



HAL
open science

Evolution under pH stress and high population densities leads to increased density-dependent fitness in the protist *Tetrahymena thermophila*

Felix Moerman, Angelina Arquint, Stefanie Merkli, Andreas Wagner, Florian Altermatt, Emanuel Fronhofer

► **To cite this version:**

Felix Moerman, Angelina Arquint, Stefanie Merkli, Andreas Wagner, Florian Altermatt, et al.. Evolution under pH stress and high population densities leads to increased density-dependent fitness in the protist *Tetrahymena thermophila*. *Evolution - International Journal of Organic Evolution*, 2020, 74 (3), pp.573-586. 10.1111/evo.13921 . hal-02466463

HAL Id: hal-02466463

<https://hal.umontpellier.fr/hal-02466463v1>

Submitted on 3 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Evolution under pH stress and high population
2 densities leads to increased density-dependent fitness
3 in the protist *Tetrahymena thermophila*

4 Felix Moerman^{1,2,3,4,*}, Angelina Arquint¹, Stefanie Merkli¹,
Andreas Wagner^{1,4,5}, Florian Altermatt^{1,2} and Emanuel A. Fronhofer^{1,2,3}

5 1. Department of Evolutionary Biology and Environmental Studies, University of Zürich,
6 Winterthurerstrasse 190, Zürich CH-8057, Switzerland

7 2. Department of Aquatic Ecology, Eawag: Swiss Federal Institute of Aquatic Science and
8 Technology, Überlandstrasse 133, Dübendorf CH-8600, Switzerland

9 3. ISEM, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France

10 4. Swiss Institute of Bioinformatics, Quartier Sorge—Bâtiment Génopode, Lausanne 1015,
11 Switzerland

12 5. The Santa Fe Institute, Santa Fe, New Mexico 87501, USA

13 * Corresponding author: felix.moerman@ieu.uzh.ch

14 **Running Title:** pH stress and convergent evolution

15 **Keywords:** eco-evolutionary feedback, microcosm, experimental evolution, density regulation,
16 artificial selection

17

Abstract

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

Abiotic stress is a major force of selection that organisms are constantly facing. While the evolutionary effects of various stressors have been broadly studied, it is only more recently that the relevance of interactions between evolution and underlying ecological conditions, that is, eco-evolutionary feedbacks, have been highlighted. Here, we experimentally investigated how populations adapt to pH-stress under high population densities. Using the protist species *Tetrahymena thermophila*, we studied how four different genotypes evolved in response to stressfully low pH conditions and high population densities. We found that genotypes underwent evolutionary changes, some shifting up and others shifting down their intrinsic rates of increase (r_0). Overall, evolution at low pH led to the convergence of r_0 and intraspecific competitive ability (α) across the four genotypes. Given the strong correlation between r_0 and α , we argue that this convergence was a consequence of selection for increased density-dependent fitness at low pH under the experienced high density conditions. Increased density-dependent fitness was either attained through increase in r_0 , or decrease of α , depending on the genetic background. In conclusion, we show that demography can influence the direction of evolution under abiotic stress.

34 **Introduction**

35 For many decades, biologists have studied the link between the abiotic environment and the
36 distribution of species on earth, trying to understand why species occur in certain environments
37 and not in others (HilleRisLambers et al., 2012; Dunson and Travis, 1991). Evolutionary biol-
38 ogists more specifically have studied the constraints and potential of species to adapt to their
39 environment and how species respond when changes in their environment occur (Bijlsma and
40 Loeschcke, 2005; Bridle and Vines, 2007). This encompasses research of adaptation to a mul-
41 titude of abiotic stressors, including salt stress (Gunde-Cimerman et al., 2006; Flowers et al.,
42 2010), heavy metal presence (Shaw, 1994; Klerks and Weis, 1987), thermal stress (Johnston
43 et al., 1990; Angilletta, 2009, chapter 9) and stress associated with drought or the water regime
44 (Kooyers, 2015; Lytle and Poff, 2004). Organisms can respond to such abiotic stress in sev-
45 eral ways. They can respond through evolutionary adaptation, by evolving genotypes which
46 match the changed abiotic conditions (Kawecki and Ebert, 2004). They can also adapt through
47 phenotypic plasticity, changing their phenotype to match the abiotic conditions (Westeberhard,
48 1989). When populations fail to either adapt quickly or to move away — to disperse (Clobert
49 et al., 2001, part 1; Clobert et al., 2012, chapter 1-2) — these populations may be driven to ex-
50 tinction locally. In order to accurately predict local population dynamics and persistence in the
51 context of evolutionary adaptations to abiotic change, it is necessary to understand the speed
52 and direction of evolution in response to changing abiotic conditions, as well as to understand
53 the constraints that such evolution faces.

54 The question of how populations can adapt through evolution to changing abiotic condi-
55 tions has a long-standing history in empirical research, both in laboratory experiments as well
56 as field studies (as reviewed in Kawecki and Ebert, 2004). Local adaptation has been recorded
57 in response to different abiotic stressors, across different habitats, and in several taxonomic
58 groups, including plants (Leimu and Fischer, 2008), fish (Fraser et al., 2011), and invertebrates
59 (Sanford and Kelly, 2011). One important environmental impact of human activities is the
60 acidification of natural waters and soils. In the past, acidification has strongly affected natural
61 environments through acid rain (Likens and Bormann, 1974; Likens et al., 1996; Burns et al.,
62 2016). It remains an important abiotic stressor because of the use of fossil fuels and ongoing

63 anthropogenic increase in atmospheric carbon dioxide. Both lead to an acidification of wa-
64 ter bodies, oceans in particular, (Caldeira and Wickett, 2003; Raven et al., 2005; Zeebe et al.,
65 2008), with potentially severe consequences for organisms therein. Consequently, recent an-
66 thropogenic pressure on the natural environment has triggered increased efforts to understand
67 if and how populations respond to human-induced climate shifts. Reviews of the literature
68 showed that some species evolve to the changing climate, whereas others do not, at least not in
69 the short term (Hoffmann and Sgro, 2011; Franks and Hoffmann, 2012). Ocean acidification
70 has sparked efforts to understand how readily species can evolve to changing pH conditions
71 (Kelly and Hofmann, 2013; Sunday et al., 2014).

72 Despite a growing body of work, evolution to pH stress is still less well studied experimen-
73 tally, compared to many other stressors. Evolutionary changes caused by pH shifts have already
74 been studied in the past, and this has typically been done comparatively or through translocat-
75 ion experiments along gradients or between locations differing in pH. For example Derry and
76 Arnott (2007) and Hangartner et al. (2011) showed that copepods and frogs are locally adapted
77 to the pH of their environment. Experimental evolution studies on adaptation to pH stress, al-
78 though existing, are limited to only few systems that include bacterial model species (Hughes
79 et al., 2007; Zhang et al., 2012; Gallet et al., 2014; Harden et al., 2015) and yeast (Fletcher et al.,
80 2017). For example Gallet et al. (2014) demonstrate how the pH-niche under pH stress evolves
81 through a transient broadening of the niche, followed by specialization. However, many of these
82 studies are focused on adaptation to digestive tracts (Hughes et al., 2007; Harden et al., 2015)
83 or oriented towards industrial application (Fletcher et al., 2017; Zhang et al., 2012). Although
84 controlled experiments can help understand evolutionary adaptation to pH stress, they are still
85 rare (Reusch and Boyd, 2013; Stillman and Paganini, 2015). In addition, existing experiments
86 do not explore important factors that can affect adaptive evolution, such as demography.

87 Abiotic conditions will alter population performance, and hence also demography. Un-
88 derstanding how demographic conditions influence evolution, specifically the evolution of
89 life-history traits, has led to an extensive body of theory and experiments (Stearns, 1977,
90 1992). This work has, for example, demonstrated the importance of density-dependent selec-
91 tion and life-history trade-offs between population growth and intraspecific competitive abil-

ity (competition-growth trade-offs; Luckinbill, 1978; Mueller and Ayala, 1981; Andrews and Rouse, 1982; Mueller et al., 1991; Joshi et al., 2001). The eco-evolutionary interaction between demographic changes due to abiotic stress, that is, ecological conditions, and adaptation to abiotic conditions, remains less well understood.

Such eco-evolutionary feedbacks highlight that ecological conditions can alter evolutionary trajectories, and, conversely, that evolutionary change can impact ecological conditions (Pelletier et al., 2009; Hendry, 2016; Govaert et al., 2019). Whereas theoretical work has already incorporated the demographic context into evolutionary questions for some time (for a review, see Govaert et al., 2019), empirical work on adaptation to novel conditions still rarely includes the effect of demography on population performance or density explicitly (for some recent examples that do, see Michel et al., 2016; Nørgaard et al., 2019).

In our study, we experimentally explored how four distinct genotypes of the model protist species *Tetrahymena thermophila* evolve when being subjected to either a low pH treatment or a neutral pH treatment (control setting). We explicitly address the question of adaptation to low-pH stress in established populations with densities close to equilibrium. We quantify how evolution changes life-history strategies in four different genetic backgrounds and highlight the importance of trade-offs in life-history traits for understanding how populations adapt to abiotic stress under conditions of high population density, and assess if populations become more similar in life-history strategy.

We can expect directional selection leading to either a maximization of growth rate, or a maximization of competition related traits. When populations experience low competition, the fastest grower likely experiences a selective advantage, and hence we can expect evolution to lead to an increase in the average growth rate. In contrast, when competition is very high due to high population density, strong competitors will likely be under positive selection. Depending on how abiotic stress alters the selection pressures, expected trends in evolution will change. If a stressful abiotic environment affects mostly growth, but does not influence competition, we might expect stronger selection for increased growth. In contrast, if a stressful abiotic environment mostly affects competition (for example, by limiting the amount of available food, or the uptake thereof), we would expect to see stronger selection for investment in competition

121 related traits at lower population densities compared to the optimal abiotic environment.

122 **Material and methods**

123 **Experiment**

124 **Study organism**

125 We used the freshwater ciliate *Tetrahymena thermophila* as a model species. Due to its small
126 body size, high population densities and short doubling time of ~ 4 h (Cassidy-Hanley, 2012;
127 Collins, 2012), *T. thermophila* is well suited for both ecological and evolutionary experiments
128 (e.g. Fjerdingstad et al., 2007; Collins, 2012; Coyne et al., 2012; Altermatt et al., 2015; Jacob
129 et al., 2016). *T. thermophila* is characterized by a high mutation rate in the macronucleus (Brito
130 et al., 2010). This high mutation rate, in combination with large population sizes (here, ranging
131 from $\sim 1 \times 10^3$ cells/mL to 2×10^6 cells/mL), makes the species an ideal model system for
132 adaptation experiments relying on mutation-driven evolution.

133 We used four clonal genotypes of *T. thermophila* obtained from the Tetrahymena Stock
134 Center at Cornell University. These 4 genotypes are strain B2086.2 (henceforth called geno-
135 type 1; Research Resource Identifier TSC_SD00709), strain CU427.4 (genotype 2; Research
136 Resource Identifier TSC_SD00715), strain CU428.2 (genotype 3; Research Resource Identifier
137 TSC_SD00178) and strain SB3539 (genotype 4; Research Resource Identifier TSC_SD00660).
138 We selected these strains because they differ strongly in both general life-history strategy and
139 their response to pH stress (see Fig. S2 in Supplementary Information section S2).

140 We maintained all cultures in axenic SSP medium consisting of proteose peptone, yeast ex-
141 tract and glucose (Cassidy-Hanley, 2012; Altermatt et al., 2015). To avoid bacterial or fungal
142 contamination, we complemented the medium with $10 \mu\text{g/mL}$ Fungin, $250 \mu\text{g/mL}$ Penicillin
143 and $250 \mu\text{g/mL}$ Streptomycin. We added these antibiotics at the start of all bioassays, at the
144 start of the evolution experiment, and at every medium replacement during the evolution exper-
145 iment (three times per week). At the beginning of the evolution experiment, we cryopreserved
146 the ancestor genotypes in liquid nitrogen and later revived them for bioassays (following the

147 protocol described by Cassidy-Hanley, 2012). Ancestors are from here on referred to as ANC.
148 During the experiment, we maintained cultures at 30 °C, on a shaker rotating at 150 rpm.

149 **Evolution experiment**

150 We prepared 32 50 mL Falcon® tubes containing 20 mL of SSP medium with antibiotics. For
151 each of the four genotypes, we inoculated eight tubes with 100 µL of high-density *T. ther-*
152 *mophila* culture and let them grow for three days to ensure that populations were well estab-
153 lished before starting the evolution experiment. After these three days, we divided the eight
154 replicates of each genotype into two groups, a low pH treatment (from here on abbreviated
155 as LpH) and a neutral pH treatment (hereafter called NpH). At day one of the experiment,
156 we removed 10 mL of culture from all 32 replicate populations and replaced it with 10 mL
157 of SSP medium with antibiotics for the NpH treatment, and with 10 mL of pH-adjusted SSP
158 medium with antibiotics for the LpH treatment. The pH of the pH-adjusted medium used for
159 these 10 mL replacements was prepared by adding 1 M HCl solution to the medium until a pH
160 of 4.5 was reached (1.6 mL of 1 M HCl per 100 mL of SSP medium, for the relationship be-
161 tween added HCl and pH, see Supporting Information section S1). We repeated this regime of
162 medium removal and replacement on every first, third and fifth day of the week for a total of six
163 weeks. Consequently, the pH of the medium for LpH populations was gradually reduced over
164 a period of two weeks, after which it was kept approximately stable at 4.5 for the remainder of
165 the experiment.

166 **Genotype revival and common garden conditions**

167 In order to perform all population growth assays of evolved (LpH and NpH) and ancestral
168 (ANC) populations at the same time, we revived the ancestor populations from liquid nitrogen
169 storage. We transferred revived cells to SSP-medium with antibiotics for recovery. We then
170 prepared a common garden treatment. We inoculated common garden cultures for the LpH,
171 NpH and ANC populations (50 mL Falcon® tubes with 20 mL of SSP medium with antibiotics)
172 with 100 µL culture and transferred them to a shaker for 72 h, in order to control for potential
173 plastic or parental effects. This should ensure that any observed phenotypic changes are the

174 result of either de novo mutations, or of highly stable epigenetic effects.

175 **Population growth assessment**

176 After culturing all populations in the same environment (common garden), we assessed popula-
177 tion growth at low pH (pH 4.5) and neutral pH (pH 6.5) of the assay medium for the ANC (four
178 genotypes, each replicated four times per assay medium pH treatment), and evolved (LpH and
179 NpH) populations (29 surviving populations per assay medium pH treatment) for a total of 90
180 cultures. We placed these cultures in an incubator, and grew them for seven days. Most popula-
181 tions reached equilibrium density well before the end of these seven days (between 20 and 100
182 hours after populations started growing; see also section S10 in the Supporting information),
183 which allows us to obtain precise measurements of growth rates and population equilibrium
184 densities.

185 **Data collection and video analysis**

186 We sampled populations both during the evolution experiment and during the population
187 growth assessments, to quantify (i) population density during evolution, (ii) intrinsic rates of
188 increase (r_0), and (iii) intraspecific competition coefficients (α) for the ANC, LpH and NpH
189 populations. These r_0 and α estimates were obtained through fitting of a population growth
190 model, as described below in the section "Population growth model fitting". During the evo-
191 lution experiment, we sampled three times per week prior to medium replacement. For the
192 population growth rate assessments of the evolved and ancestral populations, we sampled a
193 total of 10 time-points over a course of the seven days, with more frequent sampling early in
194 the growth phase (four times over two days) to adequately capture the population dynamics.
195 For sampling and analysis, we followed a previously established method of video analysis to
196 extract information on cell density and morphology of our evolved and ancestral populations,
197 using the BEMOVI R package (Pennekamp et al., 2015).

198 Our population sampling method is adapted from well-established protocols (Fronhofer
199 and Altermatt, 2015; Fronhofer et al., 2017). Briefly, 200 μ L of culture was sampled from the
200 population, and if cell density was too high for video analysis, diluted 1/10 or 1/100, because

201 excessive cell density decreases the accuracy of cell recognition during video analysis. We then
202 transferred the culture to a system of microscope slides with fixed capacity, so that a standard
203 volume (34.4 μL) of culture could be measured for all videos. Next, we took a 20 s video
204 at 25 fps (total of 500 frames) using a Leica M165FC stereomicroscope with top-mounted
205 Hamamatsu Orca Flash 4.0 camera. We analyzed our videos using the BEMOVI R package
206 (Pennekamp et al., 2015) to extract the relevant information. Parameters used for video analysis
207 can be found in the Supporting Information (section S3).

208 **Statistical analyses**

209 All statistical analyses were performed using the R statistical software (version 3.5.1) with the
210 ‘rstan’ (version 2.18.2) and ‘rethinking’ (version 1.5.2) packages (McElreath, 2015).

211 **Population growth model fitting**

212 In order to analyze population growth dynamics of ancestral and evolved populations, we fit
213 a continuous-time version of the Beverton-Holt population growth model (Beverton and Holt,
214 1993). As recently discussed by Fronhofer et al. (2018, see also chapter 5 in Thieme 2003),
215 using this model provides a better fit to microcosm data compared to less mechanistic models
216 (for example an r - K population growth model, which captures the density-regulation of mi-
217 crocosms less well) and readily allows for a biological interpretation of its parameters. The
218 Beverton-Holt model is given by the equation

$$\frac{dN}{dt} = \left(\frac{r_0 + d}{1 + \alpha N} - d \right) N \quad (1)$$

219 with the intraspecific competitive ability (α) being

$$\alpha = \frac{r_0}{Kd} \quad (2)$$

220 Here, N corresponds to population size, r_0 corresponds to the intrinsic rate of increase, α to the
221 intraspecific competitive ability (hereafter referred to as competitive ability), and d to the death
222 rate of individuals in the population. The K parameter in equation (2) represents the equilib-

223 rium population density. We adapted Bayesian statistical models from Rosenbaum et al. (2019)
224 to estimate parameter values for r_0 , α , d , and K using the rstan package and trajectory match-
225 ing, that is, assuming pure observation error (see <https://zenodo.org/record/2658131> for
226 code). We chose vaguely informative priors, that is, we provided realistic mean estimates, but
227 set standard deviation broad enough to not constrain the model too strongly, for the logarithmi-
228 cally (base e) transformed parameters with $\ln(r_0) \sim normal(-2.3, 1)$, $\ln(d) \sim normal(-2.3, 1)$
229 and $\ln(K) \sim normal(13.1, 1)$.

230 **Analysis of parameter estimates r_0 , α , and K**

231 In a next step, we analyzed the population growth parameter estimates to determine how our
232 experimental treatments affected them. As intrinsic rates of increase (r_0) integrate birth and
233 death rates and are more reliably estimated than its components (narrower posterior distribu-
234 tions), we here focussed on intrinsic rates of increase and excluded the death rate from further
235 analyses (see also Tab. S10 for summarized posteriors).

236 To analyse the parameter estimates (r_0 , α , and K), we constructed separate linear models
237 for each genotype, and fit logarithmically (\ln) transformed parameters r_0 , α and K as a function
238 of a) the pH of the assay medium, b) general evolution across pH treatments, that is, difference
239 between ANC populations, on the one hand, and evolved populations, on the other hand, c)
240 evolution to specific pH treatments (that is, differences between ANC, LpH and NpH) and d)
241 interactions between pH of the medium and evolutionary changes. This resulted in 16 statistical
242 models for each of the response variables and each of the four genotypes (see Tab. S3 in
243 Supporting Information section S7 for details). Information on priors can be found in the
244 Supporting Information (section S4). Following McElreath (2015, chapter 14), we did not only
245 use our mean parameter estimates, but took their uncertainty into account by modelling both
246 means and errors of the parameters obtained during Beverton-Holt model fitting.

247 We then compared the models using the deviance information criterion (DIC), a Bayesian
248 implementation of the Akaike information criterion (Gelman et al., 2014) and averaged the
249 posterior predictions of the 16 models based on DIC weights. Next, we calculated the relative
250 importance (RI) of the explanatory variables by summing for each explanatory variable the

251 respective model weights in which this variable is included.

252 **Correlation between r_0 and α**

253 In order to detect potential correlations between intrinsic rate of increase (r_0) and competitive
254 ability (α), we performed a Bayesian correlation analysis using the logarithmically transformed
255 estimates of r_0 and α and fitting a multivariate normal distribution. We again used both mean
256 estimates and their errors to account for errors caused by population growth model fitting.
257 To account for plastic effects associated with the pH of the assay medium, we performed the
258 correlation analysis separately for low pH and neutral pH of the assay medium, while pooling
259 the data for all four genotypes and treatments (ANC, LpH, and NpH). Pertinent computer code
260 can be found in the Supporting Information (section S5).

261 **Variation in life-history traits**

262 We asked whether evolutionary history altered between-genotype variation in life-history traits
263 (r_0 , α and K) at low and neutral pH of the assay medium. We first calculated for each group
264 (ANC, LpH and NpH) the mean of the natural logarithm of r_0 , α and K over all 4 genotypes,
265 and subsequently calculated the absolute difference between this mean and the observed trait
266 values (r_0 , α and K) of all replicate populations (logarithmically transformed). We then used
267 Bayesian models to calculate whether these differences varied between the treatments (Evolved
268 (general evolutionary change, difference between ANC and all evolved lines), LpH and NpH).
269 To account for potential genotype effects, we also included both models with and without
270 random effects per genotype (random genotype intercepts), leading to a total of 6 models per
271 trait, as shown in Tab. S4 in the Supporting Information section S7. After fitting the models, we
272 compared the models using the Watanabe-Akaike information criterion (WAIC), a generalized
273 form of the Akaike information criterion used for comparison of Bayesian statistical models
274 (Gelman et al., 2014). We then calculated relative parameter importance using WAIC weights.

275 **Density-dependent fitness calculation**

276 To assess how the observed convergence in life-history strategy might have arisen, we calcu-
277 lated the population growth rate (r) for the LpH and for ANC populations over all observed
278 population densities during the evolution experiment and integrated over these values to calcu-
279 late a weighted density-dependent fitness estimate. We then used Bayesian models to fit these
280 density-dependent fitness values as a function of a) population origin (ANC or LpH), b) cen-
281 tered intrinsic rate of increase (r_0), and c) an interaction term between r_0 and population origin.
282 Centered r_0 represents the intrinsic rate of increase, rescaled to have its mean at zero, and was
283 calculated by subtracting the mean r_0 from all r_0 values. In this analysis, we also included a
284 random intercept for the different genotypes (details in Tab. S5 in section S7 of the Supporting
285 Information). We fit all five models, starting from the intercept model to the full interaction
286 model. Subsequently, we ranked these models using the WAIC criterion and calculated the
287 relative importance of all explanatory variables based on WAIC weights. The corresponding
288 analysis for the NpH populations can be found in Supporting information section S9.

289 **Results**

290 We subjected replicate populations of four different genotypes to either low pH (LpH) condi-
291 tions or neutral pH conditions (NpH), while keeping population densities high over the course
292 of the evolution experiment. Fig. 1 shows the population densities as observed during the
293 experiment. We then tested whether and how evolution changed life-history strategies in all
294 four different genetic backgrounds. Fig. 2 shows the data and model predictions for changes
295 in life-history traits. Next, we tested how life-history traits were correlated and how this may
296 have constrained evolutionary changes. The correlation in life-history traits is depicted in Fig.
297 3. We then tested for changes in variation of life-history strategy between populations (shown
298 in Fig. 4). Lastly, we tested how evolution of life-history strategies affected density-dependent
299 fitness under the observed densities during the evolution experiment. Fig. 5 shows data and
300 model predictions of density-dependent fitness under low pH conditions.

301 Evolution of life-history traits

302 During the 42 days of the evolution experiment, population densities ranged from approxi-
303 mately 1×10^3 cells/mL to 2×10^6 cells/mL (see Fig. 1) and fluctuated around the population
304 equilibrium density due to stochastic variation in death and division rates. Observed densities
305 varied strongly depending on treatment and genetic background. Out of 32 evolving popula-
306 tions, three went extinct during the experiment, all in the low pH treatment (one population
307 each for genotype 1, 2 and 3).

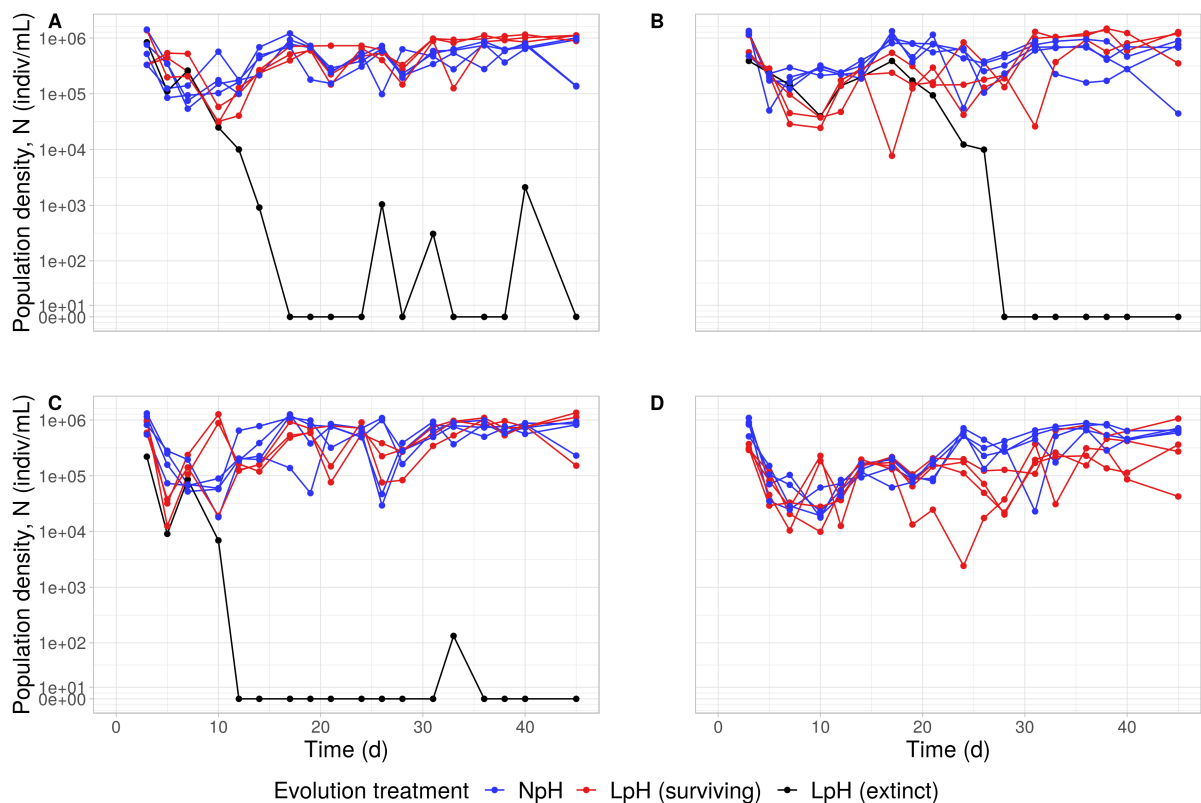


Figure 1: Density dynamics of the replicate populations over the course of the evolution experiment. The y-axis shows the population density (axis is pseudo-logarithmically transformed, to account for 0 values in the dataset), the x-axis the time since the beginning of the experiment in days. Each set of dots connected by a line represents data from a single replicate population. Red and blue symbols correspond to data from populations that survived to the end of the experiment from the LpH (populations evolved under low pH conditions) and NpH (populations evolved under neutral pH conditions) treatments, respectively. Black symbols correspond to data from LpH populations that went extinct. Panel A shows the density dynamics for genotype 1, panel B for genotype 2, panel C for genotype 3 and panel D for genotype 4.

308 After the experimental evolution phase, we found that all four genotypes showed strong
309 plastic effects associated with the pH of the assay medium (see also Tab. S6 in the Supporting

310 Information section S8). Low pH of the assay medium consistently decreased intrinsic rate of
311 increase (r_0), led to lower competitive ability (α), and, as a consequence of this decrease in
312 α , to increased equilibrium population densities (K) as shown in Fig. 2. This effect of low
313 pH was especially pronounced for r_0 and α , where the relative importance values associated
314 with pH of the medium were typically close to one for all four genotypes (see also Tab. S6
315 in the Supporting Information section S8). The effect of low pH was less pronounced for the
316 equilibrium population density (K), specifically for genotype 2.

317 We additionally found signatures of evolutionary change. These were less consistent than
318 the plastic effects, that is, they differed between the genotypes. Evolution led to an increase
319 in r_0 for genotypes 2 and 4 (Fig. 2B,D). However, for genotype 2 this increase only occurred
320 in the LpH populations. For genotype 4 we mostly observed a general change in all evolving
321 populations and only to a lesser degree specific changes in the LpH and NpH treatments.

322 LpH led to increased equilibrium population density (K) for genotype 1 and genotype 4
323 (Fig. 2E, H), and a decreased equilibrium population density (K) for genotype 3 (Fig. 2G). As
324 equilibrium density is an emergent trait, the changes in K were driven both by the changes in r_0
325 described above and by changes in α . Evolution led to lower competitive ability (α) for LpH
326 genotype 1 populations (Fig. 2I), to increased competitive ability (α) for evolved genotype 2
327 populations (Fig. 2J), to no clear change for genotype 3 (Fig. 2K), and to increased competitive
328 ability (α) for evolved and especially NpH for genotype 4 populations (Fig. 2L). Overall, we
329 detected evolutionary changes in all traits (r_0 , α and K), although direction and strength of
330 change strongly differed between genotypes.

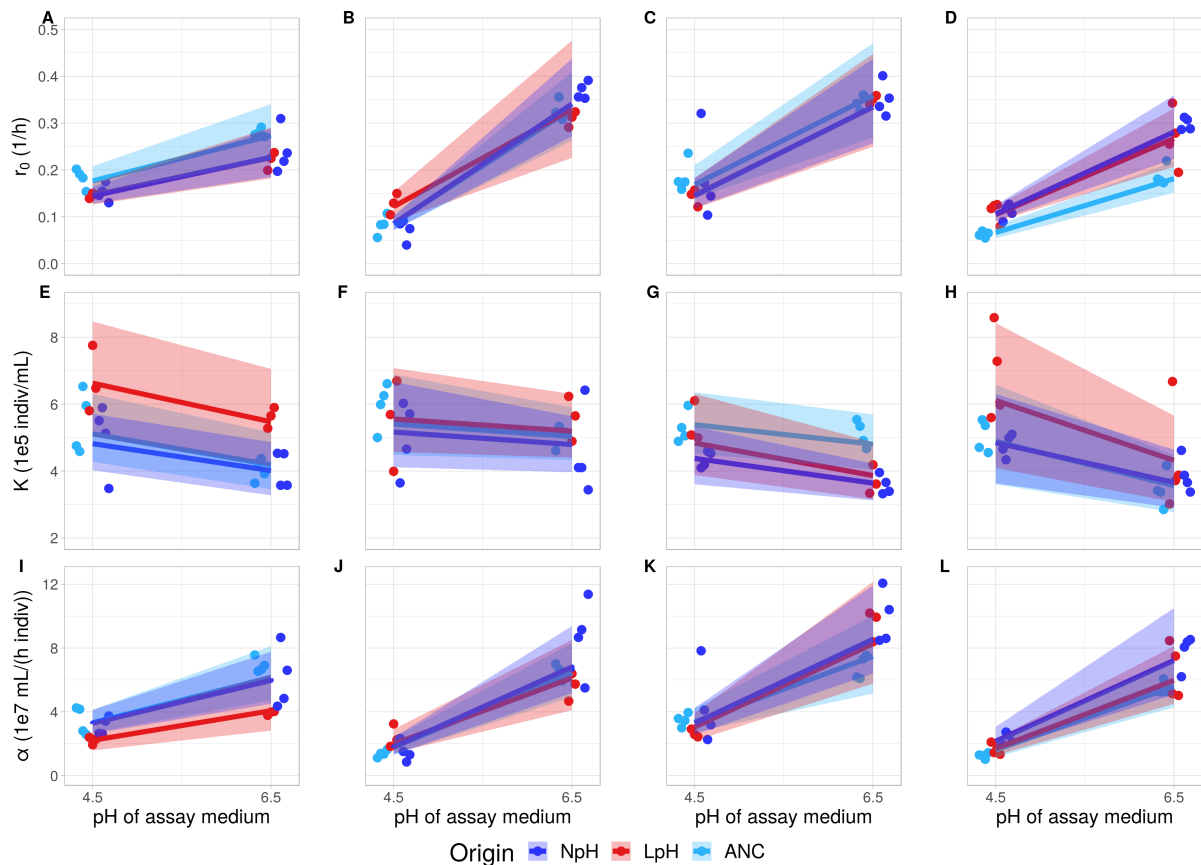


Figure 2: Evolutionary trends in intrinsic rate of increase (r_0 ; A-D), equilibrium population density (K ; E-H) and competitive ability (α ; I-L) for the 4 different genotypes. Each circle represents an estimate of r_0 , K or α (posterior means) from the Beverton-Holt model for one replicate population. Lines and shaded areas represent the averaged posterior model predictions based on DIC weights (means and 95 % probability interval). Light blue = ANC (ancestor populations), dark blue = NpH (populations evolved under neutral pH conditions), red = LpH (populations evolved under low pH conditions).

331 **Variation and covariation in r_0 and α**

332 The intrinsic rate of increase (r_0) and competitive ability (α) were positively correlated both at
 333 low pH and neutral pH of the assay medium (Fig. 3). However, the correlation was markedly
 334 stronger at low pH ($R^2 = 0.95$) than at neutral pH ($R^2 = 0.61$). Variation in these two quantities
 335 was also larger at low pH compared to neutral pH (Fig. 3).

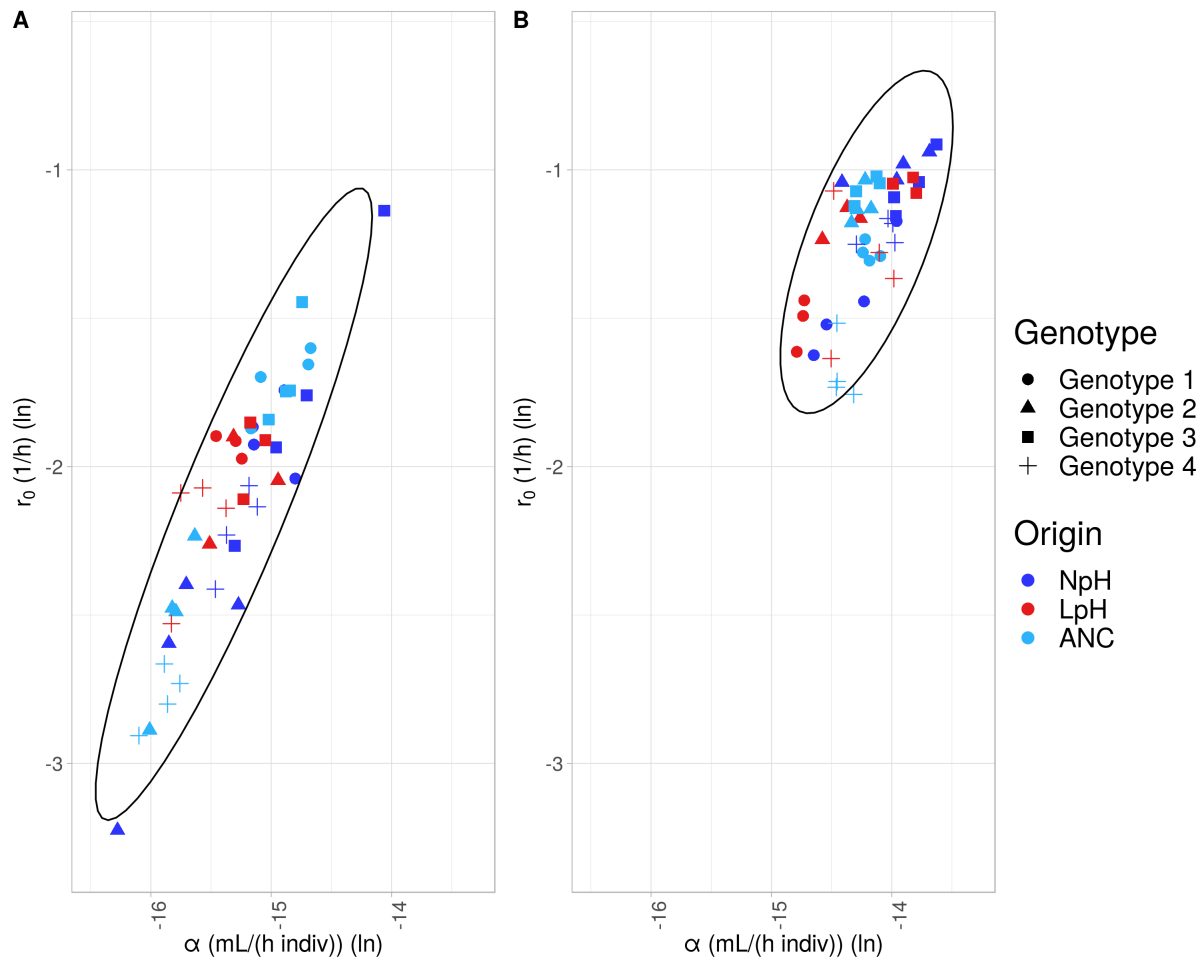


Figure 3: Correlation between the intrinsic rate of increase (r_0) and competitive ability (α) at low pH (A), and at neutral pH (B) of the assay medium. Symbols represent the different genotypes (see legend); Light blue = ANC (ancestor populations), dark blue = NpH (populations evolved under neutral pH conditions), red = LpH (populations evolved under low pH conditions). Ellipses represents 95% probability intervals.

336 At a low pH of the assay medium, r_0 and α showed lower variation for the LpH popula-
 337 tions compared to the ANC and NpH populations (Fig. 4 panels A-B and I-J; see also Tab. S7
 338 in Supporting Information section S8). We did not detect differences in terms of equilibrium
 339 population density (K). At a neutral pH of the assay medium, we did not detect differences in
 340 variation for the intrinsic rate of increase (r_0), slightly more variation in equilibrium population
 341 density (K), and strongly higher variation in competitive ability (α) of both the LpH and NpH
 342 populations compared to the ANC. Note that despite the high relative importance of the evolu-
 343 tion variables (Evolved (general evolutionary change), LpH and NpH) for r_0 at neutral pH, the
 344 effect size associated with these variables was close to zero. The high relative importance stems
 345 from the differences in how the different genotypes responded to the pH treatments, which was

346 captured in the random effects (Fig. 4 panels C-D and K-L). In summary, we found a correla-
 347 tion between r_0 and α both at low and neutral pH and found that LpH populations converged
 348 in life-history strategy, in the sense that LpH populations became more similar in life-history
 349 strategy compared to the ANC populations.

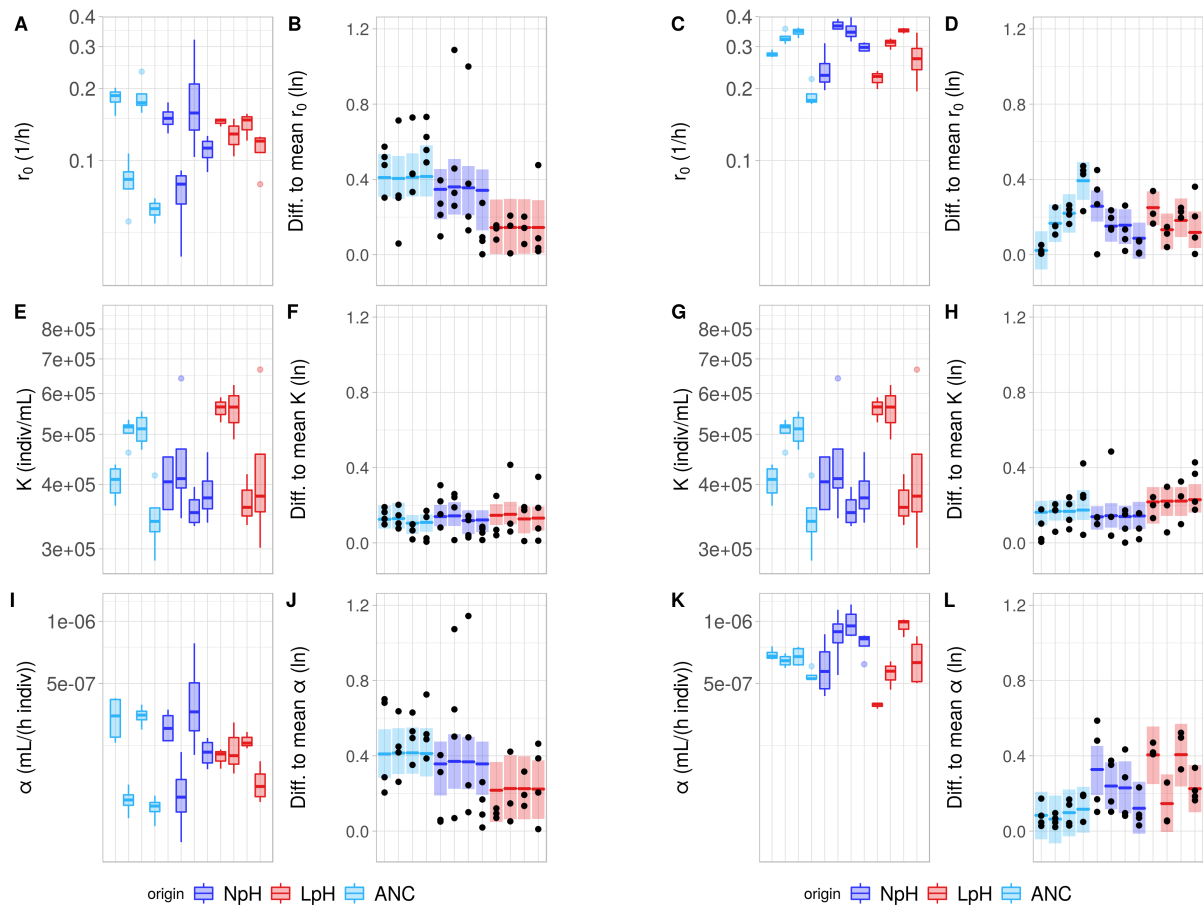


Figure 4: Left half of the figure (panels A,B,E,F,I,J) shows data for growth curves measured at low pH of the assay medium, right half (panels C,D,G,H,K,L) for growth curves measured at neutral pH of the assay medium. Traits shown are intrinsic rate of increase (r_0 ; A-D), carrying capacity (K ; E-H) and competitive ability (α ; I-L). Panels A, C, E, G, I and K show r_0 , K and α estimates (1 box plot is 1 genotype). Panels B, D, F, H, J and L show averaged model predictions (mean and 95 % probability interval) of difference between r_0 , K and α estimates and mean per treatment (ANC, LpH or NpH; boxes) and individual datapoints (black dots). Light blue = ANC (ancestor populations), dark blue = NpH (populations evolved under neutral pH conditions), red = LpH (populations evolved under low pH conditions).

350 **Density-dependent fitness**

351 While the evolutionary shifts of the individual population growth parameters were highly vari-
352 able as described above, we found that under low pH of the assay medium these different
353 changes led to an increase in the overall density-dependent fitness of the LpH populations
354 compared to the ANC population (see also Tab. S8 in the Supporting Information section S8).
355 No such increase in density-dependent fitness was observed for the NpH population compared
356 to the ANC populations (see also Supporting information section S9). In both the ANC and
357 LpH populations, density-dependent fitness increased with the intrinsic rate of increase (r_0).
358 The smaller range of r_0 - and α -values for the LpH population (Fig. 5 C and Fig. 4 panels A,B
359 and I,J) shows the convergence of r_0 discussed above. As exemplified in Fig. 5A-B, density-
360 dependent fitness can increase whether r_0 increases or decreases due to correlated changes in
361 competitive ability α . In ancestral populations where the intrinsic rate of increase (r_0) was
362 initially high (Fig. 5A), competitive ability (α) was also high due to the strong correlation be-
363 tween α and r_0 . Consequently density regulation acted strongly in these populations, leading to
364 very slow population growth (r) under high density conditions. Given that densities were typi-
365 cally high during the evolution experiment (Fig. 1; Fig. 5A), lowering r_0 allowed for increased
366 growth at higher densities and hence an increase in density-dependent fitness. If r_0 was initially
367 very low (Fig. 5B), density regulation did not act very strongly, because competitive ability (α)
368 was also very low, and as a population's intrinsic rate of increase (r_0) became higher, the pop-
369 ulation's fitness increased for all density values, leading to an increase in density-dependent
370 fitness as well. In essence, we found that the observed convergence in life-history traits led to
371 an average increase in density-dependent fitness at low pH for the LpH populations.

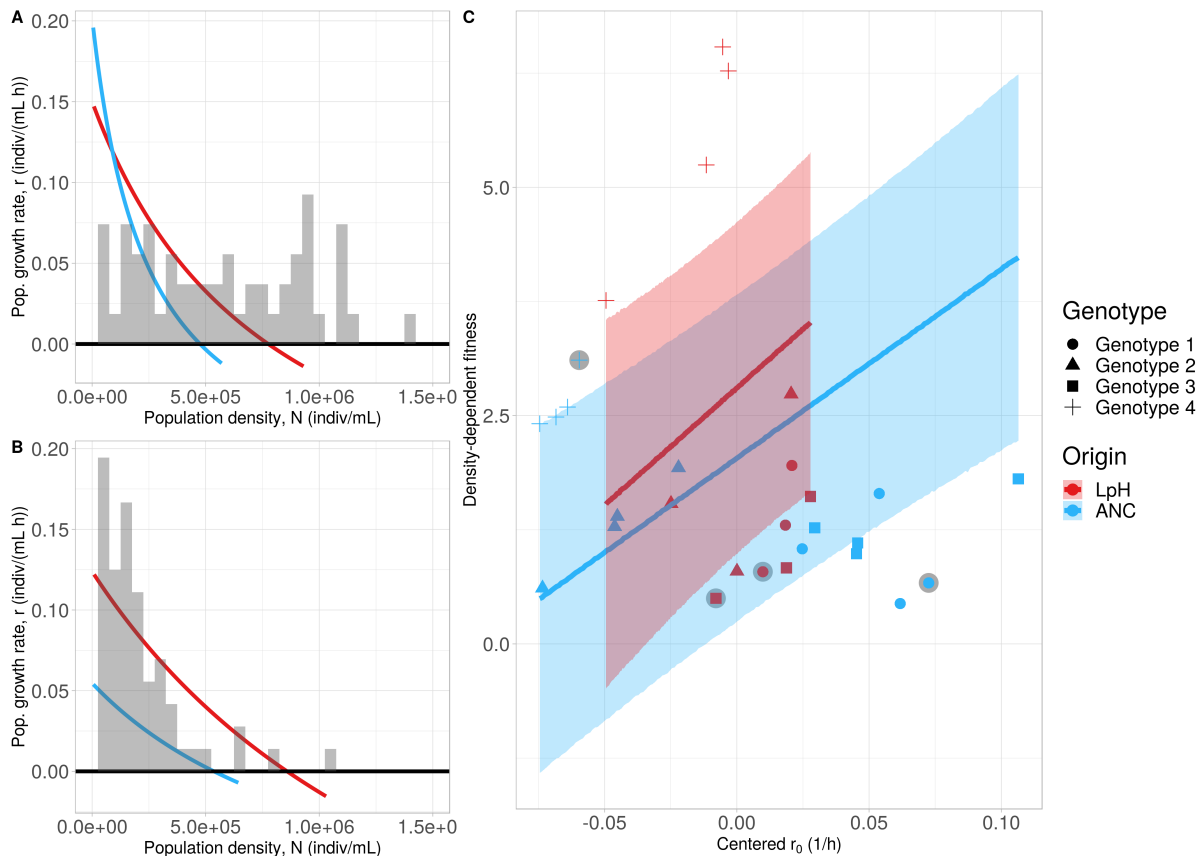


Figure 5: Density regulation functions for selected populations where the intrinsic rate of increase (r_0) evolved to decrease (panel A – genotype 1) or increase (panel B – genotype 4) in LpH populations (populations evolved under low pH conditions). Light blue lines show the density regulation functions for the ANC populations (ancestral populations), red lines for the LpH populations. Grey bars show a histogram of the observed population densities during the evolution experiment for the corresponding genotype. C) Density-dependent fitness depending on the (centered) intrinsic rate of increase (r_0). Symbols correspond to data from LpH (red) or ANC (blue) populations (shape represents genotype, see legend). Symbols surrounded by a grey disc represent the example populations of panels A-B. Lines and shaded areas represent the weighted posterior predictions and the 95% probability intervals for the four genotypes. A visual representation of the density regulation function of all replicate populations can be found in the Supporting Information Fig. S4 in section S6.

372 Discussion

373 In this experiment, we investigated the evolutionary response of the model protist *Tetrahymena*
 374 *thermophila* to pH stress under high population densities. Instead of maximizing the intrinsic
 375 rate of increase (r_0) we found that evolution of four different genotypes under low pH and
 376 high population density led to a convergence of life-history strategy, that is, genotypes became
 377 more similar in life-history strategy (see below). This observation stems, on the one hand, from

378 the high population density (demography) the populations experienced during our experiment,
379 and, on the other hand, from the genetic architecture of life-history traits, where we found that
380 intrinsic rate of increase (r_0) and competitive ability (α) were positively correlated, especially
381 under stressful conditions.

382 Evolution can help populations adapt to changing environments (Kawecki and Ebert, 2004).
383 Depending on the rate and severity of such change, populations need to respond quickly, as
384 they may otherwise be driven to extinction. Past experiments have demonstrated that evolution
385 can lead to adaptation to an abiotic stressor within few generations (Bell and Gonzalez, 2011;
386 Padfield et al., 2016; Harmand et al., 2018). However, evidence from experimental evolution
387 of such adaptation to pH stress remains relatively limited in many species, and is still more
388 commonly studied using comparative work (Reusch and Boyd, 2013; Stillman and Paganini,
389 2015, with the notable exception of bacterial evolution experiments, as discussed above). Our
390 results show that populations of the freshwater protist *T. thermophila* can adapt to such stress,
391 even under conditions of strong competition due to high population densities.

392 Whereas our finding that evolution can alter population performance under abiotic stress
393 agrees with the existing literature (Leimu and Fischer, 2008; Fraser et al., 2011; Kelly and
394 Hofmann, 2013), our results on the direction of evolution were less expected. Specifically, the
395 observed evolutionary changes in the intrinsic rates of increase (r_0 , Fig. 2) showed opposite
396 directions depending on the genetic background. Many evolution experiments are conducted
397 by serially transferring populations into fresh medium (for examples see Lenski and Travisano,
398 1994; Bell and Gonzalez, 2011; Bono et al., 2017). In such experiments, population densities
399 are low during much of the period of evolution, or at least a distinct phase of selection hap-
400 pens under low density conditions. Under these demographic condition, selection mainly acts
401 on the intrinsic rate of increase (r_0) to maximize fitness (Mueller and Ayala, 1981). In con-
402 trast, although we use a similar approach of propagating our populations in this experiment,
403 population densities were kept much higher (always above 50 % of population equilibrium
404 density), leading to strongly different demographic conditions. A growing body of work on
405 eco-evolutionary dynamics and feedbacks (Pelletier et al., 2009; Hendry, 2016) shows that it
406 is important to consider the ecological context, here, the demographic conditions, under which

407 evolution occurs.

408 This ecological context may affect how selection acts and thus alter evolutionary trajecto-
409 ries. Our results show that when populations evolve under high population densities, we do not
410 find generally increased intrinsic rates of increase (r_0). We suggest that this pattern is driven by
411 the combination of genetic architecture, that is, the linkage between intrinsic rate of increase
412 (r_0) and competitive ability (α), constraining evolutionary trajectories (Fig. 3), and by selection
413 for maximizing fitness under pH stress (abiotic conditions) and high population density (biotic
414 factor). Firstly, evolution is constrained in the sense that the intrinsic rate of increase (r_0) is
415 positively correlated with competitive ability (α ; see also Mueller and Ayala, 1981; Reznick
416 et al., 2002; Fronhofer et al., 2018, for a different view see Joshi et al. 2001). This implies
417 that fast growing genotypes will compete more strongly within the population than slow grow-
418 ing genotypes for available resources when densities increase, which is expected to slow down
419 population growth rate at higher densities.

420 This slowdown in population growth rate (r) can clearly be seen in Fig. 5 (and Fig. S4 in the
421 Supporting Information section S6), where genotypes that show initially a high intrinsic rate
422 of increase (r_0 ; high intercept) also show a strong density-dependent decrease in population
423 growth rate (strong curvature). In contrast, populations with lower r_0 show less steep declines
424 in population growth rate. Secondly, since stress associated with low pH strongly decreased
425 population growth rates, LpH populations experienced more difficulty to recover in popula-
426 tion size after each medium replacement event compared to NpH populations, and hence were
427 subject to stronger selection for increased population growth. Given that the demographic con-
428 ditions were such that populations had to grow starting from 50 % of the equilibrium population
429 density, we expect selection to lead to a maximization of population growth rate (r) under these
430 specific densities experienced during evolution, that is, a maximization of density-dependent
431 fitness (as shown in Fig. 5C).

432 Of course, populations may sometimes undergo quasi density-independent growth, for ex-
433 ample during range shifts or repeated colonization and extinction events. However, whenever
434 densities are high, growth will be density-dependent. This will often be the case in established
435 populations, which are expected to fluctuate around their equilibrium population density. For

436 example, environmental shifts (acid rain or temperature shifts, for instance) could lead to local
437 changes affecting already well-established populations. As shown in our experiment, adap-
438 tation to abiotic stress under such demographic conditions can strongly affect trajectories of
439 evolution, leading to complex evolutionary changes when populations simultaneously need
440 to adapt to abiotic and biotic stress. In addition, as in our experiment, the direction of the
441 evolutionary trajectory may depend on the starting conditions, and populations with different
442 genetic backgrounds may evolve differently. We speculate here that under these high popula-
443 tion density conditions, we can observe convergent evolution in life-history strategy, whereas
444 under low population density conditions, we may instead expect parallel evolution where all
445 populations shift their intrinsic rate of increase (r_0) upwards at low pH. The term convergent
446 evolution has however been defined multiple times (as discussed in Blount et al., 2018; Wood
447 et al., 2005; Bolnick et al., 2018). We here follow the geometric argumentation in Bolnick
448 et al. (2018). We thus define and will use the following terminology to describe evolutionary
449 responses as follows: 1) Convergent evolution occurs when different populations develop more
450 similar phenotypes during evolution, 2) divergent evolution implies that different populations
451 develop more distinct phenotypes during evolution) and 3) parallel evolution occurs when dif-
452 ferent populations undergo phenotypic changes in the same direction during evolution. We
453 should however also note that our results suggest that within genotypes, evolution happened in
454 parallel, as all replicate populations underwent directional evolution towards either increased or
455 decreased intrinsic rate of increase (r_0), although over all genotypes, we observed convergence
456 to a strategy that optimized the density-dependent fitness of populations.

457 In agreement with our observation that evolution in response to low pH may be variable,
458 recent work has found no clear consensus on the effect of acidification on species growth rates
459 (Kelly and Hofmann, 2013; Gattuso and Hansson, 2011, chapter 6-7). Also, shorter-term eco-
460 logical experiments, despite showing a clear positive effect on photosynthesis, found that dif-
461 ferent species showed strongly differing changes in growth rates to acidification (Gattuso and
462 Hansson, 2011, chapter 6). Similarly, longer-term evolution experiments have demonstrated
463 that intrinsic rate of increase can either increase (Lohbeck et al., 2012; Schlüter et al., 2014) or
464 not (Collins and Bell, 2004) for populations evolved under conditions of increased CO₂. On

465 a speculative note, our experiment suggests that demographic conditions may be a potential
466 explanatory factor for such divergent results. Taking into account the demographic context and
467 other potentially confounding eco-evolutionary interactions may help to clarify these factors in
468 future work.

469 In conclusion, we found that demography affected adaptation to low pH in the protist *T.*
470 *thermophila*, leading to a convergence in life-history strategies and increased high-density fit-
471 ness. Our work shows that taking into account demography may be key to understanding
472 evolutionary trajectories. In an eco-evolutionary context, quantifying density-regulation func-
473 tions, that is, population growth rates as a function of population density, may be a useful way
474 forward. Furthermore, although we observe convergent evolution in life-history strategy on
475 a phenotypic level, it remains unclear whether this evolution is also convergent on a genetic
476 level. As noted by Wood et al. (2005), when the genetic basis of traits is simple, convergent
477 evolution often also has a genetic basis, but when the genetic basis is more complex, there are
478 typically multiple paths available leading to similar phenotypic changes. An interesting avenue
479 for future research could be to further study how the observed trade-off between intrinsic rate
480 of increase (r_0) and intraspecific competitive ability (α) translate to the genetic level, as we
481 see a clear trade-off between these traits, that seems phenotypically rather constrained. If such
482 a trade-off also exists on a genetic level, understanding this link may yield new expectations
483 concerning convergent and parallel evolution of populations, both in presence and absence of
484 abiotic and biotic stress.

485 **Acknowledgements**

486 The authors thank Samuel Hürlemann for help with laboratory work. Funding is from the URPP
487 Evolution in Action and the Swiss National Science Foundation, Grant No PP00P3_179089.
488 This is publication ISEM-YYYY-XXX of the Institut des Sciences de l'Evolution – Montpel-
489 lier. We would also like to acknowledge support by Swiss National Science Foundation grant
490 31003A_172887 and by European Research Council Advanced Grant No. 739874. We would
491 like to thank the editorial staff of Evolution and the two referees for the helpful and constructive

492 comments on a previous version of this manuscript.

493 **Author contributions**

494 FM, FA and EAF designed the experiment. FM, AA and SM performed the experimental work.
495 Statistical analyses were done by FM and EAF. FM, FA, AW and EAF interpreted the results.
496 FM, FA and EAF wrote the first version of the manuscript and all authors commented the final
497 version.

498 **References**

- 499 Altermatt, F.; Fronhofer, E. A.; Garnier, A.; Giometto, A.; Hammes, F.; Klecka, J.; Legrand,
500 D.; Mächler, E.; Massie, T. M.; Pennekamp, F.; Plebani, M.; Pontarp, M.; Schtickzelle,
501 N.; Thuillier, V.; and Petchey, O. L. 2015: Big answers from small worlds: a user's guide
502 for protist microcosms as a model system in ecology and evolution. *Methods Ecol. Evol.*
503 6(2):218–231.
- 504 Andrews, J. H. and Rouse, D. I. 1982: Plant Pathogens and the Theory of *r*- and *K*-Selection.
505 *Am. Nat.* 120(3):283–296.
- 506 Angilletta, M. J. 2009: *Thermal Adaptation: A Theoretical and Empirical Synthesis*. OUP
507 Oxford.
- 508 Bell, G. and Gonzalez, A. 2011: Adaptation and Evolutionary Rescue in Metapopulations
509 Experiencing Environmental Deterioration. *Science* 332(6035):1327–1330.
- 510 Beverton, R. and Holt, S. 1993: *On the Dynamics of Exploited Fish Populations*. Springer.
- 511 Bijlsma, R. and Loeschcke, V. 2005: Environmental stress, adaptation and evolution: an
512 overview. *J. Evol. Biol.* 18(4):744–749.
- 513 Blount, Z. D.; Lenski, R. E.; and Losos, J. B. 2018: Contingency and determinism in evolution:
514 Replaying life's tape. *Science* 362(6415):eaam5979.

- 515 Bolnick, D. I.; Barrett, R. D. H.; Oke, K. B.; Rennison, D. J.; and Stuart, Y. E. 2018:
516 (Non)Parallel Evolution. *Annual Review of Ecology, Evolution, and Systematics*, Vol 49,
517 (edited by D. J. Futuyma), volume 49, pages 303–330. Annual Reviews, Palo Alto.
- 518 Bono, L. M.; Smith, L. B.; Pfennig, D. W.; and Burch, C. L. 2017: The emergence of perfor-
519 mance trade-offs during local adaptation: insights from experimental evolution. *BMC Mol.*
520 *Biol.* 26(7):1720–1733.
- 521 Bridle, J. R. and Vines, T. H. 2007: Limits to evolution at range margins: when and why does
522 adaptation fail? *Trends Ecol. Evol.* 22(3):140–147.
- 523 Brito, P. H.; Guilherme, E.; Soares, H.; and Gordo, I. 2010: Mutation accumulation in *Tetrahy-*
524 *mena*. *BMC Evol. Biol.* 10:354.
- 525 Burns, D. A.; Aherne, J.; Gay, D. A.; and Lehmann, C. M. B. 2016: Acid rain and its environ-
526 mental effects: Recent scientific advances. *Atmos. Environ.* 146:1–4.
- 527 Caldeira, K. and Wickett, M. E. 2003: Anthropogenic carbon and ocean pH. *Nature*
528 425(6956):365.
- 529 Cassidy-Hanley, D. M. 2012: *Tetrahymena* in the Laboratory: Strain Resources, Methods for
530 Culture, Maintenance, and Storage. *Methods in Cell Biology*, volume 109, pages 237–276.
531 Elsevier.
- 532 Clobert, J.; Baguette, M.; Benton, T. G.; and Bullock, J. M. 2012: *Dispersal Ecology and*
533 *Evolution*. Oxford University Press, USA, Oxford, first edition edition.
- 534 Clobert, J.; Danchin, E.; Dhondt, A. A.; and Nichols, J. D. 2001: *Dispersal*. Oxford University
535 Press, Oxford; New York.
- 536 Collins, K. 2012: *Tetrahymena Thermophila*. Academic Press.
- 537 Collins, S. and Bell, G. 2004: Phenotypic consequences of 1,000 generations of selection at
538 elevated CO₂ in a green alga. *Nature* 431(7008):566.

- 539 Coyne, R. S.; Stovert, N. A.; and Miao, W. 2012: Whole Genome Studies of *Tetrahymena*.
540 *Tetrahymena Thermophila*, (edited by K. Collins), volume 109, pages 53–81. Elsevier Aca-
541 demic Press Inc, San Diego.
- 542 Derry, A. M. and Arnott, S. E. 2007: Adaptive Reversals in Acid Tolerance in Copepods from
543 Lakes Recovering from Historical Stress. *Ecol. Appl.* 17(4):1116–1126.
- 544 Dunson, W. and Travis, J. 1991: The Role of Abiotic Factors in Community Organization. *Am.*
545 *Nat.* 138(5):1067–1091.
- 546 Fjerdingstad, E. J.; Schtickzelle, N.; Manhes, P.; Gutierrez, A.; and Clobert, J. 2007: Evolution
547 of dispersal and life history strategies – *Tetrahymena* ciliates. *BMC Evol. Biol.* 7(1):133.
- 548 Fletcher, E.; Feizi, A.; Bisschops, M. M. M.; Hallström, B. M.; Khoomrung, S.; Siewers, V.;
549 and Nielsen, J. 2017: Evolutionary engineering reveals divergent paths when yeast is adapted
550 to different acidic environments. *Metab. Eng.* 39:19–28.
- 551 Flowers, T. J.; Galal, H. K.; and Bromham, L. 2010: Evolution of halophytes: multiple origins
552 of salt tolerance in land plants. *Functional Plant Biol.* 37(7):604–612.
- 553 Franks, S. J. and Hoffmann, A. A. 2012: Genetics of Climate Change Adaptation. *Annual*
554 *Review of Genetics*, Vol 46, (edited by B. L. Bassler), volume 46, pages 185–208. *Annual*
555 *Reviews*, Palo Alto.
- 556 Fraser, D. J.; Weir, L. K.; Bernatchez, L.; Hansen, M. M.; and Taylor, E. B. 2011: Extent and
557 scale of local adaptation in salmonid fishes: review and meta-analysis. *Heredity* 106(3):404–
558 420.
- 559 Fronhofer, E. A. and Altermatt, F. 2015: Eco-evolutionary feedbacks during experimental range
560 expansions. *Nat. Commun.* 6:6844.
- 561 Fronhofer, E. A.; Govaert, L.; O’Connor, M. I.; Schreiber, S. J.; and Altermatt, F. 2018: The
562 shape of density dependence and the relationship between population growth, intraspecific
563 competition and equilibrium population density. bioRxiv page 485946.

- 564 Fronhofer, E. A.; Nitsche, N.; and Altermatt, F. 2017: Information use shapes the dynamics of
565 range expansions into environmental gradients. *Glob. Ecol. Biogeogr.* 26(4):400–411.
- 566 Gallet, R.; Latour, Y.; Hughes, B. S.; and Lenormand, T. 2014: The Dynamics of Niche Evolu-
567 tion Upon Abrupt Environmental Change. *Evolution* 68(5):1257–1269.
- 568 Gattuso, J.-P. and Hansson, L. 2011: *Ocean Acidification*. OUP Oxford.
- 569 Gelman, A.; Hwang, J.; and Vehtari, A. 2014: Understanding predictive information criteria
570 for Bayesian models. *Stat. Comput.* 24(6):997–1016.
- 571 Govaert, L.; Fronhofer, E. A.; Lion, S.; Eizaguirre, C.; Bonte, D.; Egas, M.; Hendry, A. P.;
572 Martins, A. D. B.; Melián, C. J.; Raeymaekers, J. A. M.; Ratikainen, I. I.; Saether, B.-E.;
573 Schweitzer, J. A.; and Matthews, B. 2019: Eco-evolutionary feedbacks—Theoretical models
574 and perspectives. *Funct. Ecol.* 33(1):13–30.
- 575 Gunde-Cimerman, N.; Oren, A.; and Plemenitaš, A. 2006: *Adaptation to Life at High Salt*
576 *Concentrations in Archaea, Bacteria, and Eukarya*. Springer Science & Business Media.
- 577 Hangartner, S.; Laurila, A.; and Räsänen, K. 2011: Adaptive divergence of the moor frog (*Rana*
578 *arvalis*) along an acidification gradient. *BMC Evol. Biol.* 11:366.
- 579 Harden, M. M.; He, A.; Creamer, K.; Clark, M. W.; Hamdallah, I.; Martinez, K. A.; Kresslein,
580 R. L.; Bush, S. P.; and Slonczewski, J. L. 2015: Acid-Adapted Strains of *Escherichia coli*
581 K-12 Obtained by Experimental Evolution. *Appl. Environ. Microbiol.* 81(6):1932–1941.
- 582 Harmand, N.; Gallet, R.; Martin, G.; and Lenormand, T. 2018: Evolution of bacteria special-
583 ization along an antibiotic dose gradient. *Evol. Lett.* 2(3):221–232.
- 584 Hendry, A. P. 2016: *Eco-evolutionary Dynamics*. Princeton University Press.
- 585 HilleRisLambers, J.; Adler, P. B.; Harpole, W. S.; Levine, J. M.; and Mayfield, M. M. 2012:
586 Rethinking Community Assembly through the Lens of Coexistence Theory. *Annual Review*
587 *of Ecology, Evolution, and Systematics*, Vol 43, (edited by D. J. Futuyma), volume 43, pages
588 227–248. Annual Reviews, Palo Alto.

- 589 Hoffmann, A. A. and Sgro, C. M. 2011: Climate change and evolutionary adaptation. *Nature*
590 470(7335):479–485.
- 591 Hughes, B. S.; Cullum, A. J.; and Bennett, A. F. 2007: Evolutionary adaptation to environmen-
592 tal pH in experimental lineages of *Escheria coli*. *Evolution* 61(7):1725–1734.
- 593 Jacob, S.; Wehi, P.; Clobert, J.; Legrand, D.; Schtickzelle, N.; Huet, M.; and Chainé, A. 2016:
594 Cooperation-mediated plasticity in dispersal and colonization. *Evolution* 70(10):2336–2345.
- 595 Johnston, I. A.; Clarke, A.; Laws, R. M.; and Franks, F. 1990: Cold adaptation in marine or-
596 ganisms. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*
597 326(1237):655–667.
- 598 Joshi, A.; Prasad, N. G.; and Shakarad, M. 2001: *K*-selection, *alpha*-selection, effectiveness,
599 and tolerance in competition: density-dependent selection revisited. *J. Genet.* 80(2):63–75.
- 600 Kawecki, T. J. and Ebert, D. 2004: Conceptual issues in local adaptation. *Ecol. Lett.*
601 7(12):1225–1241.
- 602 Kelly, M. W. and Hofmann, G. E. 2013: Adaptation and the physiology of ocean acidification.
603 *Funct. Ecol.* 27(4):980–990.
- 604 Klerks, P. L. and Weis, J. S. 1987: Genetic adaptation to heavy metals in aquatic organisms: A
605 review. *Environmental Pollution* 45(3):173–205.
- 606 Kooyers, N. J. 2015: The evolution of drought escape and avoidance in natural herbaceous
607 populations. *Plant Sci.* 234:155–162.
- 608 Leimu, R. and Fischer, M. 2008: A Meta-Analysis of Local Adaptation in Plants. *PLoS One*
609 3(12):e4010.
- 610 Lenski, R. E. and Travisano, M. 1994: Dynamics of adaptation and diversification: a
611 10,000-generation experiment with bacterial populations. *Proc. Natl. Acad. Sci. U.S.A.*
612 91(15):6808–6814.

- 613 Likens, G. E. and Bormann, F. H. 1974: Acid Rain: A Serious Regional Environmental Prob-
614 lem. *Science* 184(4142):1176–1179.
- 615 Likens, G. E.; Driscoll, C. T.; and Buso, D. C. 1996: Long-Term Effects of Acid Rain: Re-
616 sponse and Recovery of a Forest Ecosystem. *Science* 272(5259):244–246.
- 617 Lohbeck, K. T.; Riebesell, U.; and Reusch, T. B. H. 2012: Adaptive evolution of a key phyto-
618 plankton species to ocean acidification. *Nat. Geosci.* 5(5):346–351.
- 619 Luckinbill, L. S. 1978: *r* and *K* Selection in Experimental Populations of *Escherichia coli*.
620 *Science* 202(4373):1201–1203.
- 621 Lytle, D. A. and Poff, N. L. 2004: Adaptation to natural flow regimes. *Trends in Ecology &*
622 *Evolution* 19(2):94–100.
- 623 McElreath, R. 2015: *Statistical Rethinking: A Bayesian Course with Examples in R and Stan*.
624 Chapman and Hall/CRC, Boca Raton, 1st edition.
- 625 Michel, J.; Ebert, D.; and Hall, M. D. 2016: The trans-generational impact of population
626 density signals on host-parasite interactions. *BMC Evol. Biol.* 16.
- 627 Mueller, L. D. and Ayala, F. J. 1981: Trade-off between *r*-selection and *K*-selection in
628 *Drosophila* populations. *Proc. Natl. Acad. Sci. U.S.A.* 78(2):1303–1305.
- 629 Mueller, L. D.; Guo, P. Z.; and Ayala, F. J. 1991: Density-dependent natural selection and
630 trade-offs in life history traits. *Science* 253(5018):433–435.
- 631 Nørgaard, L. S.; Phillips, B. L.; and Hall, M. D. 2019: Infection in patchy populations: Con-
632 trasting pathogen invasion success and dispersal at varying times since host colonization.
633 *Evol. Lett.* 3(5):555–566.
- 634 Padfield, D.; Yvon-Durocher, G.; Buckling, A.; Jennings, S.; and Yvon-Durocher, G. 2016:
635 Rapid evolution of metabolic traits explains thermal adaptation in phytoplankton. *Ecol. Lett.*
636 19(2):133–142.

- 637 Pelletier, F.; Garant, D.; and Hendry, A. P. 2009: Eco-evolutionary dynamics. *Proc. R. Soc.*
638 *B-Biol. Sci.* 364(1523):1483–1489.
- 639 Pennekamp, F.; Schtickzelle, N.; and Petchey, O. L. 2015: BEMOVI, software for extracting
640 behavior and morphology from videos, illustrated with analyses of microbes. *Ecol. Evol.*
641 5(13):2584–2595.
- 642 Raven, J.; Caldeira, K.; Elderfield, H.; Hoegh-Guldberg, O.; Liss, P. S.; Riebesell, U.; Sheperd,
643 J.; Turley, C.; and Watson, A. 2005: Ocean Acidification due to Increasing Atmospheric
644 Carbon Dioxide. Royal Society Policy Document.
- 645 Reusch, T. B. H. and Boyd, P. W. 2013: Experimental Evolution Meets Marine Phytoplankton.
646 *Evolution* 67(7):1849–1859.
- 647 Reznick, D.; Bryant, M. J.; and Bashey, F. 2002: *r*- and *K*-selection revisited: the role of
648 population regulation in life-history evolution. *Ecology* 83(6):1509–1520.
- 649 Rosenbaum, B.; Raatz, M.; Weithoff, G.; Fussmann, G. F.; and Gaedke, U. 2019: Estimating
650 Parameters From Multiple Time Series of Population Dynamics Using Bayesian Inference.
651 *Front. Ecol. Evol.* 6:234.
- 652 Sanford, E. and Kelly, M. W. 2011: Local Adaptation in Marine Invertebrates. *Annu. Rev. Mar.*
653 *Sci.* 3(1):509–535.
- 654 Schlüter, L.; Lohbeck, K. T.; Gutowska, M. A.; Gröger, J. P.; Riebesell, U.; and Reusch,
655 T. B. H. 2014: Adaptation of a globally important coccolithophore to ocean warming and
656 acidification. *Nat. Clim. Chang.* 4(11):1024–1030.
- 657 Shaw, A. 1994: Adaptation to Metals in Widespread and Endemic Plants. *Environ. Health*
658 *Perspect.* 102:105–108.
- 659 Stearns, S. C. 1977: The Evolution of Life History Traits: A Critique of the Theory and a
660 Review of the Data. *Annu. Rev. Ecol. Syst.* 8(1):145–171.
- 661 ———. 1992: *The Evolution of Life Histories*. Oxford University Press, Oxford, New York.

- 662 Stillman, J. H. and Paganini, A. W. 2015: Biochemical adaptation to ocean acidification. *J.*
663 *Exp. Biol.* 218(12):1946–1955.
- 664 Sunday, J. M.; Calosi, P.; Dupont, S.; Munday, P. L.; Stillman, J. H.; and Reusch, T. B. H.
665 2014: Evolution in an acidifying ocean. *Trends Ecol. Evol.* 29(2):117–125.
- 666 Thieme, H. R. 2003: *Mathematics in Population Biology*. Princeton University Press.
- 667 Westeberhard, M. 1989: Phenotypic Plasticity and the Origins of Diversity. *Annu. Rev. Ecol.*
668 *Syst.* 20:249–278.
- 669 Wood, T. E.; Burke, J. M.; and Rieseberg, L. H. 2005: Parallel genotypic adaptation: when
670 evolution repeats itself. *Genetica* 123(1-2):157–170.
- 671 Zeebe, R. E.; Zachos, J. C.; Caldeira, K.; and Tyrrell, T. 2008: Carbon Emissions and Acidifi-
672 cation. *Science* 321(5885):51–52.
- 673 Zhang, J.; Wu, C.; Du, G.; and Chen, J. 2012: Enhanced acid tolerance in *Lactobacillus casei*
674 by adaptive evolution and compared stress response during acid stress. *Biotechnol. Biopro-*
675 *cess Eng.* 17(2):283–289.