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Adaptation of *Paramecium caudatum* to variable conditions of temperature stress

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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,

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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)