

# **The effects of population size and environmental composition on the utilization of an unaccustomed niche**

A Thesis submitted to

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Indian Institute of Science Education and Research, Pune

Dr. Homi Bhabha Road, Pashan, Pune 411008, India

**Sarthak Pravin Malusare**

**20141062**

**Biology department, IISER Pune**

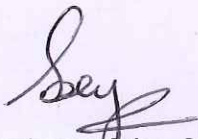
**Under the supervision of**

**Dr. Sutirth Dey**

**Biology department, IISER Pune**

# CERTIFICATE

This is to certify that this dissertation entitled "The effects of population size and environmental composition on the utilization of an unaccustomed niche" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Sarthak Pravin Malusare at IISER Pune under the supervision of "Name of the Supervisor, Designation, Department" during the academic year 2018-2019



Signature of the Supervisor

(Dr. Sutirth Dey)

Date: 18 March 2019



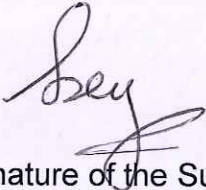
Signature of the student

(Sarthak Malusare)

Date: 18 March 2019

# DECLARATION

I hereby declare that the matter embodied in the report entitled "The effects of population size and environmental composition on the utilization of an unaccustomed niche" are the results of the work carried out by me at the Department of Biology, IISER Pune, under the supervision of Dr. Sutirth Dey and the same has not been submitted elsewhere for any other degree.



Signature of the Supervisor

(Dr. Sutirth Dey)

Date: 18 March 2019



Signature of the student

(Sarthak Malusare)

Date: 18 March 2019

## **Abstract:**

Population size influences various evolutionary outcomes. However, we have a very limited understanding of the effects of population sizes on niche evolution. In this study, we investigate how population sizes influence the utilization of an unaccustomed niche present alongside a habitual niche. We subjected *Escherichia coli* populations of two different population sizes to selection in a mixture of an unaccustomed and a habitual niche for approximately 480 generations. The selection environments consisted constant lines experiencing only one habitual niche and fluctuating lines experiencing all the habitual niches in four possible combinations in terms of predictability and speed of fluctuations, along with a constantly present unaccustomed niche. We found that all populations adapted to the unaccustomed niche with large populations adapting significantly greater than the small populations. Interestingly, the identity of the selection environment did not influence the adaptation to the unaccustomed niche. Moreover, large populations even adapted to their habitual niche better than the small populations in both the constantly selected and fluctuating lines. Also, predictability and speed of fluctuations did not affect the adaptation to the habitual niche. Therefore, our findings suggest that population size is an important parameter which must be taken into account when studying the ecological processes like niche utilization and expansion.

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Then there are my classmates with whom the five years in IISER Pune were such fun.

Last but not the least I would like to thank my parents who have always supported and guided me in all the important decisions in life.



## **Introduction:**

An important factor when studying how populations evolve over time is the total number of individuals present in these populations. Population size plays an important role in shaping its evolutionary trajectories by influencing the amount of genetic variation (Sniegowski and Gerrish, 2010a). Likewise, population size also affects the efficiency of natural selection (Petit and Barbadilla, 2009) and the effects of random genetic drift responsible for adaptation to a given environment. The phenomenon of clonal interference which affects the rate of adaptation (Charlesworth and Eyre-Walker, 2006) and the repeatability of evolutionary processes (Bailey et al., 2017) is also modulated by the population sizes of organisms.

All else being equal, large populations typically harbor more genetic variation (Sniegowski and Gerrish, 2010a). Due to this, the probability of encountering a rare large effect mutation is higher in larger populations than in the smaller populations (Desai and Fisher, 2007; Orr, 2007a; Sniegowski and Gerrish, 2010a; Wilke, 2004). Therefore the evolution of large populations is expected to be driven by rare large-effect mutations (Sniegowski and Gerrish, 2010a). As opposed to this, the evolution of small populations is expected to be driven by common small effect mutations (Sniegowski and Gerrish, 2010a). Also, as the population size increases, natural selection becomes more and more efficient at fixing beneficial mutations and discarding the deleterious mutations emerging in the population (Chavhan et al., 2019; Petit and Barbadilla, 2009). Due to this, it is expected that larger populations evolve faster and adapt better than the smaller populations to the given environment.

But, large effect mutations are generally associated with large deleterious pleiotropic effects (Lande, 1983; Orr and Coyne, 1992). This suggests that the larger populations evolving in a given environment are more susceptible to facing larger deleterious pleiotropic effects upon environmental change. A previously unpublished study from our lab has addressed the question of whether larger populations face higher costs of adaptation. We evolved both large and small populations in galactose and thymidine for around 480 generations. Along with these we also had a fluctuating environment treatment wherein both large and small populations faced both galactose and thymidine.

All the evolved lines were then assayed in both galactose and thymidine. We demonstrated that larger populations with a higher extent of specialization in the selection environment also face greater costs of adaptation in the novel carbon source. In contrast to this, larger populations facing fluctuations across galactose and thymidine (constant lines showed fitness tradeoff between these two carbon sources) were shown to evade such fitness tradeoffs completely. Therefore, we can effectively say that larger populations can readily access rare large effect mutations which provide a fitness advantage to all the components of a fluctuating environment.

Interestingly, in nature most populations of organisms face fluctuations in their niches, be it environmental fluctuation or fluctuations in the available resources. Fluctuations can be characterized by the predictability and the speed of fluctuation. In general, the literature suggests that both the predictability (Alto et al., 2013; Hughes et al., 2007) and the speed of fluctuation (Ancel, 1999; Cohan, 2005) can affect the evolutionary trajectories of evolving populations. Populations facing predictable fluctuations generally adapt to all the component environments (Hughes et al., 2007; Leroi et al., 1994) while adaptation to unpredictable fluctuation is quite stochastic (Hughes et al., 2007; Ketola et al., 2013; Turner and Elena, 2000). The speed of fluctuations also affects the adaptation of the evolving populations with changes in the phenotypic plasticity of the populations (Ancel, 1999).

The stability of the selection environment is also essential for the evolution of specialists or generalists (Kassen, 2002). Stable environments generally favor the evolution of specialists with narrow niche breadths and fluctuating environments favor the evolution of generalists with wider niche breadths (Kassen, 2002). Therefore the evolution of niche width would also depend on the stability and the identity of the environment.

An important topic in evolutionary ecology is the study of the ecological niche and its properties. The ecological niche of an organism is defined as the environmental conditions and resources the organism requires in order to survive and reproduce. The dynamics of diversification, niche expansion and ultimately the existence of a given species depends on how the species utilize the available niches. Therefore, how niche utilization evolves at evolutionary time scales is a topic of interest in both ecological and

evolutionary studies. Many studies have investigated the limitations to niche breadth expansion, favoring the evolution of a single niche specialist with narrow niche breadths (Fry, 1996; Whitlock, 1996). Previous studies have also investigated the exploitation of a new and challenging nutritional opportunity (referred to as unaccustomed niche in this study) over evolutionary time scales when another (habitual) nutritional option is simultaneously available in the environment (Friesen et al., 2004; Jasmin and Kassen, 2007; Saxer et al., 2010). However, there has been no report of simultaneous adaptation to both components (unaccustomed and habitual) of the niche (reviewed in (Kassen, 2014)). A possible reason behind this consistent observation regarding simultaneous adaptation to both the niche components could be a scarcity of accessible genetic variation owing to small population sizes.

Now consider large populations that are selected in a mixture of a habitual and an unaccustomed niche. By the virtue of having access to rare large effect mutations, which are beneficial in both the available niches, adaptation in both niches seems a plausible outcome. But by the 'rule of declining adaptability', large scope of adaptation to an unaccustomed niche may cause adaptation to only the unaccustomed niche (Alto et al., 2013; Hughes et al., 2007). Adaptation to the unaccustomed niche may, therefore, have deleterious pleiotropic effects leading to maladaptation in the habitual niche. This may not be the case with smaller populations. As smaller populations are driven by common small effect mutations, smaller populations may face limitations while adapting to both the available niches simultaneously and may only show very limited adaptation to a single niche.

To test these hypotheses, we conducted bacterial evolution experiments at two starkly different population sizes (see Materials and Methods), which enabled us to study the effect of population size on the unaccustomed niche. We further investigated how the identity of the habitual component of the niche influenced the adaptation to the unaccustomed component. If the habitual component keeps fluctuating over generations while the unaccustomed component remains constant, adaptation to the latter can be more likely as compared to a case where both the components remain constant. To test this possibility, we made the habitual component fluctuate over time in

some of our treatments but remain constant in the others. Since predictability and speed of environmental fluctuations could be a potentially important factor influencing adaptation (Karve et al., 2018), we also varied the predictability and speed of fluctuations factorially in our experiment (see Materials and Methods). We used sodium acetate as the unaccustomed niche because despite supporting observable bacterial growth to a small extent, this carbon source could not support batch culture for more than five transfers.

## **Materials and methods:**

We used bacterial experimental evolution to address our questions regarding the effects of population size and the predictability and speed of environmental fluctuations on the utilization of an unaccustomed niche.

### ***Ancestral Strain:***

The founding ancestral strain for the entire experimental selection lines was a single colony of a kanamycin resistant strain of *Escherichia coli* MG1655 ( $\Delta$  lac Y :: Kan). Therefore, we added 0.05 mg/ml kanamycin to all the culture media used in our study to diminish the likelihood of contamination.

### ***Environmental treatments:***

All the environmental treatments were an M9-based minimal media with a pre-decided carbon source depending on the environmental treatment (see Appendix for the detailed composition of the culture media). The main selection experiment was carried out in 96 well plates with a culture volume of 300  $\mu$ l. The populations in the culture plates were maintained at 37<sup>0</sup> C with continuous shaking at 150 rpm.

### ***Standardizations:***

The standardizations for identifying suitable carbon sources were carried out with the ancestral genotype. We tested for the ability of the ancestral genotype to show detectable growth in the carbon source in question (see Table S1). On the basis of the observations made during the standardizations, we selected Arabinose (A), Galactose (G), Sorbitol (S), and Thymidine (T) as the habitual carbon sources.

We found that although Sodium acetate could support detectable growth of the ancestral genotype over 24 hours, it could not support batch culture over more than 5

bottlenecks, even when the bottleneck ratio was as lenient as 1:10. Therefore we chose Sodium acetate (N) as the unaccustomed niche.

We carried out experimental evolution in the following (Table 1 and Table 2) selection environmental treatments at two different population sizes:

The two population sizes used were termed as **large** (bottleneck ratio of 1/10) and **small** (bottleneck ratio of 1/10<sup>4</sup>). The final population size (N<sub>f</sub>) was identical across the population size treatments (Fig. 1).

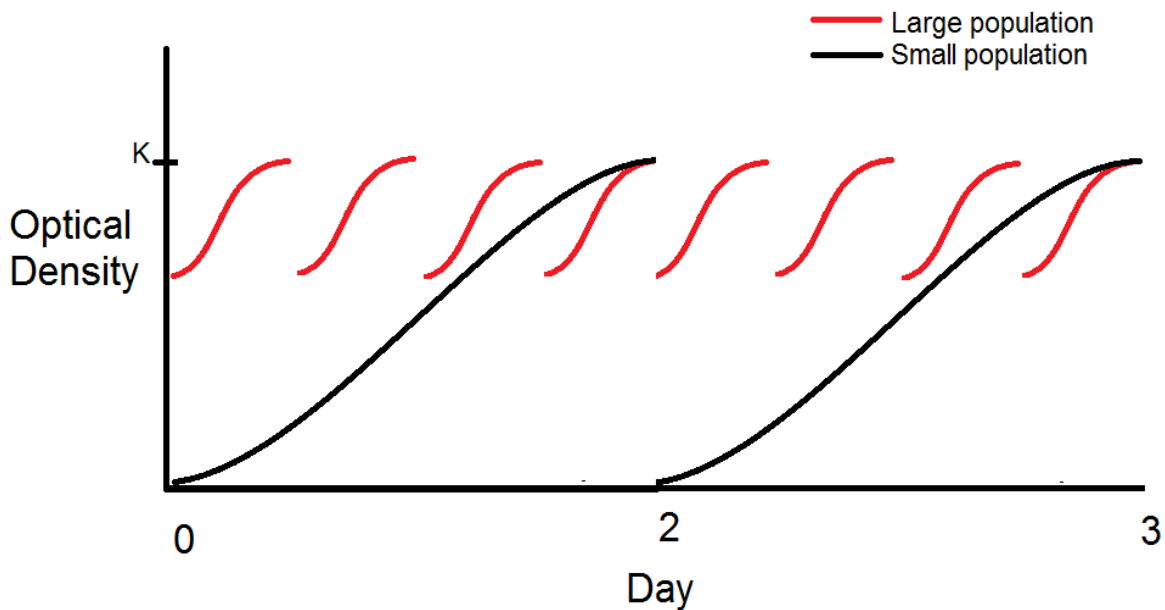


Fig. 1: **Schematic for the bottlenecking procedure of large and small populations:** Small populations were only transiently as large as the large populations when they reached their maximum carrying capacity (K).

Table 1: Selection lines with constant environments (habitual + unaccustomed niches)

Arabinose (A), Galactose (G), Sorbitol (S), Thymidine (T), Sodium acetate (N) :

Population size	Stability of environment	Constituent environment	Abbreviation
Large	Constant	A+N	AL
Large	Constant	G+N	GL
Large	Constant	S+N	SL
Large	Constant	T+N	TL
Large	Constant	N	NL
Small	Constant	A+N	AS
Small	Constant	G+N	GS
Small	Constant	S+N	SS
Small	Constant	T+N	TS
Small	Constant	N	NS

For predictable environments, the environment fluctuated across the four habitual carbon sources in a fixed sequence (A → G → S → T). For unpredictable environments the environment fluctuated across the four habitual carbon sources in a random sequence.

Table 2: Selection lines with fluctuating environments (habitual + unaccustomed niches)

(P: predictable, U: unpredictable, F: Fast (13.3 generations), S: Slow (40 generations))

Population size	Stability of environment	Constituent environment	Predictability	Speed of fluctuation	Abbreviation
Large	Fluctuating	(A or G or S or T) + N	(P)	(F)	PFL
Large	Fluctuating	(A or G or S or T) + N	(P)	(S)	PSL
Large	Fluctuating	(A or G or S or T) + N	(U)	(F)	UFL
Large	Fluctuating	(A or G or S or T) + N	(U)	(S)	USL
Small	Fluctuating	(A or G or S or T) + N	(P)	(F)	PFS
Small	Fluctuating	(A or G or S or T) + N	(P)	(S)	PSS
Small	Fluctuating	(A or G or S or T) + N	(U)	(F)	UFS
Small	Fluctuating	(A or G or S or T) + N	(U)	(S)	USS

We used 6 independently evolving replicates per treatment, thus leading to a total of 96 independently evolving populations in the experiment.



All the large populations experienced a bottleneck of 1/10 every 12 hours wherein 30  $\mu$ l of the bacterial cultures were inoculated in 270  $\mu$ l of minimal medium to form a total of 300  $\mu$ l. In 12 h, these large populations underwent approximately 3.32 generations of growth. On the other hand, all the small populations experienced a bottleneck of 1/10<sup>4</sup> every 48 hours wherein these populations were serially diluted 4 times by 1/10 dilution (30  $\mu$ l of the bacterial cultures were inoculated in 270  $\mu$ l of minimal medium to form a total of 300  $\mu$ l.) to attain a final dilution of 1/10<sup>4</sup>. In 48 hours, these small populations underwent approximately 13.28 generations.

The experiment lasted for approximately 480 generations which took 72 days for completion.

We stored cryostocks at three equally spaced intervals (160 generations) in the selection experiment. The cryostocks were made by adding 210  $\mu$ l of bacterial cultures to 90  $\mu$ l of 50% glycerol.

Lines which were selected only in Sodium acetate minimal medium underwent extinction regardless of the population size (large or small). Therefore 12 Sodium acetate evolving populations had to be terminated (these twelve populations were not among the 96 populations described above). This further strengthens the notion that Sodium acetate acted as an unaccustomed carbon source.

### ***Assays for measuring fitness:***

At the end of the experiment, we assayed the fitness of all the populations in our experiment (96 in total) in five different environments:

1. Arabinose minimal medium
2. Galactose minimal medium
3. Sorbitol minimal medium
4. Thymidine minimal medium
5. Sodium acetate minimal medium

Prior to the fitness assays, in order to remove the lingering effects of the selection conditions faced just before the preparation of the cryostocks, the populations were revived in glucose minimal medium for 18-20 h. All the assays were carried out at a single population size (dilution of 1/100; total volume 300  $\mu$ l) so as to study the effect of population size due to evolution at large and small population sizes. All the assays were carried out in 96 well plates that were incubated for 24 hours in 37<sup>0</sup> Celsius with continuous shaking at 150 rpm. Therefore, the physical conditions of the assays were identical to that of the selection for all the lines in our study.

The assays were carried out in six different measurement blocks with one randomly picked replicate of each treatment type being assayed at the same time in the same plate. For a given treatment, only one replicate was assayed on any given day. We used the plate reader (Synergy HT Biotek microplate reader) to measure the optical density at 600 nm every 20 minutes for 24 hours. The resulting 73 readings gave rise to high-resolution growth curves, which were used to determine fitness.

For calculating the maximum growth rate as an estimate of fitness, the maximum slope of a moving window of 10 data points from the growth curve was used (Chavhan et al., 2019; Karve et al., 2015, 2016).

### ***Statistical Analysis:***

The relative fitness of a given evolved lined was estimated by normalizing its absolute fitness with the ancestral fitness in the environment in question. As all the lines had descended from a single common ancestor, we did not have a biological replicate of the common ancestor. Therefore, we took the mean of three measurements of ancestral fitness values as the ancestral value.

### ***Statistical tests for all the lines assayed in the unaccustomed niche (Sodium acetate):***

We determined if adaptation to the unaccustomed niche was affected due to the differences in the population sizes or the selection environment (the habitual niche).

Therefore, we used a mixed model ANOVA with 'population size' (two levels: large and small) and 'selection environmental treatment' (eight levels: Arabinose, Galactose, Sorbitol, Thymidine, PF, PS, UF, US) crossed with each other, and 'day of assay' as the random factor (Miliken and Johnson, 1984).

*Statistical tests for the constant lines assayed in their habitual niches:*

We also determined if the constant lines had adapted significantly to the two niches provided to them (habitual and un-accustomed). To this end, we used single sample t-test for comparison against the ancestral fitness (mean fitness= 1).

*Statistical tests for the constant lines assayed in their habitual niche:*

We determined if adaptation to the habitual niche was affected due to the differences in the population sizes or the selection environment (the identity of the habitual niche). Therefore, we used a mixed model ANOVA with 'population size' (two levels: large and small) and 'selection environmental treatment' (four levels: Arabinose, Galactose, Sorbitol, Thymidine) crossed with each other, and 'day of assay' as the random factor (Miliken and Johnson, 1984).

The populations evolving in a fluctuating environmental regime faced all four habitual carbon sources, one at a time. Therefore, the component habitual niche of the fluctuating lines was composed of all four habitual carbon sources. To measure the adaptation of these lines in their habitual niche, we calculated the geometric mean of the fitness across all four habitual carbon sources for all the fluctuation lines (Orr, 2007a).

*Statistical tests for fluctuating lines assayed in habitual carbon source:*

We determined if adaptation to the habitual niche was affected due to the differences in the population sizes, predictability, and the speed of environmental fluctuation. Therefore, we used a mixed model ANOVA with 'population size' (two levels: large and small), 'predictability' two levels: predictable and unpredictable) and 'speed of fluctuation' (two levels: fast and slow) treatments crossed with each other, and 'day of assay' as the random factor (Miliken and Johnson, 1984).

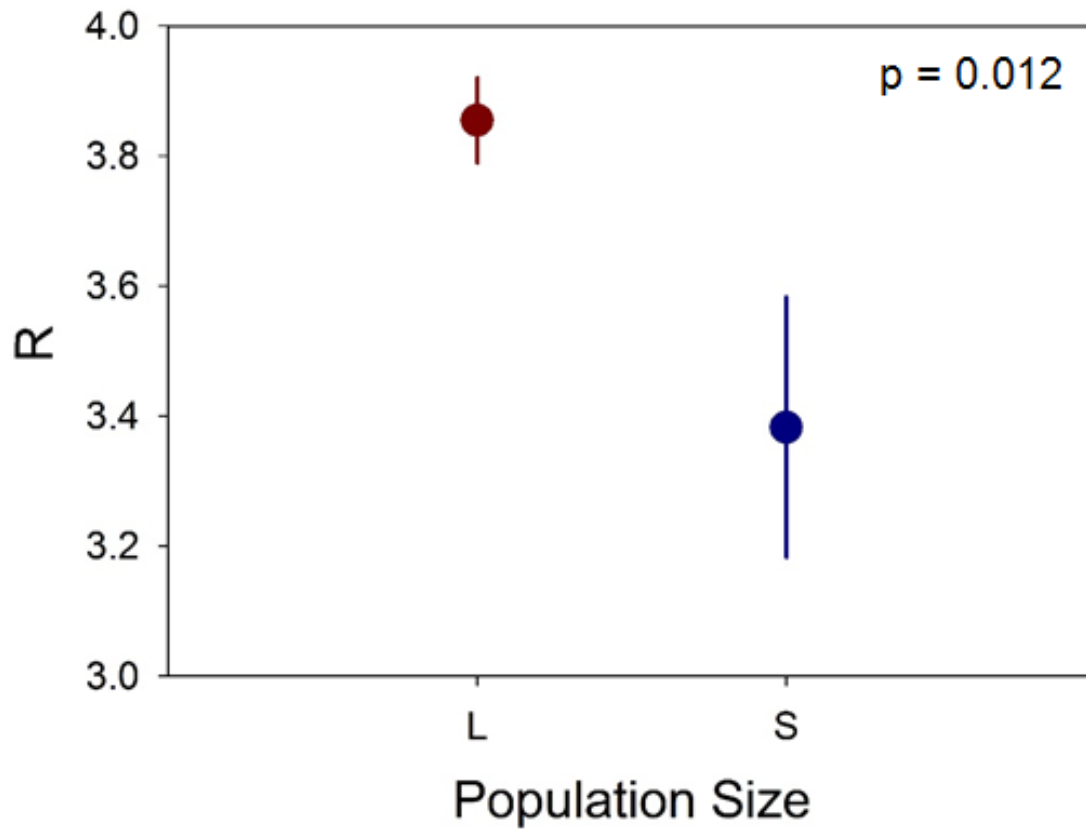
## Results:

### ***Population size had a significant effect on adaptation to the unaccustomed niche:***

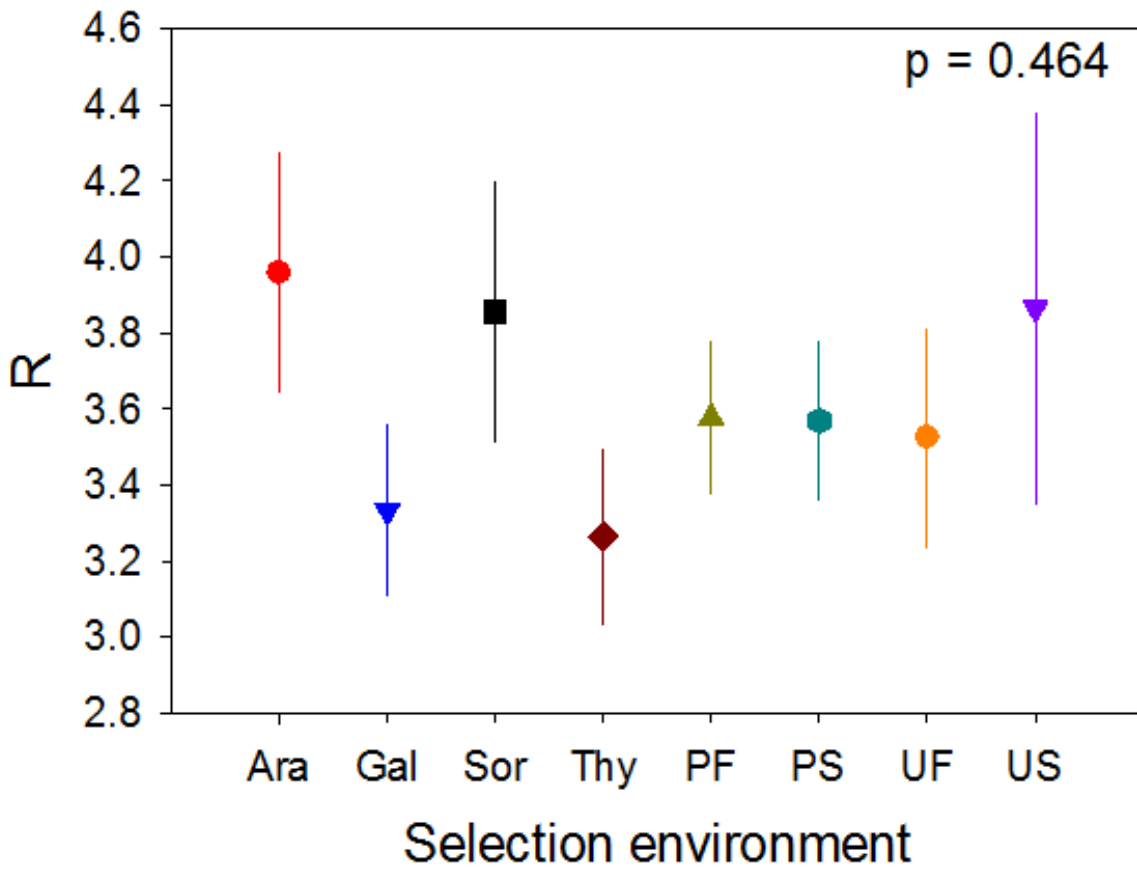
Mixed model ANOVA (Table: 3) revealed that there was a significant effect of population size treatment on the adaptation in the unaccustomed niche (Sodium acetate) with larger populations adapting significantly higher ( $F_{1,75} = 6.622$ ,  $p = 0.012$ ) to the novel niche with respect to the smaller populations (Fig. 2). The selection environment did not play a significant role ( $F_{7,75} = 0.963$ ,  $p = 0.464$ ) in the adaptation to the unaccustomed niche (Fig. 3). This reveals that the adaptation to the novel niche was not affected by the identity of the habitual niche. Also, the interaction of 'population size' and 'selection environment' did not have a significant effect ( $F_{7,75} = 0.199$ ,  $p = 0.985$ ), which suggests that only the differences in the population sizes were responsible for higher fitness of the larger populations in the novel niche (Fig. 4).

Table 3: ANOVA for all lines assayed in unaccustomed niche (Sodium acetate):

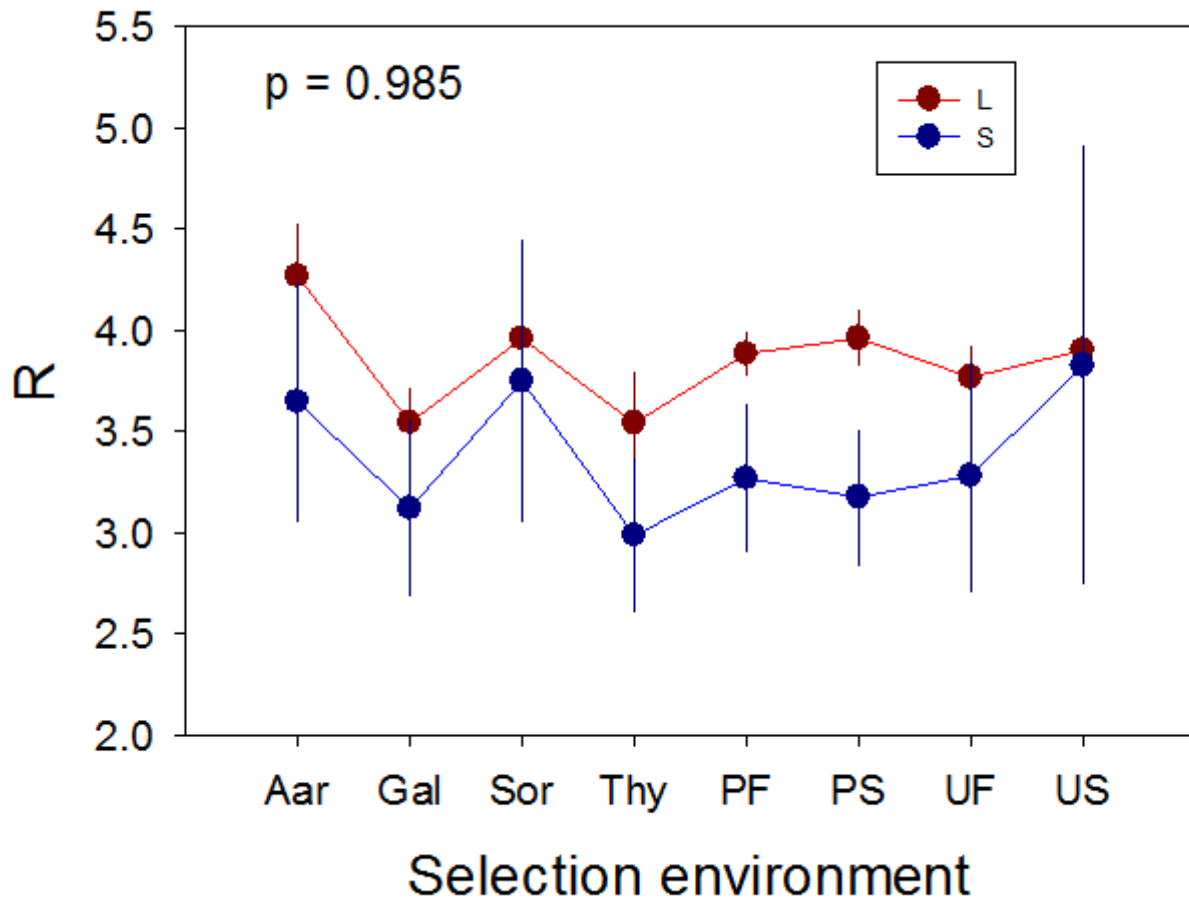
Treatment	Effect (F/R)	Degrees of Freedom	F	p	Partial eta-squared
Intercept	Fixed	1	189.68	$4 \times 10^{-5}$	0.974316
population size	Fixed	1	6.6221	0.012	0.081131
selection environment	Fixed	7	0.9631	0.4643	0.082479
population size x selection environment	Fixed	7	0.1997	0.9846	0.018294
day	Random	5	8.1997	$3 \times 10^{-6}$	0.353439
Error		75			



**Figure 2. Fitness in terms of maximum growth rate (R) in the unaccustomed niche (Sodium acetate) analyzed over all the experimental lines (mean  $\pm$  SEM (N=48)). Large populations (L) adapt significantly greater than the small populations (S) (see the text for details).**



**Figure 3. Fitness in terms of maximum growth rate (R) in the unaccustomed niche (Sodium acetate) with respect to the selection environments habitual environment) ((mean ± SEM (N=12)). The selection environment did not have a significant effect on the adaptation to the unaccustomed niche.**



**Figure 4. Fitness in terms of maximum growth rate (R) in the unaccustomed niche (Sodium acetate) with respect to the selection environments (mean  $\pm$  SEM (N=6)).** The interaction of ‘population size’ and ‘selection environment’ did not have a significant effect on adaptation to the unaccustomed niche.

***Population size affected adaptation of constant lines to their habitual niches:***

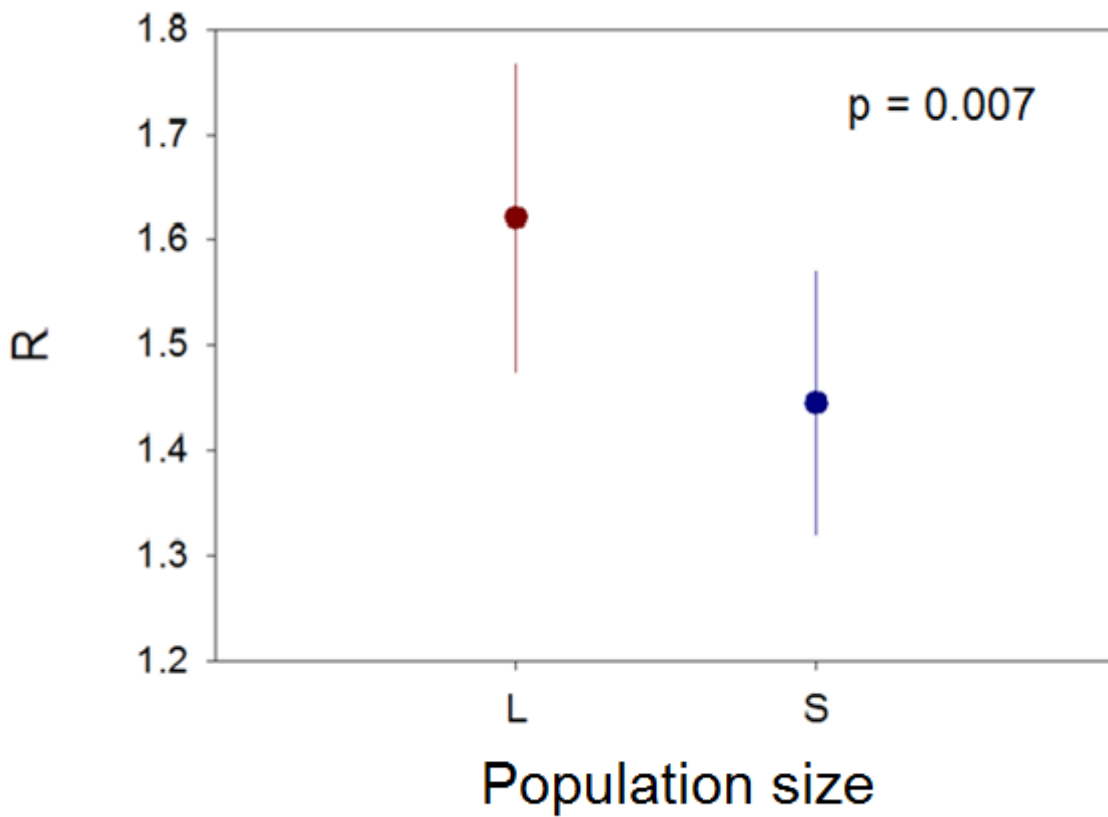
We performed a mixed model ANOVA (Table: 4) to check for the effects of population size and the selection environment (habitual niche) and the interaction between them. The population size treatment had a significant effect ( $F_{1, 35} = 8.10$ ,  $p = 0.007$ ) on the fitness in the habitual niche (Fig. 5). The selection environment also affected the fitness in the habitual niche significantly ( $F_{3, 35} = 136.08$ ,  $p < 10^{-5}$ ) (Fig. 6). Importantly, there was no significant interaction between population size and selection environments ( $F_{3, 35}$



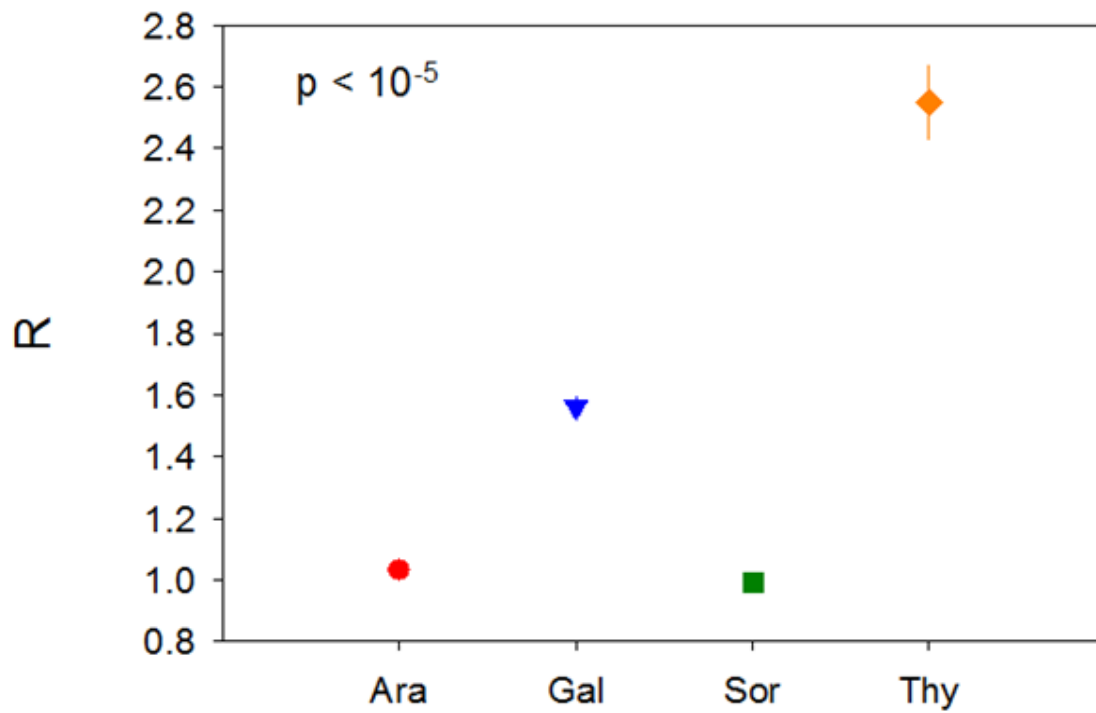
= 1.53,  $p = 0.223$ ). As shown in Fig. 7, the mean reaction norms of the fitness of the large and small populations do not cross each other, reflecting no significant interaction between the population size and the selection environment treatments.

Table 4. ANOVA for constant lines assayed in the selection environment (habitual niche).

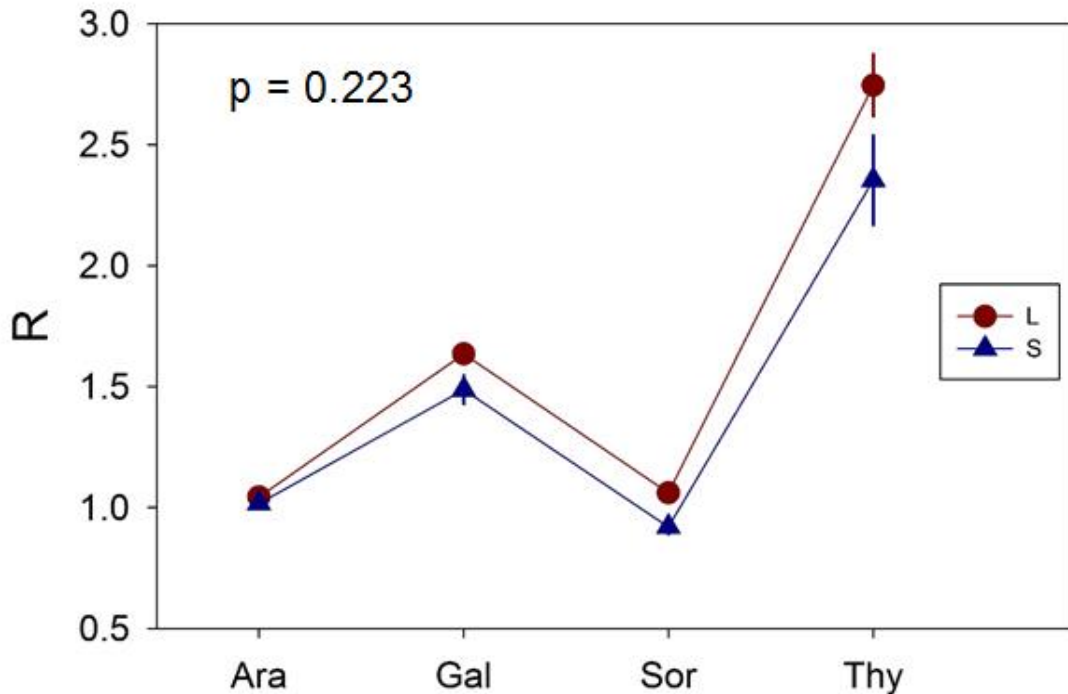
Treatments	Effect(F/R)	Degrees of Freedom	F	p	Partial eta-squared
Intercept	Fixed	1	11156.78	$1 \times 10^{-9}$	0.999552
population size	Fixed	1	8.097839	0.0074	0.187894
selection environment	Fixed	3	136.3686	$< 10^{-5}$	0.92119
population size x selection environment	Fixed	3	1.53239	0.2233	0.116098
day	Random	5	0.218044	0.9524	0.030208
Error		35			



**Figure 5. Fitness in terms of maximum growth rate (R) in the habitual niche (mean  $\pm$  SEM (N=24)). Large populations (L) adapt significantly greater than the small populations (S).**



**Figure 6. Fitness in terms of maximum growth rate (R) in the habitual niche ((mean  $\pm$  SEM (N=12)). Selection environment also affected the fitness in the habitual niche significantly.**



**Figure 7. Fitness of the constant lines assayed in their habitual niches (mean  $\pm$  SEM (N=6)).** No significant interaction between the population size and selection environments.

***Large populations adapted to both the available niches in all the cases, but small populations could not:***

The analysis using single sample t-tests (Table: 5) for the differences between the evolved lines and the ancestral lines revealed that both large and small populations adapted significantly to the unaccustomed niche. Whereas all the large populations had also adapted significantly to their respective habitual niches, this was true only for two out of four small populations in the constant lines. Specifically, the small population lines selected in Galactose and Thymidine showed significant adaptation to their respective habitual niche. However, the small populations selected in Arabinose failed to show any

significant adaptation to Arabinose. In fact, the small populations selected in sorbitol showed significant maladaptation in sorbitol.

Table 5: Mean fitness and p values of constant lines in habitual niche.

Selected population	Adaptation to Habitual Niche	Mean R	P value (Habitual niche)	Adaptation to Unaccustomed niche	Mean R	P value (Unaccustomed niche)
AL	Yes	1.044	0.039	Yes	4.27	$5 \times 10^{-5}$
AS	No	1.017	0.363	Yes	3.649	0.006
GL	Yes	1.635	$6 \times 10^{-6}$	Yes	3.544	$2 \times 10^{-5}$
GS	Yes	1.487	$4 \times 10^{-4}$	Yes	3.12	0.004
SL	Yes	1.061	0.006	Yes	3.961	$1 \times 10^{-5}$
SS	No (Maladaptation)	0.92	0.038	Yes	3.751	0.01
TL	Yes	2.745	$4 \times 10^{-5}$	Yes	3.541	$2 \times 10^{-4}$
TS	Yes	2.353	$7 \times 10^{-4}$	Yes	2.987	0.003

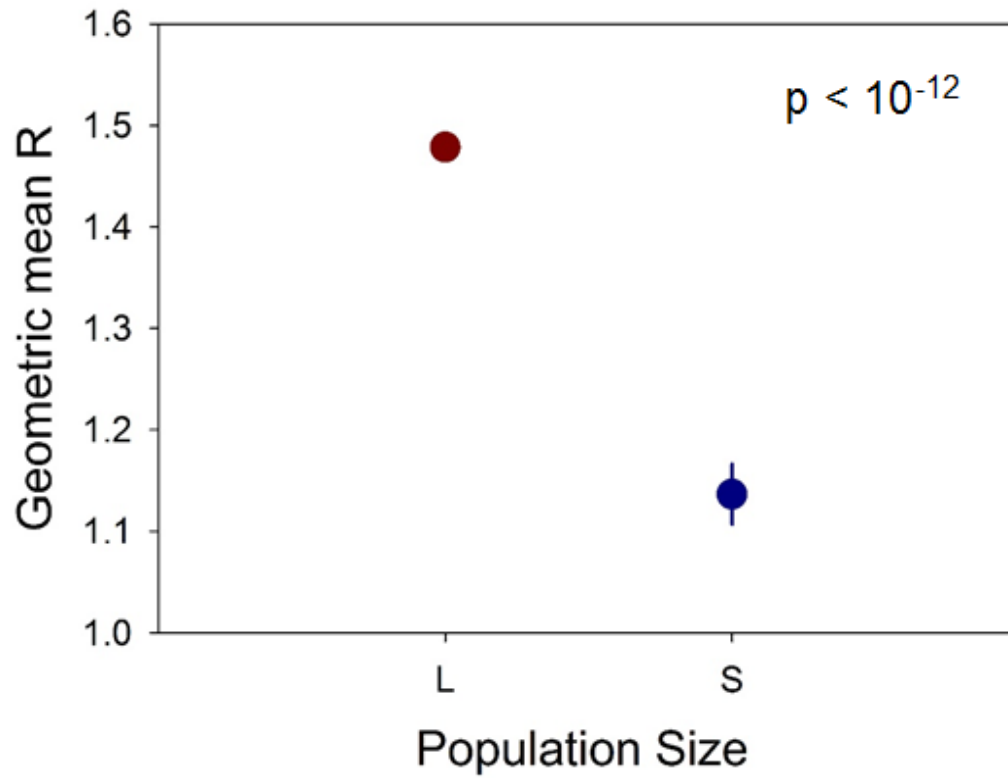
***Population size significantly affected the adaptation of fluctuating lines to their habitual niche:***

During the selection experiment, fluctuating population lines had faced all four habitual niches one after the other, randomly or predictably, with slow or fast fluctuations, along with the constantly present unaccustomed niche. To calculate the average fitness across all the four habitual niches faced by these lines, the geometric mean of the fitness in four habitual niches was calculated (Orr, 2007b). Mixed model ANOVA (Table: 6) on the geometric means revealed that population size had a very significant effect ( $F_{1, 35} = 104.43$ ,  $p < 10^{-12}$ ) on the adaptation to the habitual niches faced by the evolving populations, with the larger populations evolving higher geometric mean fitness in their

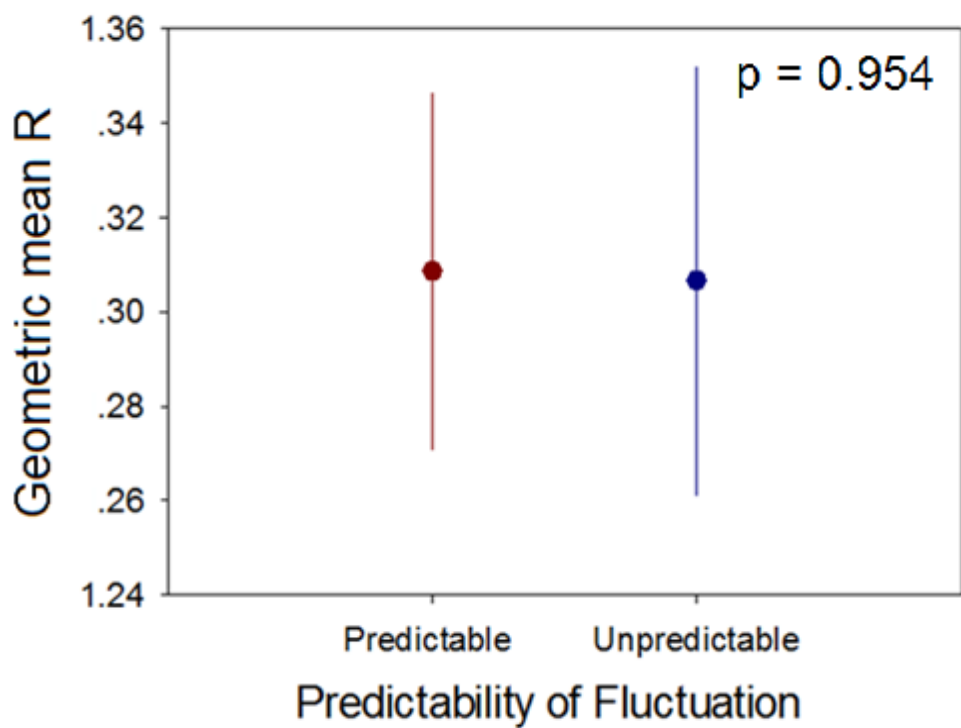
habitual niches than the smaller populations (Fig. 8 ). Both the predictability ( $F_{1, 35} = 0.00$ ,  $p = 0.953608$ ) (Fig. 9) and the speed of fluctuations ( $F_{1, 35} = 0.53$ ,  $p = 0.473$ ) (Fig. 11) did not have significant effect on the adaptation to the habitual niches. Moreover, there were no interactions between the main effects (Fig. 10, 12, 13).

Table 6. ANOVA for fluctuating lines assayed in habitual carbon source

Treatments	Effect (F/R)	Degrees of Freedom	F	p	Partial eta-squared
Intercept	Fixed	1	10537	$2 \times 10^{-9}$	0.999526
Population size	Fixed	1	104.4	$5 \times 10^{-12}$	0.748984
predictability	Fixed	1	0.003	0.954	$9.81 \times 10^{-5}$
speed of fluctuation	Fixed	1	0.526	0.473	0.014806
Population size x predictability	Fixed	1	1.191	0.283	0.032904
Population size x speed of fluctuation	Fixed	1	0.409	0.527	0.01155
Predictability x speed of fluctuation	Fixed	1	0.23	0.635	0.006521
Pop size x predictability x speed of fluctuation	Fixed	1	$5 \times 10^{-4}$	0.983	$1.39 \times 10^{-5}$
day	Random	5	0.579	0.716	0.076386
Error		35			

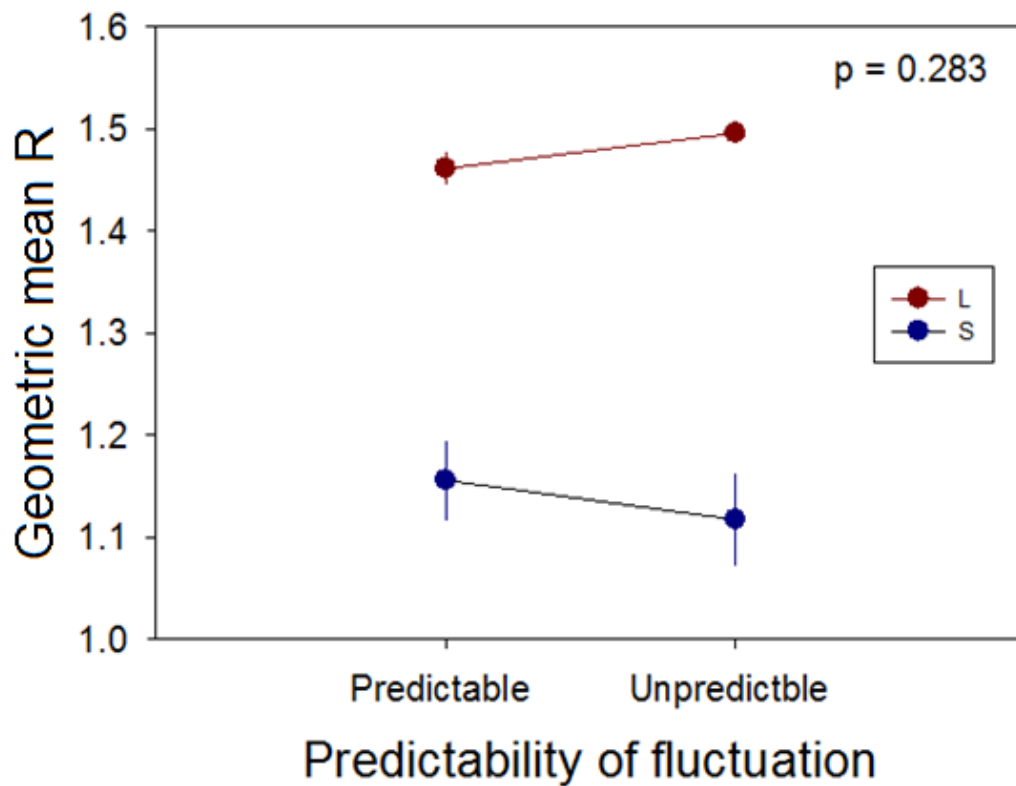


**Figure 8. Geometric mean fitness over the fluctuating habitual niche (mean  $\pm$  SEM (N=24)). Large populations (L) adapt significantly greater than the small populations (S).**

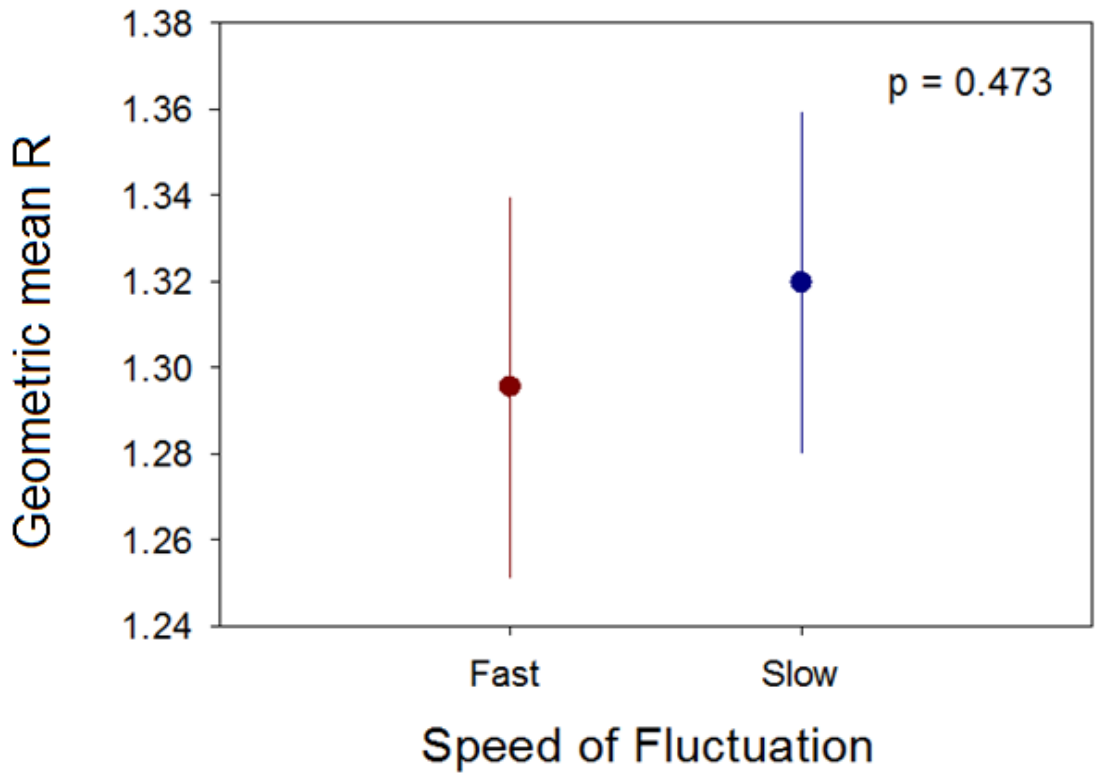


**Figure 9. Geometric mean fitness over the fluctuating habitual niche with respect to the predictability of fluctuation (mean  $\pm$  SEM (N=24)).** Predictability of fluctuation did not have a significant effect adaptation to the habitual niches.

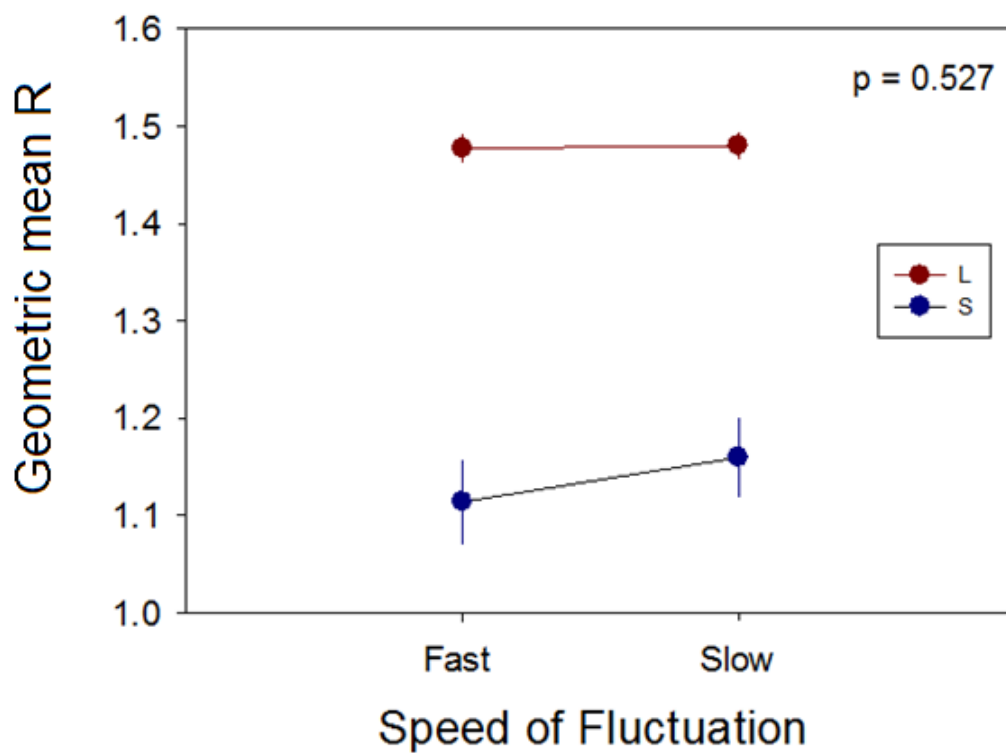




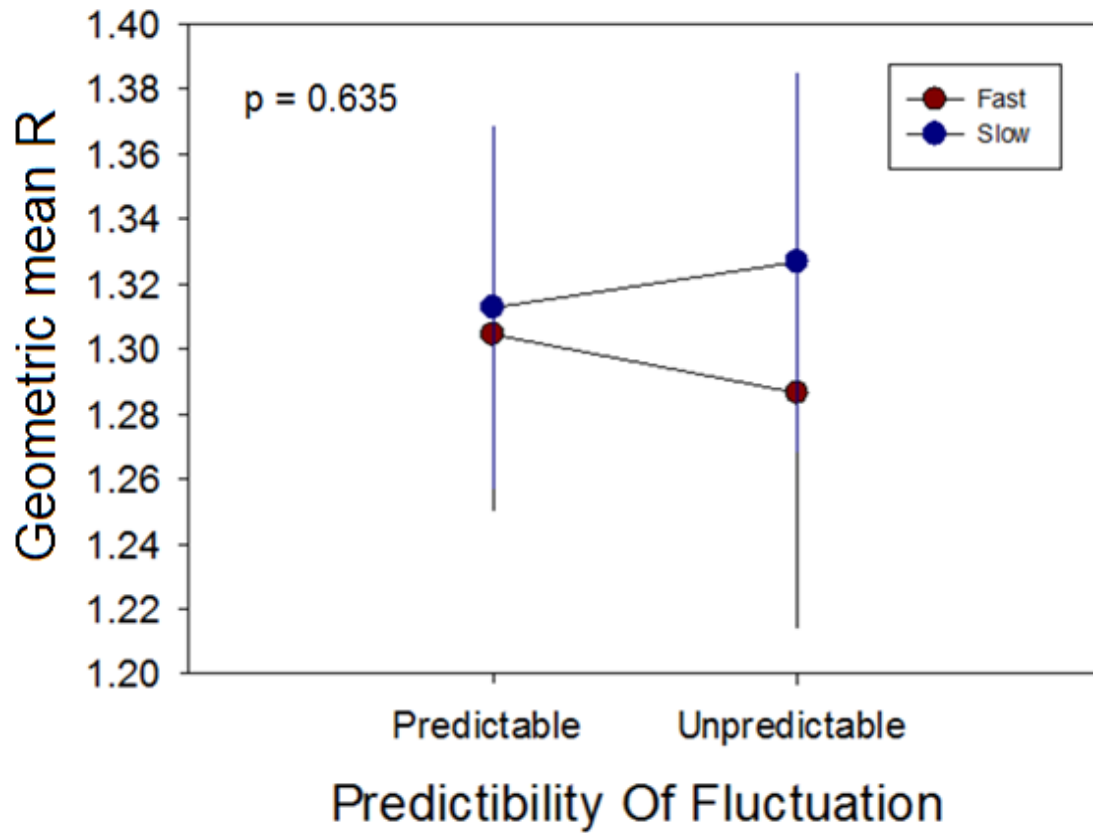
**Figure 10. Geometric mean fitness over the fluctuating habitual niche with respect to the predictability of fluctuation (mean  $\pm$  SEM (N=12)). No significant interaction between the population size and predictability of fluctuation.**



**Figure 11. Geometric mean fitness over the fluctuating habitual niche with respect to the speed of fluctuation (mean  $\pm$  SEM (N=24)). The speed of fluctuation did not have a significant effect adaptation to the habitual niches.**



**Figure 12. Geometric mean fitness over the fluctuating habitual niche with respect to the speed of fluctuation (mean  $\pm$  SEM (N=12)). No significant interaction between the population size and speed of fluctuation.**



**Figure 13. Geometric mean fitness over the fluctuating habitual niche with respect to the speed of fluctuation (mean  $\pm$  SEM (N=12)). No significant interaction between the predictability of fluctuation and the speed of fluctuation.**

## **Discussion:**

### ***Adaptation to the unaccustomed niche:***

Since the ancestral populations had very poor growth on sodium acetate, there was a lot of scope for adaptation to this environment. However, asexual populations are primarily dependent on mutations as a source of variation. Since our large populations had access to more mutations, they are also predicted to adapt better in the unaccustomed niche (Chavhan et al., 2019; Desai and Fisher, 2007; Sniegowski and Gerrish, 2010b). We found that all our experimental populations adapted significantly to sodium acetate (Fig. 2).

Importantly, even though sodium acetate was not able to sustain our bacterial populations when it was the only source of carbon, we observed that the populations could still adapt to this unaccustomed component when another (habitual) carbon source was available to allow sufficient bacterial growth for evolution to take place.

Surprisingly, the identity of the habitual niche did not significantly influence adaptation to the unaccustomed niche (Fig. 3, 4). This could potentially be explained by the large scope for adaptation in sodium acetate. The so-called 'rule of declining adaptability,' which applies generally across environments, states that the speed of adaptation in asexual systems varies negatively with their current fitness (Couce and Tenailon, 2015).

### ***Fitness changes in constant lines in their habitual niches:***

We found that adaptation to the habitual niche within the constant lines was significantly greater in the large populations as compared to the small ones (Fig. 5). Furthermore, in contrast to the previous studies that investigated adaptation in an environment containing multiple nutritional niches (Friesen et al., 2004; Jasmin and Kassen, 2007; Saxer et al., 2010), most of our constant lines adapted significantly to both the niches. Interestingly, all the larger populations in the constant lines adapted to both the niches

but only two out of four of the smaller populations in the constant lines could do so (Table 3). This observation can also be explained by the greater availability of genetic variation to the larger populations.

Why was a simultaneous adaptation to multiple niches within the environment not observed in the previous studies (Friesen et al., 2004; Jasmin and Kassen, 2007; Saxer et al., 2010)? Although we cannot provide a definitive answer to this question here, we can comment about the possible causes behind the differences between the observations of these previous studies and our results. The sizes of all the populations reported in these three studies were greater than the small populations in our study. Therefore, population size cannot explain why simultaneous adaptation was observed in our study but not in the previously reported studies. We note that the environmental pairs used in our study were different from the ones used in these three studies. Mutations conferring adaptation to the two niches in these studies might be incredibly rare. Also, the pathways involved in the assimilation of the two niches may be very distinct from each other and far off on the metabolic landscape of the organism. These differences in terms of environmental pairs could possibly explain the above discrepancy.

### ***Fitness changes in fluctuating lines in the habitual niche:***

In the fluctuating lines, the habitual niche changed in all the four possible combinations in terms of predictability and speed of fluctuations between the four carbon sources (arabinose, galactose, sorbitol, and thymidine) along with a constant supply of the unaccustomed niche (sodium acetate). We tested for differences in the geometric mean of fitness across the four components due to differences in terms of the predictability and the speed of fluctuations. We opted to use the geometric mean of fitness instead of arithmetic mean because adaptation to environmental fluctuations is expected to optimize the former and not the latter (Orr, 2007b). Surprisingly no significant effect in terms of either the predictability (Fig. 9) or the speed of habitual niche fluctuation (Fig. 11) was observed. This could potentially be explained by the consistent presence of a stable niche (sodium acetate) that offered a large scope for adaptation. Over the course

of selection, populations adapted to the unaccustomed niche. Due to this the populations always had refuge against any selection which may lead to differences between populations facing predictability or the speed of habitual niche fluctuation treatments

The only factor which had a significant effect in the adaptation of the fluctuation lines to their habitual niches was the population size, with the large populations adapting significantly higher than the small populations (Fig. 8).

Overall, our study establishes that population size is an important parameter which should be taken into account while studying how organisms utilize their niches.

### ***Future directions***

The presence of two different niches for the bacterial populations opens up the possibility of the formation of two subpopulations, one specializing to the habitual niche and the other specializing to the unaccustomed niche (Friesen et al., 2004; Saxer et al., 2010). Specialization across two different environments demands a negative correlation in fitness across them (Fry, 1996). Therefore, we can test if divergent specialization has resulted in two subpopulations in our experiments by testing for the presence of such negative correlations. An alternative to the evolution of specialists is the evolution of a generalist that can be adapted to both the niches without evolving a negative fitness correlation between them. This possibility can be easily tested by isolating individual colonies from the evolved populations and assaying them in both the available niches in question. The results from this experiment would be important in order to study how population sizes may affect the diversification process which is a very important topic in ecological and evolutionary studies.

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## Supplementary Information:

Table S1: List of carbon sources used in the standardizations:

Carbon sources that supported growth	Carbon sources that show negligible growth
Glucose	Aspartame
Galactose	Sodium acetate
Imidazole	Trehalose
Raffinose	Oxalic acid
Lactic acid	Myo-inositol
Sorbitol	Maleic acid
Maltose	Lactose
Urea	Citric acid
Glycine	Aspartic acid
Mannitol	Potassium acetate
Cholic acid	
Arabinose	

## Appendix:

### Minimal Media Recipe:

(12.8g Na<sub>2</sub>HPO<sub>4</sub>-2H<sub>2</sub>O, 3g K<sub>2</sub>HPO<sub>4</sub>, 0.5g NaCl, 1.0 g NH<sub>4</sub>Cl) +H<sub>2</sub>O such that total volume is 1000 ml, 2 mL of 1M MgSO<sub>4</sub> solution, 0.1 mL of 1M CaCl<sub>2</sub> solution and 20 mL of 20% required Carbon source. Kanamycin (50 mg/ml) was present in all the solutions in the proportion 1ml of minimal medium: 1 µl of kanamycin.

### Separate statistical analysis for constant lines and fluctuating lines assayed in the unaccustomed niche:

#### *Statistical tests for constant lines assayed in the unaccustomed niche (Sodium acetate):*

We determined if adaptation to the unaccustomed niche was affected due to the differences in the population sizes or the selection environment (the habitual niche). Therefore, we used a mixed model ANOVA with 'population size' (two levels: large and small) and 'selection environmental treatment' (four levels: Arabinose, Galactose, Sorbitol, Thymidine) crossed with each other, and 'day of assay' as the random factor (Miliken and Johnson, 1984).

Treatment	Effect (F/R)	Degrees. Of Freedom	F	p	Partial eta-squared
Intercept	Fixed	1	180.6262	0.000041	0.973064
population size	Fixed	1	3.7953	0.059452	0.097828
selection environment	Fixed	3	2.3445	0.089727	0.167333
pop size*selection environment	Fixed	3	0.1517	0.927912	0.012834
day	Random	5	5.3316	0.000958	0.432355
Error			35		

*Statistical tests for fluctuating lines assayed in the unaccustomed niche (Sodium acetate):*

We determined if adaptation to the unaccustomed niche was affected due to the differences in the population sizes or the selection environment (the habitual niche). Therefore, we used a mixed model ANOVA with ‘population size’ (two levels: large and small) and ‘selection environmental treatment’ (four levels: PF, PS, UF, US) crossed

Treatment	Effect (F/R)	Degree Of freedom	F	p	Partial eta-squared
Intercept	Fixed	1	158.2772	0.000056	0.969377
population size	Fixed	1	3.0064	0.091740	0.079102
Predictability	Fixed	1	0.1850	0.669740	0.005258
Speed	Fixed	1	0.3436	0.561533	0.009721
population size*Predictability	Fixed	1	0.5475	0.464275	0.015402
population size*Speed	Fixed	1	0.0463	0.830844	0.001322
Predictability*Speed	Fixed	1	0.3764	0.543527	0.010639
population size*Predictability*Speed	Fixed	1	0.2603	0.613108	0.007383
Day	Random	5	4.1436	0.004629	0.371835
Error		35			

with each other, and ‘day of assay’ as the random factor (Miliken and Johnson, 1984)

#### Result:

From both the mixed model ANOVA analysis presented above we infer that population size had a marginal significance in adaptation to the unaccustomed niche.

For constant lines the selection environment also shows marginal significance but the interaction between population size and selection environment treatments do not show significant effect on the adaptation to the unaccustomed niche.

For fluctuating treatments lines the predictability and the speed of fluctuation treatments and also any interaction between population size, predictability and speed of fluctuation treatments do not show any significant effect on the adaptation to the unaccustomed niche.