Adaptation of Paramecium caudatum to variable conditions of temperature stress

Alison Duncan, Simon Fellous, Elsa Quillery, Oliver Kaltz

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Alison B. Duncan*, Simon Fellous, Elsa Quillery, Oliver Kaltz

Institut des Sciences de l'Evolution (ISEM), UMR 5554, Université Montpellier 2, Place Eugene Bataillon, 34095 Montpellier cedex 05, France

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Abstract

The environment is rarely constant and organisms are exposed to spatial and temporal variation that will impact life-histories. It is important to understand how such variation affects the adaptation of organisms to their local environment. We compare the adaptation of populations of the ciliate *Paramecium caudatum* exposed to constant (23 °C or 35 °C) and temporally variable temperature environments (random daily fluctuations between 23 °C or 35 °C). Consistent with theory, our experiment shows the evolution of specialists when evolution proceeds in constant environments and generalists when the environment is temporally variable. In addition, we demonstrate costs for specialists of being locally adapted through reduced fitness in novel environments. Conversely, we do not find any costs for generalists, as all populations from variable environments had equal or superior performance to specialists in their own environment. The lack of a cost for generalists is emphasised by the presence of a super generalist that has the highest performance at both assay temperatures.

Keywords: Local adaptation; Specialists; Generalists; Temperature variation; Thermal stress

1. Introduction

Environmental heterogeneity is considered a universal driver of evolutionary change and adaptation (Levins, 1968; Bell, 1997, 2010). Indeed, in nature, organisms are expected to be adapted to the environment, or environments, they experience most commonly. This manifests itself in the evolution of specialists when organisms are exposed to a mostly constant environment, and generalists when changes in environmental state are frequent (Levins, 1968; Kassen, 2002). Accordingly, evolution in spatially structured, constant environments should generate specialists that maximise fitness in their own environment. However, adaptation in one environment is predicted to arise at a cost expressed as low performance in other environments. This could happen due to antagonistic pleiotropy where genes beneficial in one environment are costly in another. Consequently, evolution of a specialist should result in patterns of local adaptation whereby locally selected residents have a higher fitness than foreign, unselected non-residents (Kawecki and Ebert, 2004).

In contrast, temporally variable environments should select for generalist strategies that enable persistence in all environments encountered. Thus, rather than maximising fitness within environments, selection for generalists should maximise average fitness over all environments. As a result, the fitness of generalists in each single environment may be less than that of respective specialists. This is comparable to a low-risk "bet-hedging" strategy, minimising the variance in performance between environments and allowing at least some average performance in all environments encountered (Stearns, 2000; Kassen, 2002).

The evolution of generalists depends on various genetic and ecological factors, such as the amount of standing genetic variation in populations and environmental structure (Kassen, 2002). An important aspect of the temporal environment is the frequency of exposure to different environments (Levins,

* Corresponding author.
E-mail address: ali.b.duncan@gmail.com (A.B. Duncan).

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1968; Bell, 2010). If frequency of exposure to one environment is common and the other rare, local adaptation to the rare environment may not occur. However, if the rare environment is particularly harsh, adaptation to it may be required to guarantee survival following its occurrence.

Microbial organisms are ideal to study adaptation to a variable environment due to their short generation time, large population sizes and the ability to manipulate their environment in a controlled fashion (Jessup et al., 2004). In this study, we use experimental microcosms to investigate the evolution of specialists and generalists in spatially and temporally variable environments. This experiment is the first to investigate how different frequencies of exposure to different environments influences the evolution of generalists. We cultured experimental populations of the ciliate *Paramecium caudatum* in two constant temperature environments (23 °C and 35 °C) and in 4 temporally variable temperature environments, with random daily fluctuations between 23 °C and 35 °C. These variable environments differed in the frequency of occurrence of temperature stress (35 °C), ranging from rare (∼25% of the time) to frequent (∼75%). After 4 months (30–40 generations), survival and replication at 23 and 35 °C were compared among populations from the different treatments. The constant environments should select for specialists. Therefore, we predicted performance of populations tested in their own environment to be superior to performance in foreign constant temperature environments (i.e., local adaptation). The variable environments should select for generalists. Thus, we expected the performance of *Paramecium* from variable treatments to have intermediate fitness to those evolved in constant environments. Further, we expected performance of *Paramecium* evolved in the different variable environments to differ in each of the constant assay environments according to the amount of time previously spent at each temperature.

2. Materials and methods

2.1. Study organisms

P. caudatum is a freshwater ciliate found in still water bodies in the northern hemisphere that feeds on bacteria and detritus within the water column (Wichterman, 1986). Reproduction is predominantly asexual through mitotic division. Under exponential growth conditions, *P. caudatum* divides 1–3 times every 24 h and optimal growth temperatures generally range between 24 and 28 °C (Wichterman, 1986). There is also evidence of within-species genetic variation in growth and survival at different temperatures (Fels and Kaltz, 2006). In our laboratory, *Paramecium* are maintained at 23 °C, in a culture medium of dried organic lettuce supplemented with the bacterium *Serratia marcescens* as food (Nidelet, 2007). Two *Paramecium* clones were used in this experiment, clone K8 and clone VEN (see (Duncan et al., 2010) for details). Each population in the selection experiment comprised 20 ml from mass cultures of each clone in a 50 ml Falcon tube.

2.2. Selection experiment

In a 120-day selection experiment, populations of *P. caudatum* were randomly assigned to one of 6 selection treatments, two constant temperature treatments (23 °C, and 35 °C ± 0.5 °C) and four variable temperature treatments (with mean temperatures of 26.2 °C, 27.8 °C, 30.2 °C or 32.8 °C). These variable temperature environments corresponded to 27%, 40%, 60% and 73% of time during the experiment spent at 35 °C, respectively. Variable mean temperatures were achieved by randomly changing tubes daily between 23 °C and 35 °C. Each tube was randomly allocated an individual sequence corresponding to its mean temperature. At weekly intervals, 3 ml of each population was removed and replaced with fresh culture medium containing 50 *P. caudatum* from source populations of the same clone kept at 23 °C. Table 1 shows mean population sizes prior to the onset of the experiment. All populations were larger than the 50 individuals added each week, indicating self-sustaining populations even at higher temperatures. The experiment contained a total of 48 replicate populations, with 4 replicates per genotype and treatment (2 genotypes × 6 selection treatments × 4 replicate populations). On day 120 of the selection experiment, two 1-ml samples were removed from each population and each transferred to a 1.5 ml Eppendorf tube with 500 µl of medium. One sample was placed at 23 °C and the other at 35 °C for a 48 h acclimation period.

2.3. Adaptation assay

We phenotyped the division of individual *Paramecium,* from each replicated population at 23 °C and 35 °C. Four *Paramecium* cells were individually isolated from each 1 ml sample that had experienced a 48 h acclimation period prior to the onset of the experiment. Each individual cell was placed in a 60 µl drop of culture medium arranged inside the lids of 24-microwell plates (Nunc™, Fisher Scientific, France). The *Paramecium* were checked for survival and division 24, 30, 36 and 48 h after start of the experiment.

2.4. Statistical analysis

We used repeated-measures ANOVA to analyse the performance of individual *Paramecium* at 23 °C and 35 °C for 48 h after onset of the experiment. Performance was measured as the $\log_2$(number of cells + 1) in each 60 µl drop through

Table 1
Mean population sizes for *Paramecium* populations in each of the selection environments prior to the onset of the experiment (±standard error).

23 °C Constant	26 °C Variable	28 °C Variable	30 °C Variable	32 °C Variable	35 °C Constant
812 (±118)	1188 (±412)	867 (±458)	565 (±221)	281 (±148)	372 (±284)

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time. Selection treatment, assay temperature and time were included in models as fixed factors and host clone, replicate population and assay microwell plate as random factors. Non-significant terms were removed from models in a stepwise fashion ($p > 0.10$).

Unlike specialists, generalists are predicted to minimise differences in fitness between environments. To test this hypothesis, we averaged $\log_2$(number of cells + 1) after 48 h for each replicate population at 23 ($\overline{x}_{23\ ^\circ C}$) and 35 °C ($\overline{x}_{35\ ^\circ C}$). Following Bell (2010), we calculated the environmental variance as Var(E) = 1/2 $(\overline{x}_{23^\circ C} - \overline{x}_{35^\circ C})^2$ for each replicate population. In an ANOVA, we tested for difference in Var(E) among selection treatments. All analysis was done using JMP 8 (SAS, 2008).

3. Results

There was a steady increase in density during the experiment for all populations at 23 °C. In contrast, populations at 35 °C increased only marginally during the first 24 h of the experiment before stopping division or death. Time series analysis revealed a significant 3-way interaction between time, assay temperature and selection temperature ($F_{5,\ 1190} = 2.73$, $p = 0.0183$). This means that differences in relative performance of populations from the different constant and variable selection treatments, at the two assay temperatures, became apparent over the course of the assay. *Paramecium* from the 23 °C constant treatment tended to grow better at 23 °C than those from the 35 °C constant treatment. The reverse was true for *Paramecium* from the 35 °C treatment, that had greater survival at 35 °C than *Paramecium* from the 23 °C constant treatment. *Paramecium* from the variable treatments varied in their responses, with their relative ranks depending on the assay temperature and identity of the variable treatment (Fig. 1). The host clone and its interactions were not significant and never explained more than 3.5% of the variance in the model.

Fig. 2 illustrates general patterns observed during the 48 h period for *Paramecium* from the different selection treatments. Crossing of reaction norms for growth of *Paramecium* from the two constant environments at the two assay temperatures is clearly shown. Performances of *Paramecium* from populations with variable selection treatments were generally equal to or greater than resident lines from the respective constant treatment. One variable treatment (populations with a mean of 26 °C) had the highest performance at both assay temperatures, indicating the presence of a super generalist.

The environmental variance in performance Var(E) did not significantly differ among selection treatments ($F_{5,\ 28} = 0.85$, $p = 0.5264$). Thus, there was no evidence that populations from variable (generalist) treatments had reduced variation in performance at 23 and 35 °C compared to those from constant (specialist) treatments. Fig. 2 illustrates this comparable variation in performance between populations from constant and variable treatments at 23 °C and 35 °C.

We projected the fitness of *Paramecium* at variable mean temperatures by combining fitness measured at 23 °C and at 35 °C. We calculated mean (geometric) fitness of variable generalists and constant specialists at different variable treatment mean temperatures. The geometric mean is a more appropriate measure of fitness across temporally variable environments (Bell, 2010). Fig. 3 shows better fitness of generalists in all variable environments, in particular for increasing mean temperatures, i.e., in environments with more frequent exposure to 35 °C. Some generalists even appear capable of invading constant specialist populations, in

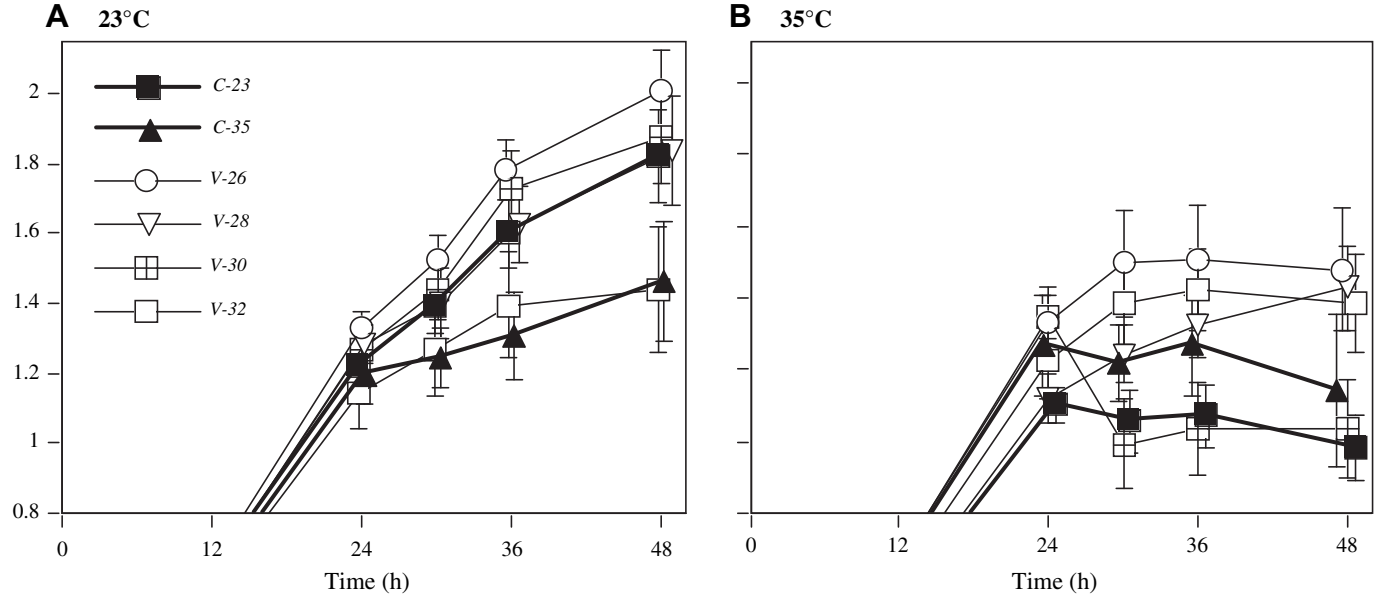

Fig. 1. Changes in mean number of paramecia through time (24, 30, 36, 48 h), measured at 23 °C (A) and 35 °C (B), starting from a single individual. Means shown for 2 constant temperature treatment origins (23 °C = *C-23*; 35 °C = *C-35*) and for 4 variable treatment origins (fluctuating between 23 and 35 °C, with means between 26 and 32 °C: *V-26*, *V-28*, *V-30*, *V-32*). Numbers are $\log_2$-transformed; thus values >1 indicate an increase in number. Error bars denote standard errors.

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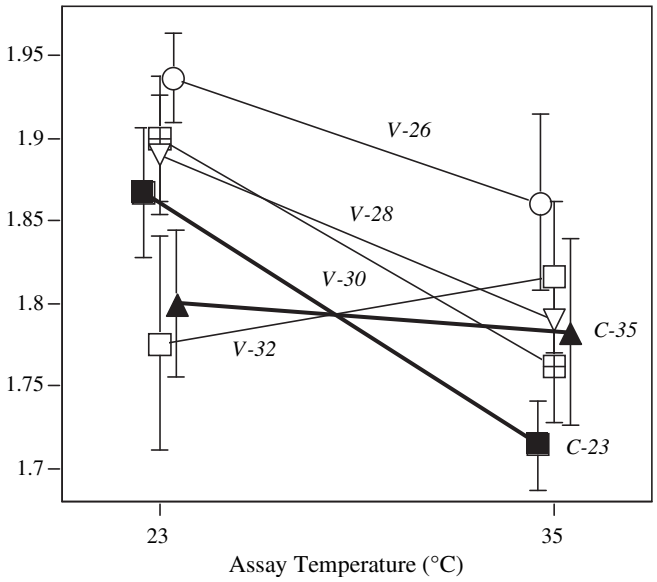

Fig. 2. Mean cumulative paramecium growth over 48 h at two assay temperatures (23 and 35 °C), as measured by the area under the curve AUC, based on $\log_2$-transformed (number of paramecia +1). Means shown for 2 constant temperature treatment origins (*C-23*, *C-35*) and for 4 variable treatment origins (*V-26*, *V-28*, *V-30*, *V-32*). Error bars denote standard errors.

particular, the stressful constant 35 °C environment. One generalist (V26, from a variable treatment with a mean of 26 °C) may even be universally best fit across all environments.

4. Discussion

We observed a crossing of reaction norms for *Paramecium* from the different constant selection treatments when tested at 23 °C and 35 °C. We will first discuss the implications of these results in terms of evolution of specialists in constant environments and then the evolution of generalists in variable environments.

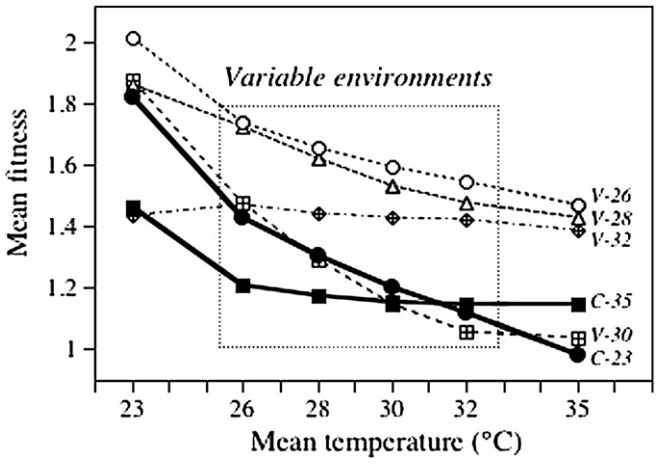

Fig. 3. Mean fitness of constant (*C-23*, *C-35*) and variable (*V-26*, *V-28*, *V-30*, *V-32)* treatment origins estimated for each of the 4 variable treatment environment temperature means. Fitness was estimated from the mean $\log_2$-transformed number of individuals at 23 or 35 °C after 48 h. For constant environments, fitness is the arithmetic mean at 23 or 35 °C, respectively. For variable environments, geometric means for performance at 23 and 35 °C were calculated and weighted for the relative frequency of time spent at the two temperatures.

4.1. Specialisation in constant environments

Populations from the two constant temperature environments (23 °C and 35 °C) were observed to have higher levels of division when assayed at their own selection temperature. This finding is consistent with the idea that spatially heterogeneous environments favour the evolution of specialists, each adapted to their own environment (Kawecki and Ebert, 2004). Experimental evolution of specialisation to different (constant) temperature regimes has also been shown, e.g., for the bacterium *Escherichia coli* and the green alga *Chlamydomonas rheinhardtii* reviewed in (Kassen, 2002). Although our results are coherent with reciprocal selection in each constant environment, this may not actually be the case. All populations had been cultured at 23 °C for a number of years prior to the onset of the experiment, such that 4 months of additional selection may not improve fitness at this temperature (see also (Bennett et al., 1992)). Accordingly, the observed cross-reaction norms may simply reflect a change in *Paramecium* cultured under temperature stress (35 °C). Thus, adaptation to 35 °C apparently comes at a cost whereby populations have reduced performance at 23 °C.

The mechanisms responsible for the observed patterns are not clear. The observed results are coherent with genetic change and evolution occurring in the different populations. Indeed, previous work has indicated a genetic basis of temperature-dependent variation in growth and survival in *P. caudatum* (Fels and Kaltz, 2006; Duncan et al., 2010). Thus, given the duration of our long-term experiment (4 months), and the population sizes at 35 °C (500–1000 individuals), this is a plausible explanation. However, it is also possible that these results are attributable to acclimation of the *Paramecium* to the different temperature environments. It is clear, however, from our results, that the 48 h conditioning period prior to the onset of the experiment did not efface local adaptation in our experiment. Previous work has shown that patterns of acclimation for *Paramecium tetraurelia* to stressful temperatures (35 °C as in our experiment) are lost within 24 h (Hennessey and Nelson, 1979). This result suggests that 48 h is sufficient to eliminate acclimation and that our results are consistent with evolution. However, another study found that patterns of acclimation for *P. caudatum* between more permissive environments (10 °C and 25 °C) remained for up to a week (Tsukuda and Takeuchi, 1984). Molecular analysis affirming genetic change in the experiment is required to distinguish between these hypotheses. In addition, true understanding of the mechanisms behind these patterns will require more detailed experiments regarding the time required for acclimation and its subsequent duration.

The responses of *Paramecium* from the different populations may hint at the underlying physiological mechanisms. At 35 °C, the resident population selected at 35 °C has a division advantage only during the first 24 h before division declines at the same rate as *Paramecium* from 23 °C. This is

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consistent with a constitutive heat stress protection mechanism, such as altered membrane structure (Sasaki et al., 2006). In contrast, at 23 °C, the resident advantage is cumulative, increasing with time, whereas growth from populations originating from 35 °C increases at first before levelling off. An absence of sustained growth for *Paramecium* from 35 °C may be attributable to the cost of the heat-stress protection mechanism when resources (food, oxygen) become limiting in the 60 µl drop. Although not expected to serve a constitutive function for heat tolerance, fitness costs of HSP overexpression have been demonstrated in various organisms (Ketola et al., 2004; Sorensen et al., 2003).

4.2. Evolution of generalists in variable environments

Our results clearly showed that *Paramecium* from variable selection environments grow reasonably well at both 23 °C and 35 °C, consistent with the evolution of a generalist strategy. However, patterns of growth for populations from the different variable treatments were not the same across populations, nor was the performance of these populations intermediate between those of the constant-temperature specialists, as would be expected for typical generalists e.g., (Legros and Koella, 2010). Indeed, environmental variance in growth between the two assay temperatures for populations from variable environments was comparable to that observed for populations from constant environments. This result is inconsistent with the evolution of a bet-hedging strategy predicted for generalists, but consistent with other empirical observations (Leroi et al., 1994; Reboud and Bell, 1997; Kassen and Bell, 1998; Barrett et al., 2005). It seems that generalist evolution here does not select for intermediate fitness across environments encountered, but instead permits optimal adaptation to different components of the environment presented (here: 23 °C and 35 °C).

Further, one novel aspect of our experiment was the use of different types of variable environments, where we manipulated the time that populations spent at 23 °C and 35 °C. We did not observe that evolution in the performance of *Paramecium* at the different variable treatments varied in the constant assay environments according to the amount of time they had spent in each. The different relative frequencies of exposure to 35 °C or 23 °C had no obvious influence on the order of performance at either assay temperature, nor on the order of the average performance over both temperatures. Combined, these results emphasise an absence of trade-off ensuing different amounts of time spent at each temperature, and no cost for being a generalist in this experiment.

Instead, our results indicate higher fitness of (some) generalists in all variable environments, in particular at increasing mean temperatures, with more frequent exposure to 35 °C. Thus, most variable treatments appear to be better adapted to variable environments than constant treatments (Fig. 3). Moreover, some of the variable treatments produced populations that may even be capable of invading the specialist populations at constant temperatures, especially at 35 °C. In fact, one variable environment (V-26) seems to have generated supergeneralists with over all superior growth at both assay temperatures, and highest projected fitness across all variable mean temperatures (Fig. 3). The absence of a cost for our generalist populations may be attributable to the evolutionary consequences of adapting in a variable environment (Lenormand et al., 2009). Fluctuations in selective pressure can induce “Evolutionary revolutions” sensu Lenormand et al. (2009). That is, when ancestral populations are fixed to a local fitness peak in an adaptive landscape (in our case populations initially adapted to 23C), stochasticity in selection can allow populations to reach a peak higher in the fitness landscape. Here, transient exposure to 35 °C could help *Paramecium* find a strategy that was initially worse at 23 °C but eventually leads to higher fitness at both temperatures. This phenomenon can be compared to "roundabout selection" as exemplified by MacLean et al. (2002).

The physiological mechanism for heat tolerance may not be the same for constant and variable temperature treatments. For example, heat shock proteins are associated with a response that is induced relatively quickly following exposure to heat stress (Feder and Hofmann, 1999) and may not last. Therefore, in temporally variable environments, increased heat stress tolerance may be mediated by selection on the HSP machinery (Ketola et al., 2004). In contrast, constitutive HSP overexpression may be less rewarding under sustained exposure to heat stress, because of the costs associated with it (Sorensen et al., 2003; Ketola et al., 2004). Thus long-term constant exposure to high temperature may even lead to lower HSP expression levels (Cavicchi et al., 1995; Bettencourt et al., 1999). Heat tolerance in our constant 35 °C treatment may therefore have another mechanistic basis, such as the altered membrane structures mentioned above (Sasaki et al., 2006; Toyoda et al., 2009). The costs associated with each mechanism may be very different, which may explain the different reaction norms for populations from constant and variable environments observed in this experiment. Alternatively, the costs we observe for specialists in foreign environments may arise following mutation accumulation of genes/alleles that are only deleterious in foreign environments (Kassen, 2002). Accumulation of such genes will not occur in variable environments because they will be consistently selected against.

4.3. Conclusions

This experiment demonstrates that specialists appear when selection occurs in constant environments, and costs associated with specialisation are consistent with theory. In contrast, evolution under variable conditions produces generalists with often superior fitness at both assay temperatures. Although we cannot be sure, we postulate that there may be different mechanisms mediating heat tolerance for generalists and specialists in our experiment. Constitutive and induced quantification of heat shock protein expression in *Paramecium* would confirm this hypothesis. Aside from the mechanism, we cannot distinguish whether the phenotypic differences between populations are genetic, or due to trans-generational acclimation. Confirmation of this hypothesis would require

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molecular analysis demonstrating genetic change in populations. Regardless of the mechanism, we demonstrate that patterns of local adaptation unravel differently in constant and variable environments.

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