Received: 19 September 2022 | Revised: 2 February 2023 | Accepted: 22 March 2023

DOI: 10.1111/jeb.14178

JOURNAL OF Evolutionary Biology eseb

SHORT COMMUNICATION

Experimental evolution of evolutionary potential in fluctuating environments

Tarmo Ketola | Ilkka Kronholm

Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland

Correspondence
Tarmo Ketola, Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland.
Email: tketola@jyu.fi

Funding information
Academy of Finland, Grant/Award Number: 278751 and 321584

Abstract

Variation is the raw material for evolution. Evolutionary potential is determined by the amount of genetic variation, but evolution can also alter the visibility of genetic variation to natural selection. Fluctuating environments are suggested to maintain genetic variation but they can also affect environmental variance, and thus, the visibility of genetic variation to natural selection. However, experimental studies testing these ideas are relatively scarce. In order to determine differences in evolutionary potential we quantified variance attributable to population, genotype and environment for populations of the bacterium *Serratia marcescens*. These populations had been experimentally evolved in constant and two fluctuating environments. We found that strains that evolved in fluctuating environments exhibited larger environmental variation suggesting that adaptation to fluctuations has decreased the visibility of genetic variation to selection.

KEYWORDS
bet-hedging, experimental evolution, fluctuating environments, genetic variation

1 | INTRODUCTION

Variation is the raw material for selection, a driver of evolution and source for economic improvements in animal and plant breeding (Lynch & Walsh, 1998). High heritability, that is, a large proportion of additive genetic variation relative to total variation, guarantees fast evolutionary changes, but at the same time, such variation should be rapidly depleted when alleles are brought to fixation (Johnson & Barton, 2005). The maintenance of genetic variation in populations has long been an enigma, as empirical estimates show that there is more variation in populations that what is predicted by theory (Johnson & Barton, 2005). It has been suggested that fluctuating environments can maintain genetic variation (Bürger & Gimelfarb, 2002), but the evidence for this has been not been conclusive. Although high genetic variation is considered advantageous for species facing changing environments by enabling rapid response to altered selection pressure, in some cases slower evolutionary changes are more beneficial (Kawecki, 2000). Sometimes it could be better not to evolve at all.

Lack of evolutionary change or a slower evolutionary response could be beneficial if the environment varies unpredictably, as a strong response to selection could be maladaptive in the next generation. This could lead to canalization and hiding genetic variation from selection (Kawecki, 2000). Unpredictable environments can also lead to the evolution of bet-hedging, where genotypes produce variable phenotypes that could secure their survival in uncertain conditions (Botero et al., 2015; Kronholm, 2022; Levins, 1968). In essence, both cases are supposed to lead to lowered heritability. However, the hallmark of the latter is increased environmental variation. This could occur either through classical bet-hedging or if

adaptation via inducible plasticity entails increased developmental instability, seen as random deviations in phenotype, as suggested by Tonsor et al. (2013). Inducible plasticity has been suggested to be theoretically very tractable adaptation to fluctuating environments (Botero et al., 2015; Kronholm, 2022; Levins, 1968). Whether evolution is slowed down due to low additive genetic variation, or due to high environmental variation in different environments and traits has gained interest from researchers over the years (Hoffmann & Merilä, 1999; Schou et al., 2020; Wilson et al., 2006).

We tested these ideas using strains of the bacterium *Serratia marcescens* resulting from a replicated ($n = 10$ populations in each treatment) evolution experiment, where we propagated populations for approximately 140 generations in stable (31°C) and in rapidly cycling thermal environments with similar mean temperature. We considered two kinds of thermal fluctuations: abrupt fluctuations generated by cycling between 24 and 38°C (45 min in each temperature), or smooth fluctuations generated by cycling sequentially from 24-31-38-31-24°C (30 min in each temperature). Thus, the two different fluctuations had different rates of temperature changes. After the experiment we measured the maximal growth rate and maximal population sizes of bacterial clones (12 per population) four times at 31°C in order to quantify, using quantitative genetic methods of clonal analysis (Lynch & Walsh, 1998), if the evolution in fluctuating environments led to increased environmental variability in strains than in those evolving in constant environments.

2 | MATERIALS AND METHODS

The strains used in this study were obtained from a previous study (Ketola & Saarinen, 2015). Briefly: an evolution experiment using the bacterium *Serratia marcescens* strain DB 11 was initiated from an overnight culture of single clone in DM25 medium. Experimental microcosms were wells of Bioscreen C 100 well plates. The ancestor clone was seeded to three plates, with 10 populations on each plate, that were assigned to three different thermal conditions: constant, abrupt fluctuations (between 24 and 38°C, 45 min in each temperature) and smooth fluctuations (between 24 and 38°C with an intermediate temperature of 31°C in each cycle, 30 min in each temperature). In our growth chambers, the temperature change between 24 and 38°C took ca. 20 min. Populations were cultured using batch culture, and every 48 h 10 µL of a population was transferred to a new well containing 400 µL of fresh DM25 medium. At each renewal population sizes were high in all treatments and replicates. The experiment was continued for 27 renewals, that is, 54 days and approximately 140 generations.

After the evolution experiment, we extracted 12 clones from each population. These clones were frozen 1:1 in 80% glycerol in a randomized order in four bioscreen 100 well plates and stored at −80°C. Growth measurements were initiated by cryo-replicating frozen clones to fresh media (Duetz et al., 2000). After 24 h the clones were replicated to new plates containing DM25 and the plates were placed in Bioscreen spectrophotometers at 31°C for 3–4 days, that is, until growth in all wells had stopped. We repeated growth measurements 4 times at 31°C. Maximal growth rate and yield were obtained from growth data (measured every 5 min, 420–580 nm) using a custom R script described previously (Ketola et al., 2013). In short, ln-transformed growth data were analysed in a 25 time point sliding window excluding data from the first five timepoints (due to vapour). To find the maximal growth rate, we fitted linear regression to the data of each 25 timepoint sliding window. The maximum slope of the regression equals the maximal growth rate. Maximum biomass yield was obtained by finding the window with the largest mean optical density over the 25 timepoints.

Maximal growth rate and yield were subjected to statistical analyses. Determination of the instability of the genotypes across repeated measurements at a constant 31°C required analysing the evolutionary treatments separately. Prior to analysis, wells, where no growth had occurred, were removed from the data. This left $n = 479$, $n = 480$ and $n = 480$ observations for the constant, smooth and abrupt treatments, respectively. Traits and inoculum size were standardized to a mean of zero. This transformation was made for each of the evolutionary treatments separately to allow testing differences in variance without an effect of trait mean on the variance. We extracted variance components arising from population, clone and replicate using a Bayesian multilevel linear model. The model was:

$$y_i \sim N\left(\mu_i, \sigma_e\right) \quad (1)$$

$$\mu_i = \alpha + \alpha_{p[i]} + \alpha_{c[i]} + \beta_m x_i$$

$$\alpha, \beta_m \sim N(0, 5)$$

$$\alpha_{p[i]} \sim N\left(0, \sigma_p\right)$$

$$\alpha_{c[i]} \sim N\left(0, \sigma_c\right)$$

$$\sigma_p, \sigma_c, \sigma_e \sim \exp(1)$$

where α is the intercept, α_p is the population effect, α_c is the clone effect, β_m is the effect of inoculum size, σ_p, σ_c, and σ_e are the standard deviations for population, clone and environmental effects respectively. The priors were weakly informative, with a normal distribution with a mean of zero and standard deviation of five for the intercept and inoculum size effect, and an exponential prior with rate 1 for population, environmental and clonal standard deviations. The model was fit using Hamiltonian Monte Carlo implemented via the Stan language and the R package brms (Bürkner, 2017). For parameter estimation, we ran four MCMC chains, with a warm-up of 2000 iterations followed by 2000 iterations of sampling. Model convergence was investigated by inspecting traceplots and the summary statistic $\hat{R}$, which was 1 for all parameters, thus, no convergence problems were found. Variances were calculated by squaring the posterior distributions of standard deviations and their differences were investigated by subtracting the posterior of the fluctuating treatments from that of the constant treatment.

Variation stemming from the clone effect quantifies the amount of genetic variation within the populations (Lynch & Walsh, 1998) and population effect variation quantifies variation across the populations indicating the propensity of populations to reach different evolutionary outcomes within evolutionary treatments or uniformity of selection pressures across replicated populations (Ketola et al., 2013; Travisano et al., 1995).

3 | RESULTS

Total variation in both traits (maximal growth rate and yield) was comparable between the evolutionary treatments, as the highest posterior density intervals for the difference in total variances between treatments were not different from zero (Figure 1).

For maximal growth rate, we did not observe differences between the clonal, or population variances (Figure 2a). Clonal variance was slightly higher in the constant treatment but the 95% highest posterior density interval for differences overlapped with zero (Figure 2a). The fluctuating treatments had somewhat higher environmental variances than the constant treatment, but only the difference between abrupt and constant treatment was different from zero (Figure 2a).

In contrast to maximal growth rate, results for yield showed larger differences and higher precision of the estimates (Figure 2b). There were no differences in clonal or population variance (Figure 2b). However, the two fluctuating treatments had higher environmental variance than the constant treatment (Figure 2b). Environmental variance was 1.9 [1.5, 2.3] times higher in the smooth treatment than in the constant treatment, and 2.2 [1.8, 2.7] times higher in the abrupt treatment than in the constant treatment.

Replicates of the ancestral clone were stored in random locations in the cryoplates, which allowed the estimation of environmental variance also for the ancestor ($n = 63$). For maximal growth rate the environmental variance was estimated to be 0.95 [0.64, 1.3], and for yield 1.0 [0.7, 1.4], which was higher than the variance of the evolved strains.

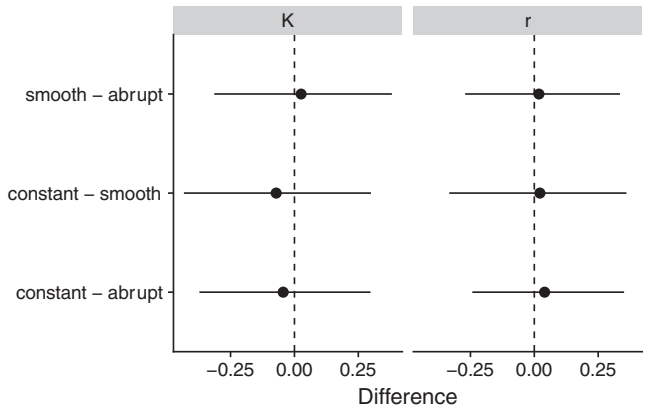

FIGURE 1 Comparison of total variation difference between evolved strains for biomass yield (*K*) and maximal growth rate (*r*).

4 | DISCUSSION

After experimental evolution, during which *Serratia marcescens* DB 11 was exposed to constant and fluctuating thermal environments, we found changes in evolutionary potential. In comparison to evolving in a constant environment, in fluctuating environments the environmental variation was higher, suggesting that fluctuating environment hindered the ability of *S. marcescens* to evolve. Total variation was comparable between the experimental strains, indicating that the observed changes were not due to changes in total variation (Figure 1).

Environmental fluctuations have been suggested to maintain a higher amount of genetic variation. However, the evidence is mixed (Kassen, 2002). In our experiment genetic variation between strains did not differ in the different evolutionary treatments. In principle, higher variation in fluctuating environments could be due to maintained polymorphism of temperature specialists (Levins, 1968). However, if selection benefits generalists or phenotypically plastic genotypes, then fluctuating environments may also result in low genetic variation (Botero et al., 2015; Kronholm, 2022). Another potential signature of weak selection, high between-population variation (Cooper & Lenski, 2010; Ketola et al., 2013; Travisano et al., 1995), which did not differ between the evolutionary treatments (Figure 2).

Bet-hedging is known also in bacteria, for example, in propensity to alter between morphotypes, and it plays important role in bacterial fitness and growth characteristics (Beaumont et al., 2009). Morphotype variation and associated growth variation are also important in natural conditions (Kunttu et al., 2009; Pulkkinen et al., 2022; Sundberg et al., 2014). Therefore, the repeatability of growth patterns, quantified as an environmental variation, can also be indicative of bet-hedging or developmental instability and could be followed when more easily recordable traits, such as morphotypes, do not exist. We observed that environmental variation was higher in strains that evolved in fluctuating environments than in strains that evolved in a constant environment. Interestingly, the ancestor had even higher environmental variation, which indicates that laboratory conditions had generally decreased the environmental variation, especially so in constant temperature. This finding could be indicative of diversity and complexity of the environment that this environmentally growing opportunistic pathogen (Flyg et al., 1980) might often encounter (see below). As measurements were conducted using plates with a balanced number of clones from all treatments in randomized order, treated similarly and measured simultaneously, we can safely exclude technical effects as an explanation.

In addition to bet-hedging, our result could be explained by the evolution of phenotypic plasticity and instability of genotypes of highly plastic individuals (Tonsor et al., 2013). Improved tolerance of thermal transitions could be obtained by inducible phenotypic plasticity but, in order to mount a plastic response at the correct time, individuals require a cue about the upcoming environment. Picking up the cue and responding strongly to it is obviously beneficial if, indeed, the environments fluctuate, and the cue is reliable. However,

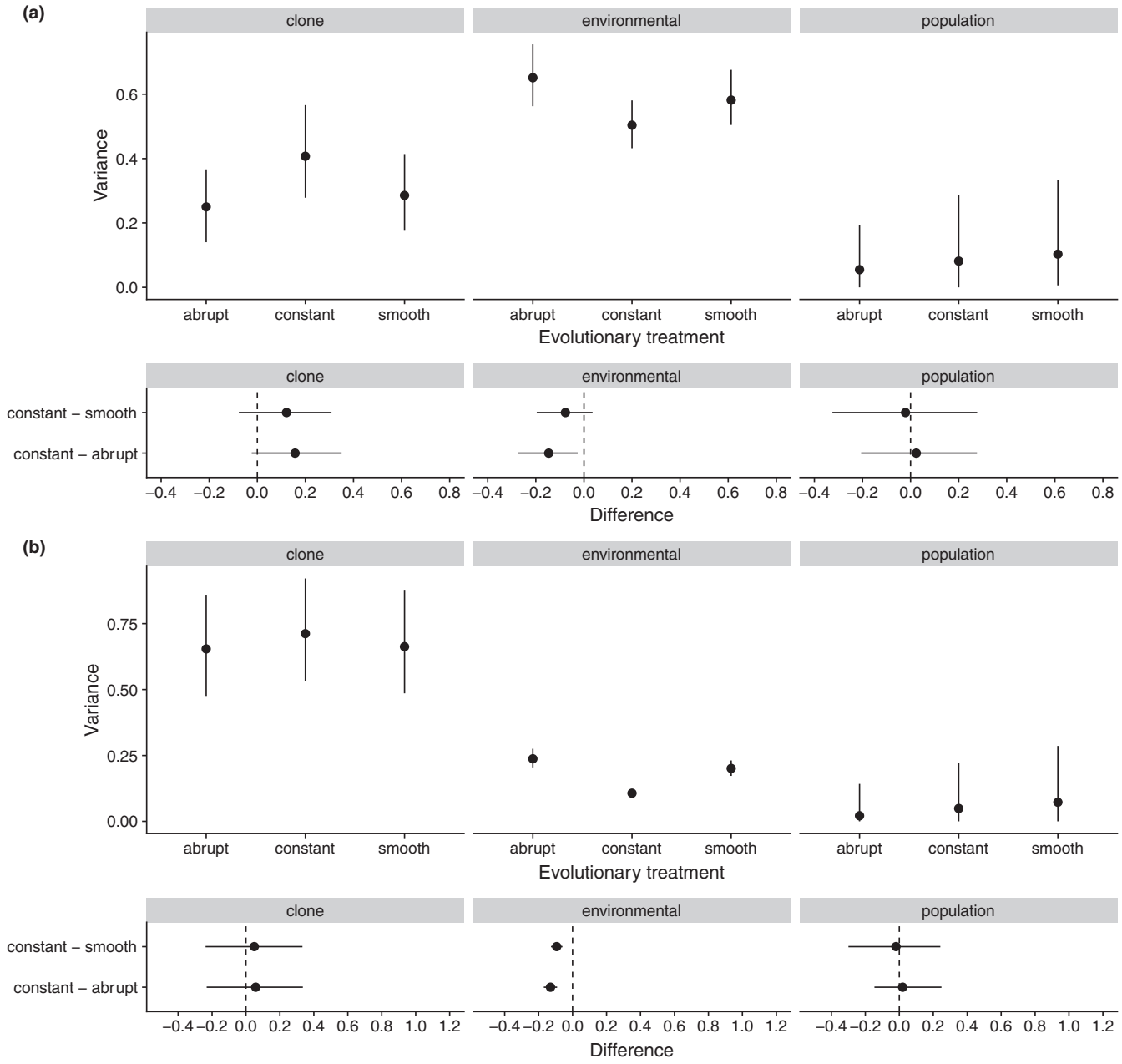

FIGURE 2 (a) Results for maximal growth rate. Top row shows the 95% HPD intervals for clone, environmental and population variance components in the different evolutionary treatments. Bottom row shows 95% HPD intervals for differences between the constant treatment and the two fluctuating treatments are shown for each variance component. Vertical dashed line indicates difference of zero. (b) Results for yield.

environments also contain, 'noise', that could act as a false cue for a response, unavoidably resulting in a suboptimal phenotype also in apparently constant environments. This instability can also effectively act against the evolution of inducible phenotypic plasticity (Tonsor et al., 2013).

Previous experimental evolution work conducted at very rapidly fluctuating environments suggests that tolerance to fast fluctuations might be due to the evolution of inducible plasticity (Ketola et al., 2004; Ketola & Saarinen, 2015; Saarinen et al., 2018). Our findings here support the earlier work and suggest potential consequences: higher environmental sensitivity, that is, environmental variation. However, regardless of the exact mechanism (bet-hedging or a side effect of phenotypic plasticity), our data show important an result: that evolution alters evolutionary potential by altering visibility of genetic variation and the population's potential for responding to new conditions.

AUTHOR CONTRIBUTIONS

Tarmo Ketola: Conceptualization (lead); data curation (lead); formal analysis (equal); funding acquisition (lead); investigation (lead); methodology (lead); project administration (lead); writing – original draft (lead); writing – review and editing (lead). **Ilkka Kronholm:**

Conceptualization (supporting); formal analysis (equal); visualization (lead); writing – original draft (supporting); writing – review and editing (supporting).

ACKNOWLEDGEMENTS

We thank Kati Saarinen for conducting the measurements, Kalevi Viipale for insightful discussions and Academy of Finland for funding (TK: 278751, IK: 321584).

CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jeb.14178.

DATA AVAILABILITY STATEMENT

Data and codes are available at Dryad: doi:10.5061/dryad.fbg79cp03

ORCID

Tarmo Ketola https://orcid.org/0000-0001-5151-7980
Ilkka Kronholm https://orcid.org/0000-0002-4126-0250

REFERENCES

Beaumont, H. J. E., Gallie, J., Kost, C., Ferguson, G. C., & Rainey, P. B. (2009). Experimental evolution of bet hedging. *Nature*, *462*, 90–93.

Botero, C. A., Weissing, F. J., Wright, J., & Rubenstein, D. R. (2015). Evolutionary tipping points in the capacity to adapt to environmental change. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 184–189.

Bürger, R., & Gimelfarb, A. (2002). Fluctuating environments and the role of mutation in maintaining quantitative genetic variation. *Genetical Research*, *80*, 31–46.

Bürkner, P.-C. (2017). Brms: An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software*, *80*, 1–28.

Cooper, T. F., & Lenski, R. E. (2010). Experimental evolution with *E. coli* in diverse resource environments. I. Fluctuating environments promote divergence of replicate populations. *BMC Evolutionary Biology*, *10*, 11.

Duetz, W. A., Ruedi, L., & Hermann, R. (2000). Methods for intense aeration, growth, storage, and replication of bacterial strains in microtiter plates. *Applied and Environmental Microbiology*, *66*, 2641–2646.

Flyg, C., Kenne, K., & Boman, H. G. (1980). Insect pathogenic properties of Serratia marcescens: Phage resistant mutants with a decreased resistance to cecropia immunity and a decreased virulence to drosophila. *Journal of General Microbiology.*, *120*, 173–181.

Hoffmann, A. A., & Merilä, J. (1999). Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology and Evolution*, *14*, 96–101.

Johnson, T., & Barton, N. (2005). Theoretical models of selection and mutation on quantitative traits. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *360*, 1411–1425.

Kassen, R. (2002). The experimental evolution of specialists, generalists, and the maintenance of diversity. *Journal of Evolutionary Biology*, *15*, 173–190.

Kawecki, T. J. (2000). The evolution of genetic canalization under fluctuating selection. *Evolution*, *54*, 1–12.

Ketola, T., & Saarinen, K. (2015). Experimental evolution in fluctuating environments: Tolerance measurements at constant temperatures incorrectly predict the ability to tolerate fluctuating temperatures. *Journal of Evolutionary Biology*, *28*, 800–806.

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., 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.

Kronholm, I. (2022). Evolution of anticipatory effects mediated by epigenetic changes. *Environmental Epigenetics*, *8*, 1–12.

Kunttu, H. M. T., Valtonen, E. T., Jokinen, E. I., & Suomalainen, L.-R. (2009). Saprophytism of a fish pathogen as a transmission strategy. *Epidemics*, *1*, 96–100.

Levins, R. (1968). *Evolution in changing environments: Some theoretical explorations*. Princeton University Press.

Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits*. Sinauer Associates, Inc.

Pulkkinen, K., Ketola, T., Laakso, J., Mappes, J., & Sundberg, L.-R. (2022). Rich resource environment of fish farms facilitates phenotypic variation and virulence in an opportunistic fish pathogen. *Evolutionary Applications*, *15*, 417–428.

Saarinen, K., Laakso, J., Lindström, L., & Ketola, T. (2018). Adaptation to fluctuations in temperature by nine species of bacteria. *Ecology and Evolution*, *8*, 2901–2910.

Schou, M. F., Kristensen, T. N., & Hoffmann, A. A. (2020). Patterns of environmental variance across environments and traits in dairy cattle. *Evolutionary Applications*, *13*, 1090–1102.

Sundberg, L.-R., Kunttu, H. M. T., & Valtonen, E. T. (2014). Starvation can diversify the population structure and virulence strategies of an environmentally transmitting fish pathogen. *BMC Microbiology*, *14*, 67.

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., Mongold, J. A., Bennett, A. F., & Lenski, R. E. (1995). Experimental tests of the role of adaptation, chance ad history in evolution. *Science*, *267*, 87–90.

Wilson, A. J., Pemberton, J. M., Pilkington, J. G., Coltman, D. W., Misfud, D. V., Clutton-Brock, T. H., & Kruuk, L. B. (2006). Environmental coupling of selection and heritability limits phenotypic evolution. *PLoS Biology*, *4*, 1270–1275.

How to cite this article: Ketola, T., & Kronholm, I. (2023). Experimental evolution of evolutionary potential in fluctuating environments. *Journal of Evolutionary Biology*, *00*, 1–5. https://doi.org/10.1111/jeb.14178