropy, multiple effects of a single gene have opposite effects on fitness, i.e., beneficial mutations in one environment may become harmful in later environments (Bono *et al.* 2017; Chen and Zhang 2020). This plays important role in fitness balancing during evolution. Natural selection can maintain antagonistic pleiotropy to enable the phenotypic plasticity of organisms in different environments (Yadav *et al.* 2015).

Environmental change can cause bottlenecks that diminish population size and genetic diversity (Hall *et al.* 2010). Small populations are assumed to face environmental changes in differently than large populations because they adapt mainly through common, beneficial mutations of small effect. (Chavhan *et al.* 2019). In particular, the adaptation of small populations can be reduced by altered environmental conditions and population processes, such as environmental stress and poor individual evolvability (Willi *et al.* 2006).

1.4 Background theories

Here, we focus on various theories of adaptation to different environmental conditions. According to a long-standing theory, individuals whose parents were in the same environment and remain in that habitat are more likely to have genes suitable for that environment than another randomly selected individual (Whitlock 1996). In this case, adaptation to one environment occurs at the cost of slower adaptation or even maladaptation to other environments. This leads to lower fitness in alternative performance environments. These costs of local adaptation are hypothesized to arise when locally adaptive alleles are antagonistically pleiotropic or neutral to fitness in an alternative environment. However, studies do not often observe this antagonistic pleiotropy, which would suggest that this kind of antagonistic pleiotropy that promotes local adaptation is rare (Bono *et al.* 2017).

In 2017, Bono et al. examined this paradox. According to their research, fitness costs are less likely to evolve during selection in fluctuating environments than in constant environments. This is because in fluctuating environments, populations experience more variation in conditions and stronger selection against costs between alternative environments. Antagonistic pleiotropy occurs when the environment imposes constant selection pressure. Adaptation costs were detected more frequently in experiments in constant environments when the heterogeneity was spatial rather than temporal (Bono *et al.* 2017). However, many experimental evolutionary studies of microbes have yet to find higher fitness costs in constant environments than in fluctuating environments. This offers the possibility that factors other than the environment's influence can also play a significant role in developing fitness costs (Chavhan *et al.* 2021).

Chavhan et al. (2021) added the interaction between population size and environmental stability to supplement Bono's theory. Previous studies have discovered an indirect link between population size and the extent of ecological specialization (Bennett and Lenski 1999, Buckling *et al.* 2000, Buckling *et al.* 2007, Jasmin and Kassen 2007a, Jasmin and Kassen 2007b, Ketola and Saarinen 2015). Large populations should adapt due to rare, beneficial mutations with large effects. Small populations, in turn, adapt through common, small-effect beneficial mutations at a slower rate than large populations. Antagonistic pleiotropy can be used to explain the positive relationship between population size and fitness cost in constant environments. The adaptation of large populations to the constant environment should lead to higher costs in the

alternative environment (Chavhan *et al.* 2020). In a fluctuating environment, the environments stability and the population size are important factors that can shape fitness costs. Chavhan et al. (2021) demonstrated that the mechanism of cost avoidance in fluctuating environments could be the enrichment of beneficial mutations in the same line by multiple selection pressures. To bring the rare beneficial mutations into line, a threshold number of new mutations is needed. Due to this, small populations cannot avoid costs in a fluctuating environment.

Here, we wanted to examine how population size affects adaptation to fluctuating temperature environments. Temperature fluctuation or speed as a changing environmental factor with population size has not been studied before. In our experiment, we tested the theory by Chavhan et al. 2021 (Figure 1). We conducted experimental evolution for 500 generations with fission yeast *S. pombe* and evaluated the fitness costs by competition experiments between experimental strains and their ancestors in different thermal environments. Our experiment had fluctuating temperatures and constant temperature environments to detect the interaction with population size.

We studied the following questions (Q) and hypotheses (H) concerning the effects of population size, evolution environment, and the interplay of the population size and the evolution environment (Figure 1):

Q1: Do larger populations adapt more efficiently (have higher fitness) than smaller populations?

H1: Larger populations adapt more efficiently (have higher fitness) than smaller populations.

Q2: Do populations have fitness costs in alternative local environments if they have evolved at fluctuating rather than constant temperatures?

H2: Populations evolved at fluctuating temperatures have less fitness costs in alternative local environments.

Q3: Does population size have opposite effects on fitness costs during evolution in fluctuating versus constant temperatures?

H3: Large populations evolved under fluctuating temperatures have less fitness costs in alternative local environments than small populations.

H4: Large populations evolved under constant temperatures have more fitness costs in alternative local environments than small populations.

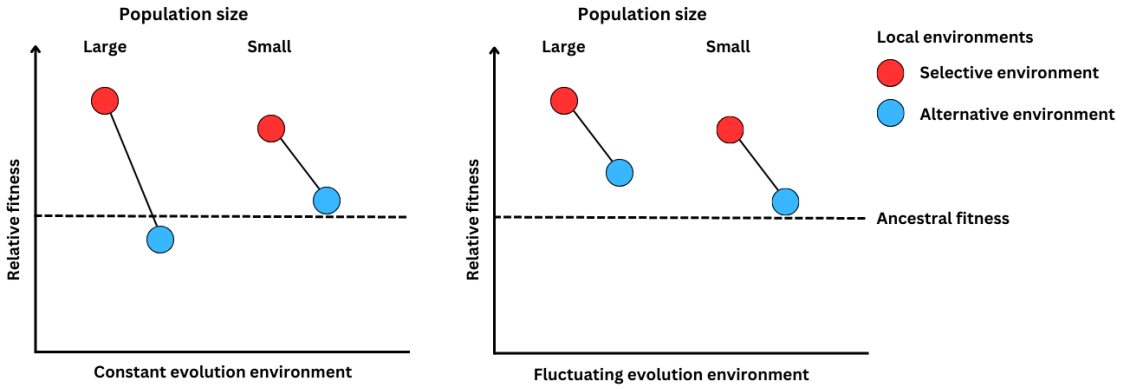

Figure 1. Picture of our hypotheses derived from Chavhan et al. (2021) for the interaction of the population size and the environmental stability during the evolution. Large populations that have evolved in fluctuating environments are assumed to have less fitness costs in alternative local environments than small populations. Reversely, large populations evolved in constant environments would have more fitness costs in alternative local environments than small populations. Ancestral fitness is marked with a dashed line. The red dot describes a selective environment, and the blue dot describes an alternative environment. If the dot (blue) is higher in the alternative environment than the ancestral line, the population has positive fitness effects and possible adaptation has occurred. When the blue dot is at the same level as the ancestor line, the populations fitness is neutral. When the blue ball is below the line, the population has negative relative fitness and possible maladaptation.

2 MATERIALS AND METHODS

2.1 Study species

Yeasts are widely used in experimental evolution due to their large population sizes and short reproduction time (Forsburg and Rhind 2006). Our study used the fission yeast *Schizosaccharomyces pombe* as a research species. *S.pombe* cells are rod-shaped and reproduced by medial fission to produce two identical daughter cells (Forsburg and Rhind 2006). Under optimal conditions, its generation time is 2-4 hours (Rosas-Murrieta *et al.* 2015). Yeasts are unicellular eukaryotes and have molecular, genetic, and biochemical characteristics that are shared with multicellular organisms. This feature makes *S.pombe* valuable for assessing the potential function of genes in complex eukaryotes (Vyas *et al.* 2021).

2.2 Strains

In our experiment, we used four haploid ancestor strains, including two different mating types (h+ and h-) and two different ade6-markers (M210 and M216). The different mating types are mat1- locus alleles. Wild-type cells can switch their mating type. Researchers commonly use strains that are unable to change their mating type. These strains can only mate if presented with a partner of the opposing mating type and nitrogen-limited conditions (Forsburg and Rhind 2006). In our experiment, we used strains that cannot change their mating type. Furthermore, we used both mating types so that the strains could be mated in future experiments.

The pair of adenine markers can be distinguished by their color: ade6-M210 marker is typically a darker shade of pink than ade6-M216 (Forsburg and Rhind 2006). The color difference between the alleles is formed when the precursor of the adenine biosynthesis pathway accumulates in the mutants and turns into a red pigment when it is oxidized (Petersen and Russell 2016). This red color is shown when grown in low adenine media (Forsburg and Rhind 2006). In our study, we used both adenine markers to separate the competing strain on selective low-adenine plates.

2.3 Evolution experiment

In the evolution experiment, 32 populations of *S.pombe* evolved in five different temperature environments and at two different population sizes. The experiment lasted for six months, equal to around 500 generations. The degree of adaptation was investigated with a competition experiment. (Figure 2).

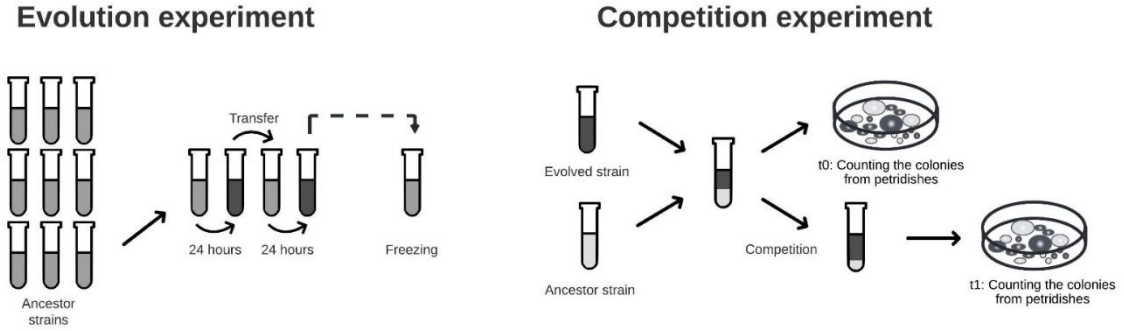

Figure 2. Simplified figure of the experimental setup (Modified from Buckling *et al.* 2009). Strains evolved in the evolution experiment. After that, the evolutionary results were examined using a competition experiment.

The most optimal growth temperature for used *S. pombe* strains is 34 °C (Figure 3). Based on the growth rate of the ancestral strain, we decided on relevant temperature environments for the evolution experiment.

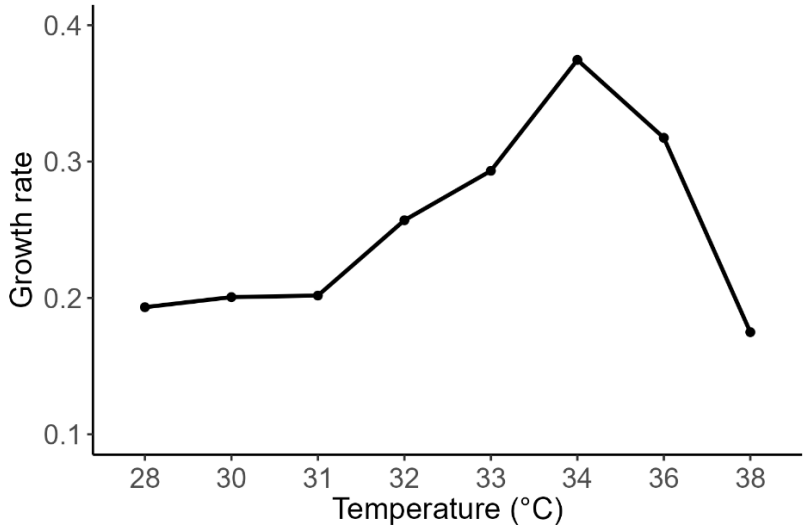

Figure 3. Thermal performance curve for growth rate of an ancestor strain at different temperatures.

Populations evolved in five different temperature treatments: constant mean (34 °C), constant extreme (38 °C), fast, intermediate, and slow fluctuation (30-38-30 °C). The frequencies were chosen based on simulation results where the frequency of environmental fluctuation relative to generation time was expected to result in adaptation using different mechanisms (Kronholm 2022) (Table 1).

Table 1. Treatments of the evolution experiment and the assumed adaptation mechanism based on the number of generations.

Evolution experiment	Cycle duration in days	Generations per cycle	Hypothetical mechanism of adaptation
Fast fluctuation (30-38-30 °C)	1	2,5	Phenotypic plasticity
Intermediate fluctuation (30-38-30 °C)	13	32	Epigenetic
Slow fluctuation (30-38-30 °C)	40	100	Genetic
Constant mean (34 °C)	-	-	Genetic
Constant extreme (38 °C)	-	-	Genetic

Four biological replicates in the experiment represented each of the four ancestors. There were large (10^7) and small (10^6) effective population sizes. All different combinations evolved in each temperature environment. The population sizes were decided based on Chavhan et al. (2021), wherein, in fluctuating environments, large populations ($\approx 10^8$), did not show fitness costs, unlike small populations ($\leq 10^7$) (Chavhan et al. 2021, Figure 1a). In our experiment, the effective population sizes did not match this prediction but differed by the same order of magnitude.

In the evolution experiment, we used haploid ancestor strains, which were frozen at -80 °C (1:1 in 80 % glycerol) before the experiment. Strains were grown separately in liquid culture in a shaking incubator at 150 rpm at 30 °C. Depending on the size of the population, it was grown in liquid culture in well plates. We used Edinburgh minimal medium with adenine and uracil amino acids (EMM+ade+ura). Large populations were grown in 24-well plates that contained 300 µL of *S.pombe* culture and 1700 µL of fresh medium. Small populations were grown in 96-well plates that contained 40 µL of culture and 210 µL of fresh medium.

After being put to the well plates, the populations evolved in growth chambers (MTM-313 Plant Growth Chamber, HiPoint Corp., Taiwan) at various temperatures. Cultures were transferred daily (24h) to a fresh medium with the same concentration. The amount of culture in each transfer was chosen to be optimal for evolution, meaning that it minimizes the number of beneficial mutations lost during transfers (Wahl *et al.* 2002). Each transfer is a bottleneck that randomly selects some individuals to continue the population, reducing the probability that a beneficial mutation will reach the fixation (Wahl *et al.* 2002; Chavhan *et al.* 2019).

The duration of the experiment was estimated based on the division rate of *S. pombe*. The average number of generations was 2.5 generations per day. The estimated total number of generations during the experiment was 464 for small populations and 507 for large populations. After the evolution experiment, the populations were frozen at -80 °C (1:1 in 80 % glycerol).

Control well containing only medium were left on the plates to minimize all bias in our results during the experiment. So, if pipetting errors occurred, this could be observed as growth in the medium. Also, after the evolution experiment, we spread the samples on low adenine plates so that the colors of the strains could be detected. Some clones were contaminated during the evolution experiment. Those samples were excluded. Contaminated populations contained only one replicate per strain, so there were still three replicates to use in competition experiments.

2.4 Competition experiment

After an evolutionary experiment, evolved strains competed with their ancestors allowing us to see if adaptations occurred. We used 32 populations from each of the five temperature environments and both small and large populations. Each evolved population had four biological replicates. Competition assays had three replicates from which we calculated the mean fitness. In total, we had 1080 competition assays. In every competing pair, the evolved strain differed from the ancestor regarding the ade6 -allele, allowing the strains to be distinguished based on color.

Before competition, strains were taken out of the freezer to defrost. All strains were first grown separately in liquid culture in a shaking incubator at 150 rpm at 30 °C. After 48 hours of growth, the cell density was measured as optical densities

to estimate the right cell concentration of the competition mix. The two competitors were mixed at an estimated ratio of 1:1 in an Eppendorf tube and pipetted on a petri dish to count the initial ratio at the beginning of the competition (t0) (see 2.5 Colony counting). Competition samples were grown on 96-well plates containing 230 µl medium and 20 µl of competition mix. The samples were grown in thermal cabinets during temperature treatment. Every 48 hours, 20 µl of the samples were transferred to fresh medium plates.

Evolved strains competed in their evolution environment and one alternative environment (Table 2). Strains evolved in mean temperature competed in all competition environments. Because the slow fluctuation cycle length was 40 days, these strains did not compete in their evolution environment but at constant mean and extreme temperatures. Competitions at constant temperatures lasted five days. Competition in intermediate fluctuation lasted 13 days (length of one cycle), and competition in fast fluctuation lasted five days, including four cycles.

Table 2. The temperature treatments of the evolved strains and the thermal environments in which they competed with the ancestor to measure the possible adaptation and maladaptations.

Evolution experiment	Competition experiment
Constant mean	Constant mean / Constant extreme / Fast fluctuation / Intermediate fluctuation
Constant extreme	Constant mean / Constant extreme
Fast fluctuation	Constant mean / Fast fluctuation
Intermediate fluctuation	Constant mean / Intermediate fluctuation
Slow fluctuation	Constant mean / Constant extreme

2.5 Colony counting

To estimate the initial colony ratio (t0), we spread competition mixes on petri dishes. After five days of propagation at 30 °C, we took pictures to calculate the number of colony-forming units (CFU) for evolved strains and ancestors. After competition treatments (t1), the competition samples were handled like in the initial time point (t0) for calculating the final CFU. The unit of adaptation was the change in proportion of the evolved strain between the initial time point and the end of the competition experiment. We counted the colonies manually.

2.6 Data analysis

Statistical analysis was conducted with Bayesian generalized linear mixed models using Hamiltonian Monte Carlo algorithms implemented in Stan (Carpenter *et al.* 2017). The analysis was performed with RStudio (R version 4.2.2). Stan was used with the brms package (Bürkner 2017). We signified parameter

values to differ if their 95% highest posterior density intervals (HPDI) did not overlap zero.

First, the relative fitness of the evolved strain compared to its ancestor was counted for each competition experiment. This was counted as an average of three replicates, and the ancestor got fitness value 1. In the model (1) we used for complete data (N=1080), the relative fitness, y_i, was predicted by temperature treatment, a_t, and its interaction with the population size, p_i, as well as the genotype of the ancestor, g_i, and the identity of the evolved strain, s_i, as a random factor. Priors for standard deviations were the half-location scale version of Students's t-distribution, with 3 degrees of freedom, location 0, and scale 10. This corresponds to a weakly informative prior. The evolution environment and the competition environment were combined as one index variable (temperature treatment) with 12 categories. The identity of the evolved strains means the specific replicate from the evolution experiment. Ancestral genotype included combinations of mating type and ade6 allele. The genotype of the evolved strains was also included in the model, as there were only four possible competition combinations relative to the ancestral genotype.

We also run separate models for small and large populations to compare competition environments and population sizes. These comparisons were made by counting posterior estimates for differences. We used a model similar to complete data but without the population size effect.

$$\begin{aligned} y_i &\sim N(\mu_i, \sigma) \\ \mu_i &= \alpha_{t[i]} + p_{[i]} + \alpha_{t[i]} \times p_{[i]} + g_{[i]} + s_{[i]} \\ \alpha_{t[i]}, p_i, g_i &\sim N(0,1) \\ s_i &\sim N(0, \sigma_s) \\ \sigma, \sigma_s &\sim hT(3, 0, 10) \end{aligned} \quad (1)$$

3 RESULTS

3.1 Population size effects

There were no differences in adaptation between small and large populations compared to the ancestor when tested with a model with population size effect over all treatments (posterior mean -0.03 and 95 % HPDI -0.11—0.04) (Table 3). Because there were some treatments where large populations seemed to do better than smaller ones (Figure 4), we tested the differences between population sizes separately (Figure 5). The difference between large and small populations was significant when populations had evolved at the constant extreme and competed at constant extreme (posterior mean 0.14 and 95% HPDI 0.01—0.27) (Figure 5a). There was also a significant difference between large and small populations when they had evolved at constant mean and competed at fast fluctuations (posterior mean 0.14 and 95% HPDI 0.02—0.27) (Figure 5b).

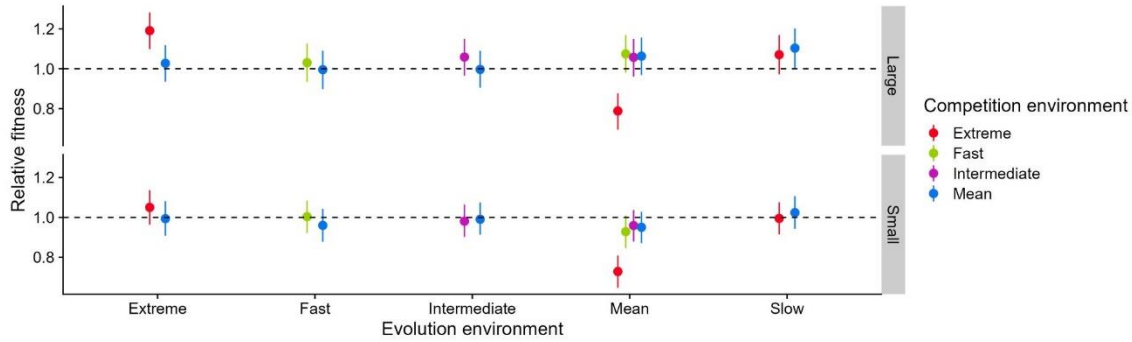

Figure 4. Relative fitness of the evolved strain compared to the ancestor. The strains that evolved in the same evolutionary environment and competed in different environments are marked next to each other to see the possible differences in relative fitness between competitive environments. The data is divided by population size. The relative fitness is shown as a posterior mean with 95 % HPDI. Ancestors are marked in the picture with a dashed line, and their fitness is set as one.

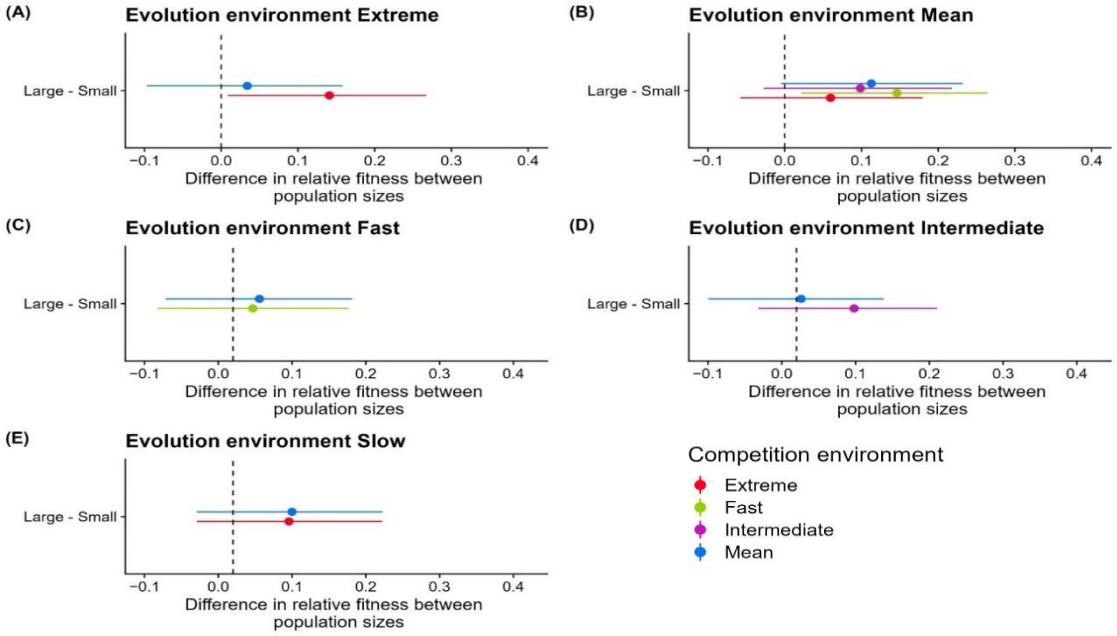

Figure 5. The difference in relative fitness between population sizes at different temperature treatments. For the statistical significance, the 95 % highest posterior density (HPDI) does not overlap with zero.

3.2 Environment effects

Populations that evolved in the extreme environment were 12 % stronger competitors than their ancestors in the extreme competition environment (posterior mean 1.12 and 95 % HPDI 1.05—1.19) (Figure 6, Table 3). When populations evolved in the fast-fluctuating environment, they were 9 % weaker

competitors than their ancestors in a constant mean competition environment (posterior mean 0.91 and 95 % HPDI 0.83—0.97). Populations that evolved in mean temperature had 28 % lower fitness than their ancestors when competing in extreme temperature environment (posterior mean 0.72 and 95 % HPDI 0.65—0.78). There was no significant difference in competitive ability between different ancestor types in the competition experiment (h-M210 was used as an intercept in the model) (Table 3).

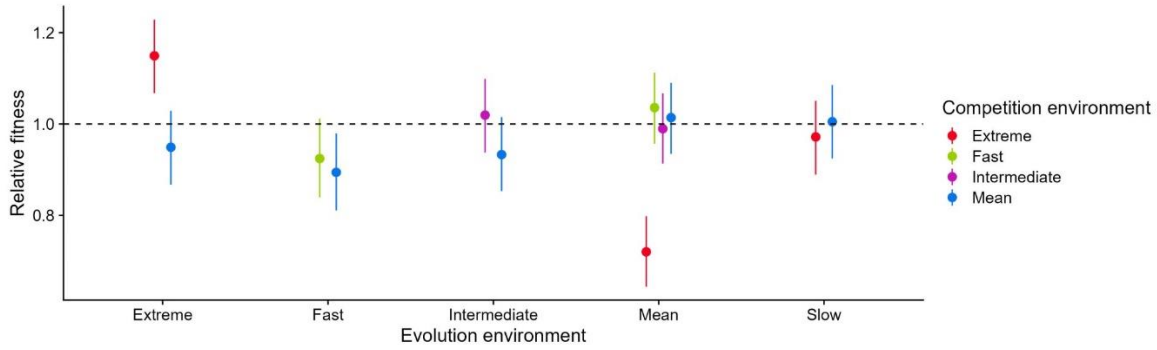

Figure 6. Relative fitness of the evolved strain compared to the ancestor over all populations. The strains that evolved in the same evolutionary environment and competed in different environments are marked next to each other to see the possible differences in relative fitness between competitive environments. The relative fitness is shown as a posterior mean with 95 % HPDI. Ancestors are marked in the picture with a dashed line, and their fitness is set as a one.

Table 3. The results of the Bayesian generalized linear mixed model. In the temperature environment column, the first letter represents the evolution treatment and the second for the competition environment. Treatment codes: constant extreme: E, constant mean: M, fast fluctuation: F, slow fluctuation: S, intermediate fluctuation: I. Ancestor effects were compared to h-M210. Interactions with population size are marked with x. For the statistical significance of the temperature treatments, the 95 % highest posterior density (HPDI) does not overlap with one. For the statistical significance of the other factors, the 95 % highest posterior density (HPDI) does not overlap with zero. Statistical significance is marked with an asterisk next to the treatment code.

Temperature treatment	Estimate	Est. Error	l-95%	u-95%
EE *	1.12	0.03	1.05	1.19
EM	0.96	0.03	0.89	1.02
FF	0.94	0.04	0.87	1.01
FM *	0.91	0.04	0.83	0.97
II	1.00	0.04	0.93	1.07
IM	0.94	0.03	0.87	1.01
ME *	0.72	0.03	0.65	0.78

Temperature treatment	Estimate	Est. Error	l-95%	u-95%
MF	1.01	0.03	0.94	1.07
MI	0.98	0.03	0.91	1.05
MM	1.00	0.03	0.93	1.06
SE	0.98	0.04	0.91	1.05
SM	1.01	0.04	0.94	1.08
Population size	-0.03	0.04	-0.11	0.04
h-M216	0.02	0.02	-0.03	0.07
h+M210	0.02	0.02	-0.03	0.07
h+M216	-0.04	0.02	-0.08	0.01
EM x Population size	0.11	0.04	0.02	0.19
FF x Population size	0.13	0.05	0.03	0.24
FM x Population size	0.12	0.05	0.02	0.22
II x Population size	0.04	0.05	-0.07	0.14
IM x Population size	0.11	0.05	0.01	0.21
ME x Population size	0.08	0.05	-0.01	0.18
MF x Population size	-0.01	0.05	-0.10	0.09
MI x Population size	0.05	0.05	-0.05	0.15
MM x Population size	0.03	0.05	-0.07	0.12
SE x Population size	0.10	0.05	0.00	0.20
SM x Population size	0.10	0.05	-0.00	0.20

3.3 Interaction of population size and environment

The interaction between population size and environment and temperature treatment was observed in Figure 4. The fitness costs were tested by the differences in the relative fitness between competition environments (Figure 7). Populations that evolved at constant extreme were better competitors at matching competition environment than in alternative mean competition environment, this was significant for large populations (posterior mean 0.16 and 95 % HPDI 0.12—0.22) and small populations (posterior mean 0.06 and 95 % HPDI 0.01—0.10) (Figure 7a). Also, populations that evolved at constant mean were better competitors in matching competition environment than in alternative extreme competition environment. This was significant for large populations (posterior mean 0.27 and 95 % HPDI 0.22—0.33) and small populations (posterior mean 0.22 and 95 % HPDI 0.18—0.26) (Figure 7b). Large populations that evolved at intermediate fluctuations were slightly better competitors in matching competition environment than in alternative mean competition environment (posterior mean 0.06 and 95 % HPDI 0.005—0.12) (Figure 7d).

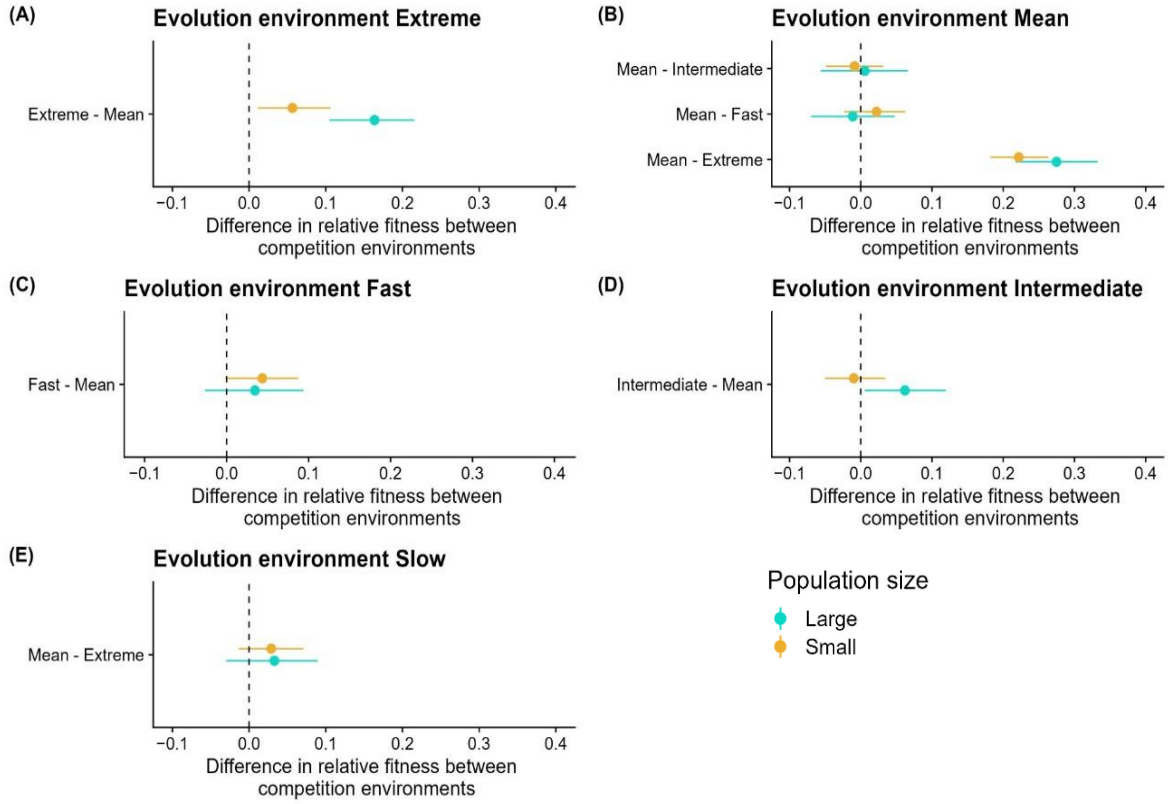

Figure 7. The difference in relative fitness between competition environments for large (green) and small (yellow) populations. For the statistical significance, the 95 % highest posterior density (HPDI) does not overlap with zero.

4 DISCUSSION

Climate change is predicted to increase the frequency of extreme weather events (Gunderson and Stillman 2015). These conditions, which are unfamiliar to the species' history, can be expected to reduce fitness and population sizes (Jiang *et al.* 2010, O'Dea *et al.* 2016). Population size also influences species adaptation (McDonough and Connallon 2023). Here, we tested the presented theory of how population size affects adaptation under fluctuating environmental conditions (Chavhan *et al.* 2021).

4.1 Population size effects

We wanted to test if larger populations adapt more efficiently than smaller populations. Large populations were assumed to adapt better than small populations because large populations have more beneficial mutations in each generation than small populations (Chavhan *et al.* 2019). Contrary to our hypothesis, population size had no significant effect on adaptation efficiency

(Figure 4) when we tested population size effect over all treatments (Table 3). Chavhan et al. (2021) literature review was used to calculate population sizes for this experiment. We did not have the exact population sizes but differences of the same magnitude. It can be questioned whether the size difference between our populations was significant enough to discover differences in adaptation. There are some suggestions as to why larger populations perform differently than expected. When larger populations adapt fast to local environments, it can make the population vulnerable to sudden environmental changes (Chavhan *et al.* 2019). Also, it has been proposed that alleles become fixed faster in populations with a small effective size, and the substitute rate of neutral alleles will be determined only by mutation rate rather than population size (Otto and Whitlock 2013).

4.2 Environment effects

According to our hypothesis, populations that evolved at fluctuating temperatures had less fitness costs in alternative local environments (Figure 6). In our results, when populations evolved in constant mean or extreme, they had more costs in alternative constant environments (Figure 6, Table 3). Populations evolved in mean temperature competed in extreme temperature had lower fitness than their ancestors, or even maladaptation. High temperatures as an environmental stress adaptation require some traits, like heat shock proteins which are energy-costly and useless for long-term adaptation to constant mean temperatures (Kristensen *et al.* 2020). Populations evolved and competed in constant extreme and were stronger competitors than their ancestors (Figure 6, Table 3). It is proposed that warm extreme temperatures shift thermal tolerance curves more than mean temperatures (Buckley and Huey 2016).

Fitness costs should occur in constant temperatures and slow fluctuations, in which case adaptation is the hypothetical mechanism (Table 1). One explanation for why symmetrical trade-offs were not observed, is that antagonistic pleiotropy is difficult to detect. For example, due to limited statistical power, perhaps that is why such a genetic trade-off has rarely been demonstrated (Anderson *et al.* 2013). Nevertheless, for example, Anderson et al. (2013) have demonstrated the existence of antagonistic pleiotropy in plants at the quantitative trait locus level.

4.3 Interaction of population size and environment

Our results showed no clear opposite effects on fitness costs between population sizes when evolved in fluctuating and constant environments. Large and small populations that evolved at constant extreme were better competitors at matching competition environment than in alternative mean environment (Figure 7a). Bennet et al. (1992) have found similar results in the *E.coli* evolution experiment, the highest temperature groups react the fastest and most extensively. Several mechanisms have been proposed to explain these findings.

It has been noted that populations that evolve in extreme temperatures perform poorly under environments of constant mean temperature.

Also, large and small populations evolved at constant mean and were better competitors in matching competition environment than in alternative extreme environment (Figure 7b). It has been shown in laboratory experiments that evolution in a constant temperature environment can lower fitness under extreme conditions (Kingsolver *et al.* 2009).

High temperatures can have adaptive effects. New mutations can appear in populations due to increased temperatures, and mutations that affect many cellular processes can increase fitness at high temperatures (Cox *et al.* 2010, Deatherage *et al.* 2017). On the other hand, mutations already present in the population may react to new environmental conditions in different ways (Cox *et al.* 2010). Evolutionary adaptation can only occur between generations, but there was little variation in the number of generations between large and small populations. However, population size does affect the pace of fixation.

4.4 Conclusion

After 500 generations, the population size did not affect the adaptation to thermal fluctuations. The strongest improvement in fitness was in the constant extreme environment. We did not have any clear results supporting the hypotheses concerning population size, evolution environment, and the interplay between population size and the evolution environment. One possible explanation is that the ancestors could have already been close to the fitness optimum, so adaptation was unnecessary. Also, adaptation to the temperature probably requires a lot of time and genetic evolution because temperature affects all the chemical reactions in a cell. Evolution of thermal tolerance has been observed in *E.coli* evolution experiments over 2,000 generations (Bennett *et al.* 1992, Bennett and Lenski 1993, Bennett and Lenski 1997), which is four times longer evolutionary time than we had. If thermal tolerance does not adapt quickly, this can weaken the ability of organisms to adapt to climate change.

In addition to longer evolutionary time, our population sizes could have been more different. To measure better the effect of the evolutionary environment on observed trade-offs, we could do additional competition experiments and have more alternative competition environments. It would also be interesting to compete with fluctuation-evolved populations in other fluctuations to see if adaptation to types of fluctuations evolves specific adaptations. It is also possible that competition experiments did not adequately measure the change in relative fitness. Fitness measurements could also be done by using optical density to estimate the evolved changes in growth rate. Also, the changes in heat shock gene expression could be measured for evolved strains compared to ancestors.

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