

1 The evolution of competitive ability for essential resources

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3 Joey R. Bernhardt^{1*}, Pavel Kratina², Aaron Pereira¹, Manu Tamminen³, Mridul K.
4 Thomas⁴, Anita Narwani¹

5
6 ¹Aquatic Ecology Department, Eawag, Überlandstrasse 133, CH-8600 Dübendorf,
7 Switzerland

8
9 ²School of Biological and Chemical Sciences, Queen Mary University of London, Mile
10 End Road, London E1 4NS, United Kingdom

11
12 ³Department of Biology, University of Turku, Natura, University Hill, 20014 Turku,
13 Finland

14
15 ⁴Centre for Ocean Life, DTU Aqua, Technical University of Denmark, Kongens Lyngby,
16 Denmark

17
18 *Correspondence to: joanna.bernhardt@eawag.ch.

19
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22
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35 Abstract

36

37 Competition for limiting resources is among the most fundamental ecological
38 interactions and has long been considered a key driver of species coexistence and
39 biodiversity. Species' minimum resource requirements, their R^* s, are key traits that link
40 individual physiological demands to the outcome of competition. However, a major
41 question remains unanswered - to what extent are species' competitive traits able to
42 evolve in response to resource limitation? To address this knowledge gap, we
43 performed an evolution experiment in which we exposed *Chlamydomonas reinhardtii*
44 for approximately 285 generations to seven environments in chemostats which differed
45 in resource supply ratios (including nitrogen, phosphorus and light limitation) and salt
46 stress. We then grew the ancestors and descendants in common garden and quantified
47 their competitive abilities for essential resources. We investigated constraints on trait
48 evolution by testing whether changes in resource requirements for different resources
49 were correlated. Competitive abilities for phosphorus improved in all populations,
50 while competitive abilities for nitrogen and light increased in some populations and
51 decreased in others. In contrast to the common assumption that there are trade-offs
52 between competitive abilities for different resources, we found that improvements in
53 competitive ability for a resource came at no detectable cost. Instead, improvements in
54 competitive ability for multiple resources were either positively correlated or not
55 significantly correlated. Using resource competition theory, we then demonstrated that
56 rapid adaptation in competitive traits altered the predicted outcomes of competition.
57 These results highlight the need to incorporate contemporary evolutionary change into
58 predictions of competitive community dynamics over environmental gradients.

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60

61

62 Introduction

63

64 Resource limitation and competition for limiting resources are among the most
65 important drivers of population growth [1], species distributions [2,3] and biodiversity
66 [4]. Resource competition theory (RCT, [1]) predicts that a few key resource traits,
67 including the minimum resource level a population requires to maintain positive
68 population growth (R^*), determine the outcome of competition over short time scales
69 [5]. However, we still do not know how these resource traits evolve as populations
70 adapt to new environments, especially in the context of organisms competing for
71 essential resources such as light and nitrogen. This is an important gap in knowledge
72 because rapid evolution may be able to alter competitive outcomes among species
73 [6,7]. Understanding how evolutionary processes influence species' traits that are
74 relevant to coexistence is therefore critical to understanding the ecological
75 mechanisms that create and maintain biodiversity [8]. Evolutionary change in one or
76 multiple competing species can increase the likelihood of coexistence by reducing
77 differences in species' competitive abilities for a given resource (i.e. reducing 'fitness
78 differences') and by altering the identity of the resource that each species finds most
79 limiting (i.e. increasing 'niche differences') [9]. Since we do not currently understand the
80 potential constraints on the adaptation of essential resource-use traits, we cannot
81 predict the degree to which evolution contributes to or prevents competitive
82 coexistence.

83

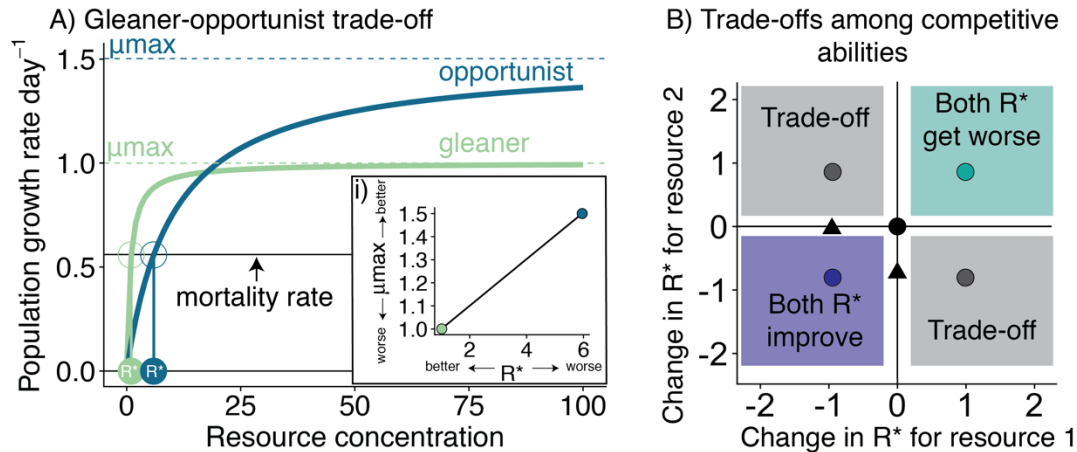
84 Resource competition often acts as a strong selective agent that drives patterns of
85 biodiversity and trait change via character divergence [10,11] and adaptive radiation
86 [12]. Competition can select for individuals that are able to consume 'alternative'
87 resources, or those that are not shared with other competitors [13]. Over time, this
88 results in adaptive trait divergence and niche differentiation [9,14]. Less well
89 appreciated is that when resources are essential, or non-substitutable, opportunities for
90 niche differentiation are limited and competition cannot be avoided by character
91 displacement because all competitors require the same limiting resources [7,15]. In this
92 case, selection favours improved competitive ability, or a reduced population-level R^*

93 for the shared limiting resource [15,16]. However, adaptation may be constrained by
94 physiological limits, genetic correlations between multiple traits [17], or lack of genetic
95 variation in resource traits [18]. These constraints may be particularly strong in the case
96 of adaptation to essential resource limitation because there are few opportunities for
97 divergence in adaptive strategies.

98

99 Trade-offs among species in competitive abilities for different resources have been
100 observed at large evolutionary scales (i.e. across clades) [19,20]. Turnover in species
101 abundances across gradients of resource ratios suggests that these trade-offs underlie
102 species distributions and patterns of biodiversity [1,21]. These trade-offs may arise due
103 to differences in the local conditions in which the traits evolved, or from biophysical or
104 genetic constraints that prevent individuals from optimizing several resource-use traits
105 simultaneously. There are at least two types of trade-offs which can govern resource
106 competition: gleaner-opportunist trade-offs (Figure 1A), (Electronic Supplementary
107 Material (ESM) Figure S1) [22,23], and trade-offs in the ability to acquire different
108 essential limiting resources (e.g. light versus nitrogen or nitrogen versus phosphorus;
109 Figure 1B) [19,20,24,25]. A gleaner-opportunist trade-off is a trade-off between a low
110 minimum resource requirement and a high maximum growth rate. A gleaner
111 phenotype grows better at low resource levels and an opportunist phenotype can take
112 advantage of high resource levels [26] (Figure 1A). Although the existence of trade-offs
113 in resource-use traits has been demonstrated on a macroevolutionary scale spanning
114 large swaths of evolutionary time, the microevolutionary processes by which they may
115 arise and the mechanisms that maintain them are still poorly understood.

116



117
 118 **Figure 1.** A) Two example Monod curves [27], describing resource-dependent
 119 population growth rates demonstrate a gleaner-opportunist trade-off (i.e. a trade-off
 120 between individuals that have high growth rates at low resource levels (green curve,
 121 gleaner), and lower growth rates at high resource levels compared to opportunists
 122 (blue curve, opportunist)). R^* , shown in circles, are the resource concentration at which
 123 population growth rate is zero. Here we show a mortality rate of 0.56/day, consistent
 124 with the dilution rate in our experiments. A gleaner-opportunist trade-off may be
 125 detected empirically by a positive relationship between μ_{max} and R^* (panel A, inset i).
 126 B) Trade-offs may arise when adaptation to one environment comes at the cost of
 127 performance in a different environment (e.g. grey dots in grey regions), here shown in
 128 terms of changes in R^* of descendant populations relative to their ancestors (black dot
 129 in centre). Alternatively, adaptation may arise via improvement in multiple traits
 130 simultaneously (e.g. purple dot, lower left quadrant), or conditional neutrality (i.e.
 131 improvements in one trait dimension, but no cost in another, black triangles).
 132 Maladaptation may occur if there are losses of performance in multiple traits
 133 simultaneously (turquoise dot, upper right quadrant).

134
 135 Ecological and evolutionary trade-offs are expected to arise from fundamental
 136 constraints on the use and acquisition of energy and materials. Organisms have fixed
 137 resource-and energy budgets with which to metabolize, grow and reproduce, such that
 138 energy and resources allocated to performing one function necessarily cannot be used
 139 for performing another independent function [28,29]. Furthermore, the observation
 140 that no single genotype or phenotype maximally performs all functions necessarily

141 implies that there are physiological constraints preventing the evolution of “Darwinian
142 demons” [30]. Despite the fact that evolving individuals eventually will face trade-offs,
143 not all local adaptation comes at a cost. First, trade-offs may not occur when multiple
144 functions can be optimized using the same energetic and resource allocations. For
145 example, this may occur when metabolic pathways affecting multiple functions are
146 highly connected and interdependent. Increasing efficiency in any part of the
147 metabolic pathway may therefore also reduce demands in the rest of the network [31].
148 In phytoplankton, this may be the case for resource requirements for light and nitrogen
149 because chloroplasts are typically very nitrogen-rich [32,33]. Similarly, proteins required
150 for nutrient uptake and metabolism are produced by phosphorus-rich ribosomes
151 [32,33]. Second, trade-offs between competitive abilities for different resources may
152 not arise if local adaptation results in energy and material budgets that are larger
153 overall - i.e. they are still approaching a fitness optimum. Finally, mutations that
154 improve fitness in a local environment may result in trade-offs due to antagonistic
155 pleiotropy [28] or in mutation accumulation for traits that are not under selection [34].
156 However, other outcomes are also possible, including neutral genetic variation or
157 synergistic pleiotropy [35,36]. Evidence that pleiotropy and mutation accumulation
158 should consistently generate trade-offs (rather than fitness-neutral or positive trait
159 change in an alternative environment) is still lacking [28].

160

161 To understand how essential resource competition traits evolve and how adaptation is
162 constrained, we used experimental evolution with a model organism, *Chlamydomonas*
163 *reinhardtii*. Experimental evolution allowed us to control the ecological conditions of
164 selection in chemostat, to isolate the effect of single limiting resources, and to
165 minimize confounding selective forces across treatments and replicates. We created
166 seven distinct selection environments in chemostats that varied either in the supply of
167 essential resources or salt concentration and quantified how populations’ resource-
168 competition traits and salt tolerances evolved. We replicated the evolutionary
169 treatments across five ancestral populations in order to quantify heterogeneity in the
170 responses to selection, and the repeatability of evolutionary outcomes [37]. Using
171 whole genome resequencing of the ancestors and descendants of the evolution

172 experiment, we confirmed that the descendants had fixed mutations over the course of
173 the experiment, and were no longer genetically identical to the ancestors, suggesting
174 that the observed phenotypic changes have a genetic basis.

175

176 We tested three predictions:

177

178 1) When populations are exposed to limitation of essential resources, selection on
179 resource-use traits should reduce R^* , the minimum resource requirement.

180 Additionally, evolutionary changes in R^* should be larger in the genotypically
181 diverse population relative to the isoclonal populations [38] because adaptation
182 from standing genetic variation can occur more rapidly [39] than adaptation
183 acting on novel mutations [40]. Lastly, we predicted that salt stress, in addition
184 to resource limitation would lead to greater adaptive trait change, particularly
185 because stress can increase rates of mutation [41].

186

187 2) Adaptive trait change is subject to trade-offs. Trade-offs between competitive
188 abilities for different resources, gleaner-opportunist trade-offs, or trade-offs
189 between resisting salt stress and having a high growth rate or low R^* may
190 constrain or structure adaptive change in resource traits [16,20] and potentially
191 cause adaptation in one environment to come at a cost to performance in
192 another environment [42] (Figure 1B). Alternatively, positively correlated
193 competitive traits may cause selection for a lower R^* for one resource to reduce
194 R^* for another (pleiotropic or correlated fitness benefits in low-resource
195 environments) [42–44].

196

197 3) We predicted that if trade-offs in resource-use traits cause traits to diverge
198 across different selection environments, this would increase the chance that
199 populations selected in different environments could competitively co-exist.

200

201 **Methods**

202

203 **Evolution experiment**

204 We obtained a strain of *C. reinhardtii* (cc1690 wild type mt+) from the Chlamydomonas
205 Center (chlamycollection.org). We selected four colonies derived from single cells
206 (hereafter referred to as Anc 2, Anc 3, Anc 4 and Anc 5, ESM Appendix B Figure S16),
207 and inoculated them into liquid COMBO freshwater medium [45]. We randomly
208 assigned seven small chemostats (28 mL) to each of the four isoclonal ancestral
209 populations (Anc 2-5) and the genotypically diverse population, cc1690. The seven
210 chemostats assigned to each of the ancestral populations were then randomly assigned
211 to one of seven treatments which we maintained for 285 days: COMBO (hereafter
212 referred to as C), nitrogen limitation (N), phosphorus limitation (P), light limitation (L),
213 salt stress (S), biotically-depleted medium (i.e. medium previously used to grow seven
214 other species of phytoplankton, which was then filtered and sterilized) (B), and a
215 combination of salt stress and biotically-depleted medium ('BS'). The C treatment had
216 COMBO medium supplied with an equable resource ratio (i.e. not highly limited in a
217 single nutrient), which allowed us to compare specific adaptations to resource-
218 limitation to adaptations to life in chemostat more generally. Here we used the term
219 'population' to refer to Anc 2, Anc 3, Anc 4, Anc 5, cc1690 (the 'ancestors') as well as
220 all of their descendant populations ('descendants'). In total, there were five ancestral
221 populations, and 32 descendant populations because three were lost to contamination.
222 Detailed information on experimental evolution methods is available in the
223 Supplementary Methods in the ESM (Appendix A).

224

225 **Determination of R^* and salt tolerance**

226 We determined the minimum resource requirements for positive population growth (R^*)
227 for each population [1] via batch culture experiments. We defined N^* as the minimum
228 nitrogen concentration and P^* as the minimum phosphorus concentration for positive
229 population growth. We define I^* as the minimum light level required for positive
230 population growth (similar to I_c in [46]). We estimated R^* by measuring population
231 growth rates at ten resource levels for each of nitrogen, phosphorus and light for three
232 days (see ESM Appendix A: Supplementary Methods: 'Competitive trait assays' for
233 more details on the resource levels, acclimation and measurements). We estimated

234 'consumption vectors' ([1]) for N and P via stoichiometry of exponentially growing
235 populations [3], and cell size by measuring single cell lengths using a high throughput
236 imager (Biotek®Cytation 5), and calculating cell biovolume assuming cells were
237 spheres using $4/3 \times \pi \times \text{radius}^3$.

238
239 In order to determine populations' R^* , we modeled resource-dependent population
240 growth via a Monod curve [1,35]. We estimated the parameters of the Monod curve
241 directly from population-level time series of chlorophyll-a relative fluorescence units
242 measured over the resource gradients. We modeled the resource-dependent rate of
243 population growth, r , during the exponential phase as:

$$244 \quad F(t) = F(0)e^{r(R)t}, \quad (1a)$$

245
246
247 where $F(t)$ is the population-level RFU at time t , and $r(R)$ is given by:

$$248 \quad r(R) = \mu_{max} \left(\frac{R}{k_s + R} \right), \quad (1b)$$

249
250
251 using nonlinear least-squares regression with the *nls.LM* function in the *minpack.LM*
252 package [36] in R. Population growth rate, r , is a function of μ_{max} , the maximum
253 population growth rate, R , the resource concentration, and k_s , the half-saturation
254 constant for population growth.

255
256 Using the estimated parameters of the Monod curve (i.e. Equation 1b), we estimated R^*
257 as:

$$258 \quad R^* = \frac{m k_s}{\mu_{max} - m}, \quad (2)$$

259
260 where m is the mortality rate, which we set to be 0.56/day to reflect the mortality
261 caused by dilution in chemostat experiments. To simplify our analyses, we used
262 Equations 1 and 2 to estimate minimum light requirements (I^*), where $R = \text{irradiance}$.

263 We also included ESM Figure S2 with parameters estimated from an Eilers-Peeters
264 curve [37] for light.

265

266 To estimate the uncertainty in the Monod curve (Equation 1) fits, we determined
267 confidence intervals around the fitted Monod curves using non-parametric
268 bootstrapping of mean-centered residuals using the *nlsBoot* function with 999
269 iterations in the *nlstools* [38] package in R. We calculated 95% confidence intervals as
270 the range between the 2.5th and 97.5th quantiles.

271

272 We defined the salt tolerance as salt concentration at which growth rates are half their
273 maximum (which occurs at a salt concentration of zero). We estimated salt tolerance by
274 modeling population growth rates during the exponential phase, r , as a function of salt
275 concentration, S , using a simplified form of the logistic function:

276

$$277 \quad r(S) = \frac{a}{1 + e^{-b(S-c)}}, \quad (3)$$

278

279 where a (the upper asymptote), is the maximum population growth rate (not salt-
280 stressed), b is the decline in growth rate with increasing salt concentration and c is the
281 salt concentration at which growth rates are half their maximum, in g/L.

282

283 **Quantifying trait change and testing for trade-offs**

284 We tested for changes in R^* between descendant and ancestral populations by
285 subtracting the ancestral trait value from the descendant trait value and quantifying
286 whether the 95% on the difference overlapped zero. We tested whether the change in
287 resource-use traits was greater in the genotypically diverse populations than the
288 isoclonal populations by comparing the 95% CI of the trait changes.

289

290 We tested for trade-offs between:

291

- 292 1. growth rates at high vs low supply of a given resource (ie. μ_{\max} vs R^* , or a
293 gleaner-opportunist trade-off) (Figure 1A),

- 294
- 295 2. competitive abilities for different resources, or competitive ability and cell size,
296 and
297
- 298 3. changes in traits in multiple dimensions (Figure 1B).
299

300 We tested for trade-offs using multiple linear regressions. We quantified competitive
301 ability for a given resource as $1/R^*$ [20], and tested for trade-offs among competitive
302 abilities for different resources (trade-off 2). In order to assess trade-offs among
303 multiple traits and cell size, we centered and scaled the variables using the *scale*
304 function in R (mean=0, standard deviation=1) so all variables could be compared on
305 the same scale. In all cases of multiple regression, we included ancestor ID as a fixed
306 effect to account for relationships among ancestors and descendants.

307

308 We tested for differences in multivariate trait change as a function of selection
309 treatment and ancestor using redundancy analysis (RDA) with the *capscale* function in
310 the R package *vegan* [50], version 2.5-4. Here we included all of the traits we
311 measured: R^* s, cell biovolume, consumption vectors (i.e. stoichiometry), and salt
312 tolerances. We used permutation tests (*anova.cca* in *vegan*) to test the statistical null
313 hypothesis that selection treatment and ancestor ID had no significant impact on any
314 independently varying linear combination of traits. We used the same approach to test
315 the effects of treatment on trait variation along the individual axes. We assessed which
316 descendant populations had diverged from their ancestors in different environments
317 using post-hoc Tukey tests using the *TukeyHSD* function in R. We conducted all of our
318 statistical analyses using R, version 3.6.1 [51].

319

320 **Quantifying genetic changes associated with selection environments**

321 DNA was extracted using a chloroform-methanol extraction and libraries were
322 prepared using the Bioo Scientific NEXTflex Rapid Illumina DNA-Seq Library Prep Kit.
323 For details and bioinformatic methods, please refer to the Supplementary Methods in
324 the ESM (Appendix A).

325

326 ***Testing the potential for altered predicted outcomes of competition***

327 We used resource competition theory [1] to predict the outcome of pairwise
328 competition for two resources: nitrogen and phosphorus. RCT predicts that two
329 populations can coexist stably if they meet three conditions: 1) their zero net growth
330 isoclines (ZNGIs) cross (i.e. populations differ in the identity of the resource that most
331 limits their growth), 2), they each consume more of the resource which most limits their
332 growth (i.e. each population has a steeper consumption vector for the resource which
333 is most limiting to it) and 3), the supply point of resources in the environment falls
334 above their ZNGIs and between the consumption vectors of the two populations. If the
335 pair of populations meets criterion 1 and 3 but not 2, theory predicts unstable
336 coexistence or priority effects. If the pair of populations meets one or none of these
337 criteria, theory predicts competitive exclusion. We compared all possible combinations
338 of the five populations in their ancestral state and after selection in the different
339 resource environments. We then assessed the proportion of these pairwise interactions
340 that would be expected to lead to unstable coexistence, stable coexistence or
341 competitive exclusion.

342

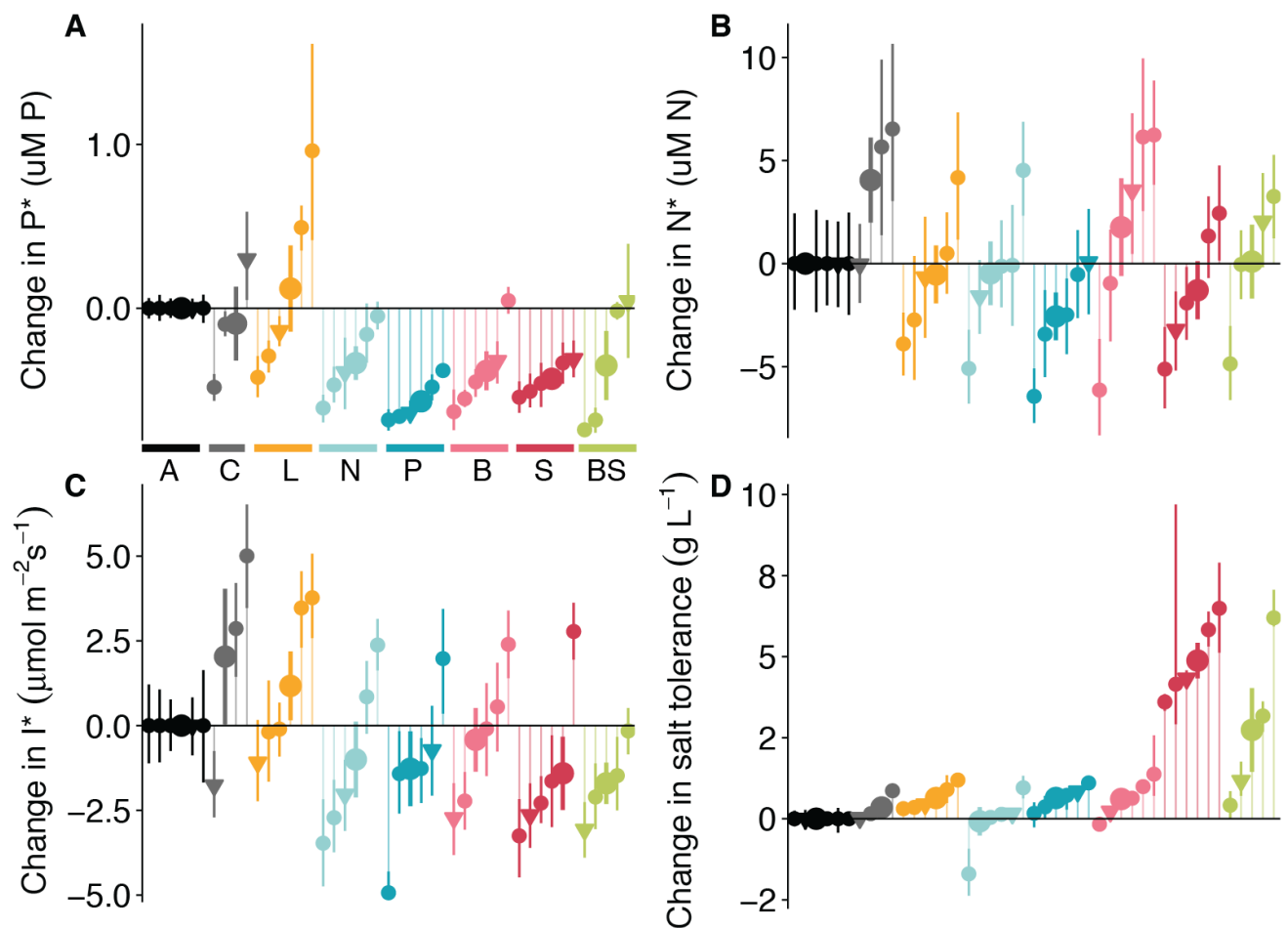
343 **Results**

344

345 ***Evolutionary changes in R^* and salt tolerance***

346 Relative to their ancestors, P^* declined in all five populations exposed to P limitation (P,
347 Figure 2A). Declines in P^* ranged from 43% to 85% across the replicate populations. In
348 response to N limitation, N^* declined in two populations (14%, 34% decline), did not
349 change in two populations and increased in one population (47% increase) (N, Figure
350 2B). I^* increased in two populations exposed to low light (L, 12%; 28% increase), and
351 did not change in the remaining three populations exposed to low light (Figure 2C).
352 Salt tolerance increased in all populations exposed to high salt (93% - 369% S and BS,
353 Figure 2D). Consumption vectors, quantified as the P:N molar ratio in the biomass of
354 populations growing exponentially, decreased in all of the populations subjected to
355 nitrogen limitation, and increased in four of the five populations exposed to

356 phosphorus limitation (ESM Figure S4, see also Figures S5-6). This suggests that
 357 populations selected under nitrogen limitation contained more nitrogen relative to
 358 phosphorus whereas populations selected under low phosphorus contained more
 359 phosphorus relative to nitrogen. Contrary to our predictions, the descendants of the
 360 genotypically diverse cc1690 population did not show more trait change than any of
 361 the isoclonal populations (triangles vs small dots in Figure 2). However, the
 362 genotypically diverse cc1690 population did match our predictions in terms of the
 363 direction of adaptive trait change in all selection environments: P* decreased under P-
 364 limitation, N* decreased under N-limitation, salt tolerance increased in the high salt
 365 environment, and I* decreased under low light, though the change in I* was not
 366 statistically significant.
 367

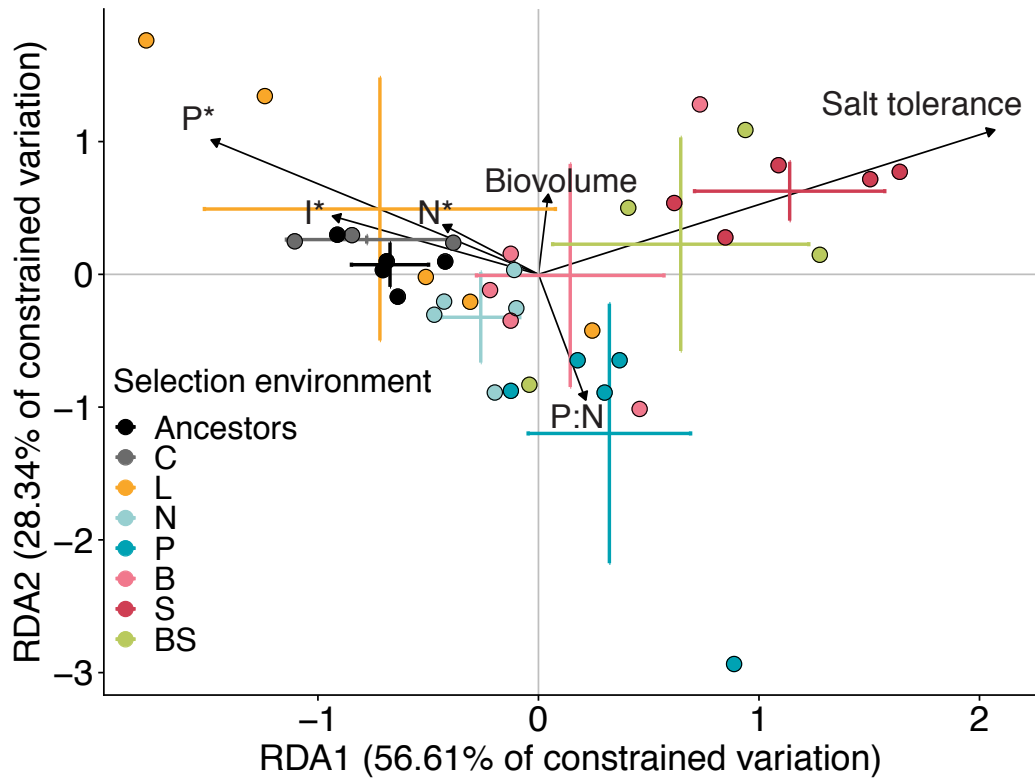


368
 369 **Figure 2.** Changes in resource competition traits relative to ancestors in seven different
 370 selection environments (A: ancestors, C: COMBO, L: light-limited, P: P-limited, N: N-

371 limited, B: biotically depleted media, S: high salt, BS: biotically depleted and high salt).
372 Small points correspond to individual populations and large points correspond to the
373 average change (error bars are ± 1 SE) of all populations in a given environment.
374 Populations represented with a triangle are the genotypically diverse populations
375 (cc1690), circles are isoclinal. Error bars on small points in A-D correspond to 95% CI
376 from non-parametric bootstrapping. Colour legend in A applies to all panels.

377
378 When considering all traits together, descendant populations diverged from their
379 ancestors, and variation in these new phenotypes was associated with selection
380 environment (Redundancy Analysis, Figure 3). We tested for constraints on adaptive
381 change by assessing whether there was significant separation between ancestors and
382 descendants on the RDA axes. RDA axes 1 and 2, which represent linear combinations
383 of selection environment and ancestor ID (PERMANOVA $p < 0.01$) explain 85% of the
384 variation associated with selection environment, and 36% of the total variation. On
385 RDA axis 1 (PERMANOVA $p < 0.001$), which is primarily associated with variation in salt
386 tolerance and P^* , populations selected in the P, S, B and BS environments were
387 significantly different (separated) from the ancestors. The salt selected populations (S
388 and BS) were also different from the COMBO (C) and low-light selected treatments (L).
389 On RDA axis 2 (PERMANOVA $p = 0.005$) which is associated with variation in P:N
390 (consumption vector slope), P is different from ancestors and the C, L, B, S, BS
391 populations. The RDA showed that most of the variation in multivariate phenotypes
392 across selection environments was associated with variation in salt tolerance and P^* ,
393 and much less independent variation was associated with N^* and I^* (Figure 3),
394 suggesting that variation in these traits may be subject to physiological or genetic
395 constraints.

396

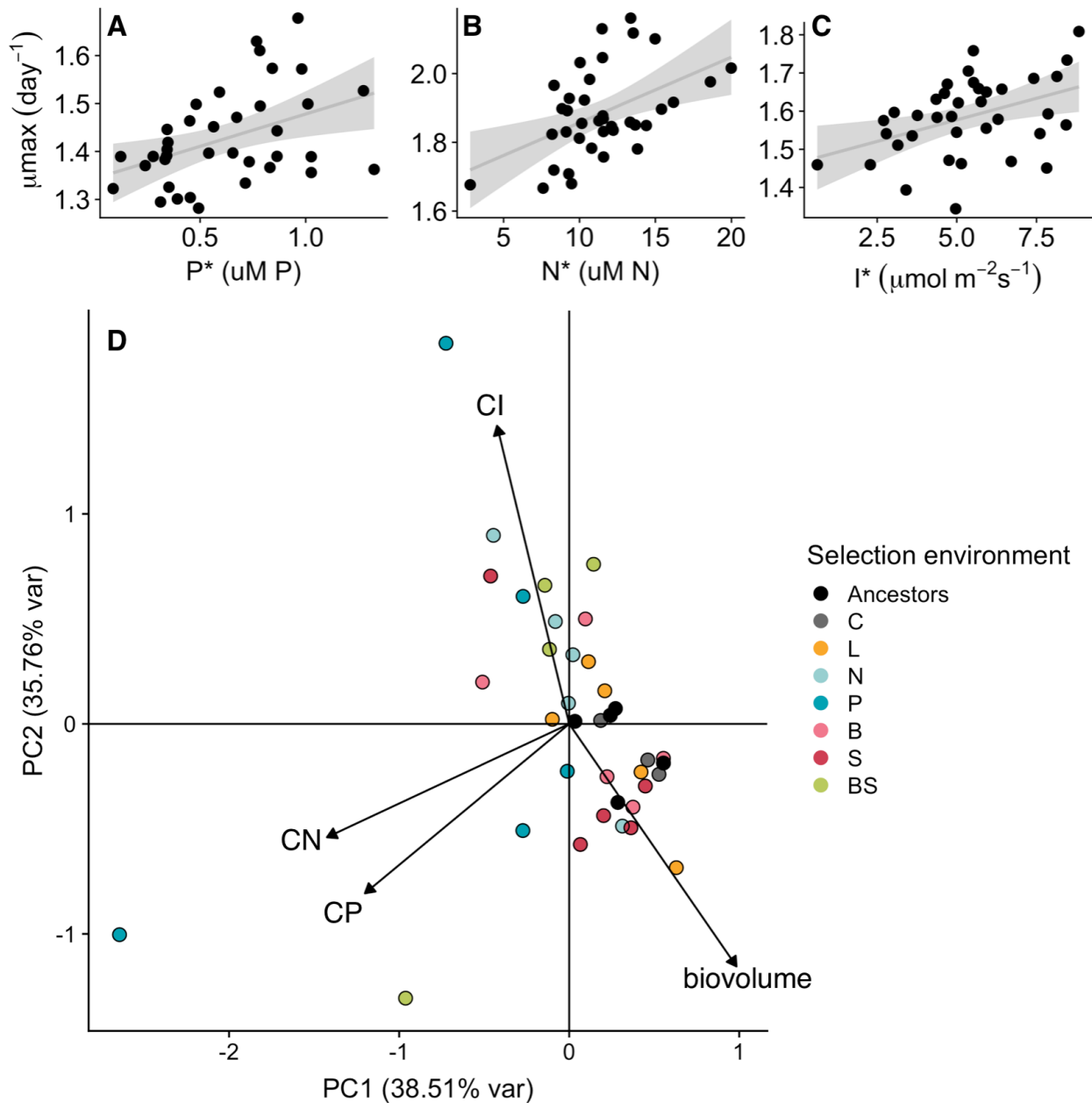


397
 398 **Figure 3.** Redundancy analysis of N^* , I^* , P^* , salt tolerance, consumption vectors and
 399 cell biovolume across selection environments. Error bars correspond to standard error
 400 around treatment means ($n = 5$ per treatment). RDA axes 1 and 2 (PERMANOVA $p <$
 401 0.01) explain 85% of the variation associated with selection environment, and 36% of
 402 the total variation.

403
 404 ***The structure of trait variance: observed trade-offs***

405 Maximum growth rate across populations increased with minimum resource
 406 requirements (R^*) for light (OLS slope = 0.021, 95% CI: 0.0027, 0.039, Adjusted $R^2 =$
 407 0.11), for nitrogen (OLS slope = 0.019, 95% CI: 0.0078, 0.031, Adjusted $R^2 = 0.30$), and
 408 for phosphorus (OLS slope = 0.12, 95% CI: 0.0094, 0.23, Adjusted $R^2 = 0.13$),
 409 indicating a trade-off between growth at high and low resource supplies (because a
 410 lower R^* indicates faster growth at minimum resource levels) (Figure 4, A-C). Across
 411 populations, competitive abilities for N and P (CN and CP) were positively associated
 412 (ESM Tables 4-6, Figure 4D, ESM Figures S13-15). After accounting for covariance with
 413 competitive abilities for other resources and ancestor ID, competitive abilities for light
 414 were negatively associated with cell biovolume, while and N and P competitive abilities

415 were not related to cell size (ESM Tables 4-6, ESM Figures S13-15). Principal
 416 components analysis of cell biovolume, competitive abilities for light, nitrogen and
 417 phosphorus showed that 74% of the variation in cell volume and competitive abilities is
 418 explained by the first two PC axes. The first two PC axes demonstrate a positive
 419 association between competitive abilities for N and P, and a possible trade-off
 420 between biovolume and competitive ability for light (Figure 4D).
 421



422

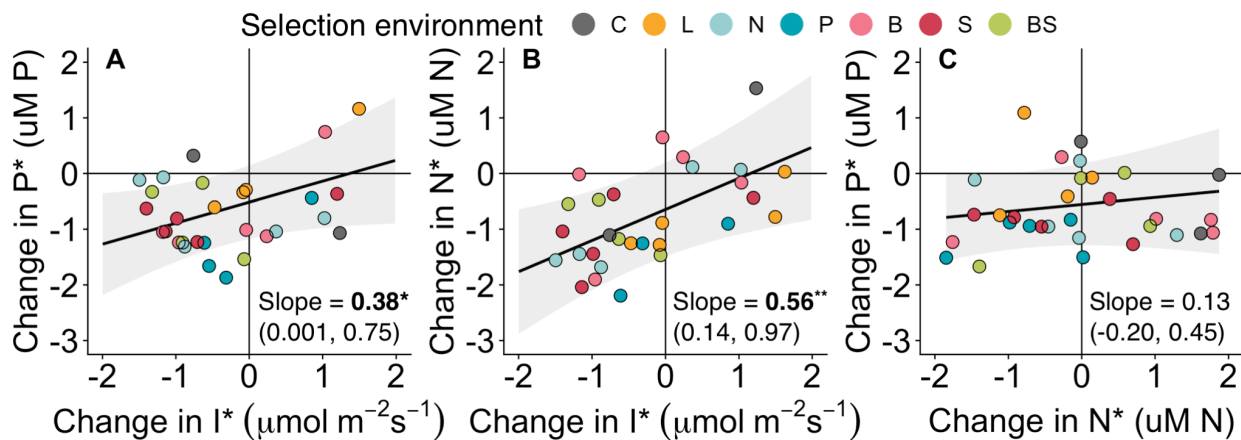
423 **Figure 4.** High maximum population growth rates (μ_{\max}) are positively associated with
 424 high minimum resource requirements for phosphorus (A), nitrogen (B) and light (C).
 425 Estimates of μ_{\max} and R^* in A-C are from Monod curves generated over independent
 426 gradients of phosphorus, nitrogen and light. D) Loadings on the first two PC axes of a
 427 PCA using competitive abilities for phosphorus, nitrogen and light ($CP = 1/P^*$, $CN =$
 428 $1/N^*$, $CL = 1/L^*$) and cell biovolume. 74.3% of the variation in resource use traits and cell
 429 size is explained by the first two PC axes.

430

431 **Correlations in changes across traits**

432 Though theory often assumes that competitive abilities for different resources are
 433 negatively related [16,52], our results did not support this finding either when
 434 considering absolute variation in competitive abilities (ESM Tables 4-6) or variation in
 435 the change in R^* relative to the ancestral populations (Figure 5; ESM Tables 1-3, ESM
 436 Figure S10). The changes in R^* for different resources never showed evidence of any
 437 trade-offs, and instead were either positively associated (Figure 5A, B) or showed no
 438 significant relationship (Figure 5C, ESM Figure S10).

439



440

441 **Figure 5.** Partial regression plots showing how changes in descendants relative to
 442 ancestors in two traits are related to each other, while holding all factors in the
 443 statistical model that are not being displayed constant (complete model results in ESM
 444 Tables 1-3). Positive slopes indicate positively associated trait changes.

445

446 **Genetic changes after selection**

447 Genetic differences between the ancestral and descendant populations were identified
448 by whole genome re-sequencing. The presence or absence of single nucleotide
449 polymorphisms (SNPs) identified within the populations were compared between the
450 ancestors and descendants for each selection treatment. The number of variable SNPs
451 ranged from 396 to 582 (ESM Table 7), roughly corresponding to mutation fixation
452 rates of $1.25e-8$ to $1.83e-8$ mutations/[locus \times generations]. Contrary to our
453 expectations, salt stress did not increase the number of fixed mutations. Selection
454 treatment had no significant effect on the total number of fixed mutations (ESM Figure
455 S11 A; ANOVA $p = 0.788$), but the effect of the ancestor was highly significant (ESM
456 Figure S11 B; ANOVA $p < 1e-7$).

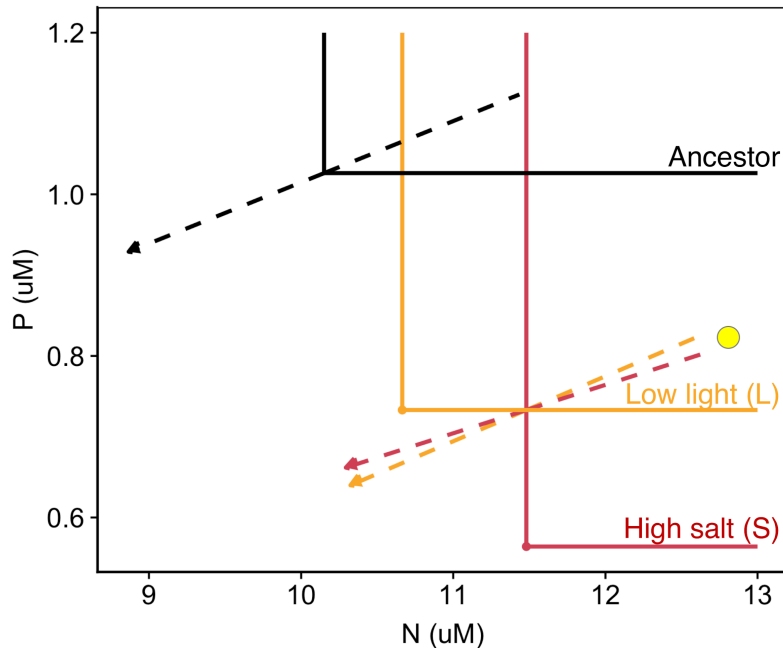
457

458 ***Evolutionary adaptation altered the predicted outcomes of competition***

459 Divergence in minimum resource requirements and consumption vectors among
460 populations of the same ancestor selected in different environments (Figure 3) was
461 sufficient in some cases to lead to predicted coexistence. One such example is
462 illustrated in Figure 6, where descendants of Ancestor 3 selected in low light and high
463 salt environments have diverged sufficiently in their P^* and N^* such that they could
464 possibly coexist. While neither descendant population could coexist with Ancestor 3,
465 the two descendant populations could coexist with one another at a supply point
466 illustrated as a yellow dot. In their ancestral state, out of all pairwise combinations of
467 our five ancestor populations, resource competition theory [1] predicts unstable
468 coexistence in four of 10 cases and competitive exclusion in five of 10 cases, and stable
469 coexistence in one of 10 cases. After selection across the range of environments in our
470 study, resource ratio theory predicts unstable coexistence in 19.9% of all possible
471 pairwise interactions (698 total), stable coexistence in 27.94% of all possible pairwise
472 interactions and competitive exclusion in 52.15% of all possible interactions (ESM
473 Figure S12). Among populations selected in the same environment (59 total), resource
474 ratio theory predicts competitive exclusion in 47.5%, stable coexistence in 32.2%,
475 unstable coexistence in 20.33%.

476

477



478
 479 **Figure 6.** Descendants of Ancestor 3 evolved in high salt (red) and low light (orange)
 480 environments have diverged in their P^* and N^* such that they can co-exist. Neither
 481 descendant could co-exist with the ancestor. Zero net growth isoclines (solid lines) and
 482 consumption vectors (dashed lines) for Ancestor 3 (black) and the descendant of
 483 Ancestor 3 selected in low light (orange) and high salt (red).

484

485 Discussion

486

487 Resource competition is among the most important processes structuring ecological
 488 communities [53], but competition theory often assumes that traits underlying
 489 competitive abilities remain fixed over ecological time scales [1,54]. Here we showed
 490 that the traits that underlie competitive abilities for essential resources can adapt
 491 rapidly in new resource-limited environments. Populations of *C. reinhardtii* often
 492 adapted to resource limitation by reducing their minimum resource requirements.
 493 When exposed to high salt, populations evolved higher salt tolerances. Not only could
 494 populations respond adaptively to new environments, but they could also adapt within
 495 approximately 285 generations. While we observed gleaner-opportunist trade-offs, we
 496 did not find evidence for trade-offs in competitive abilities for different resources.

497 Instead, adaptive changes in competitive ability for one resource were often positively
498 associated with improvements in competitive ability for another. Since the ancestral
499 and descendant populations were maintained under identical conditions when
500 quantifying their traits, the changes we observed were heritable. We documented
501 genetic changes as fixed single nucleotide polymorphisms in each of descendant
502 lineages (ESM Appendix B Figure S16, Appendix C Table 7), which likely contributed to
503 the heritable phenotypic changes we observed. Due to a lack of annotational
504 information on the genes in which mutations were fixed, our ability to infer the
505 connection between genotype and phenotype is limited. Future studies investigating
506 the roles of gene expression regulation and epigenetic modification in contributing to
507 heritable trait change of resource requirements would provide additional insights [55].
508

509 The magnitude of evolutionary change varied among resource competition traits.
510 When considering all the populations together, adaptive change in P^* was large (up to
511 85% decrease relative to ancestors), while adaptive change in N^* was more limited,
512 and change in I^* was sometimes maladaptive. It is possible that the lack of adaptive
513 change in N^* and I^* was because the ancestral populations in our experiment were
514 already at or near a fitness optimum. Although there were no consistent differences in
515 the magnitude of adaptive trait changes when comparing the genotypically diverse
516 populations to the isoclonal populations, in all selection environments the direction of
517 trait change was adaptive in the genotypically diverse populations. However, the
518 absence of replicated genetically diverse populations within each treatment limits our
519 ability to generalize the effects of genotypic diversity on evolutionary outcomes in a
520 given environment.

521
522 Trade-offs in resource-use traits have been invoked to explain changes in dominance
523 across supply ratio gradients and the coexistence of as many species as resources
524 [1,19]. Trade-offs in competitive abilities for nitrogen and phosphorus [20], iron and
525 light [24], and light and nitrogen [25] have been documented among and within
526 species of phytoplankton. These trade-offs may arise due to local adaptation, or due to
527 biophysical constraints on the acquisition or metabolism of different resources [19,52].

528 Individuals may invest resources into two main types of cellular machinery: uptake or
529 assembly machinery. Uptake machinery is composed of nutrient uptake proteins and
530 chloroplasts, which are both relatively nitrogen-rich, and both of which may scale with
531 cell size because uptake and photosynthesis must take place at the cell surface.
532 Assembly machinery, primarily composed of ribosomes, is relative phosphorus-rich and
533 may also depend on cell size, as smaller cells tend to grow faster (“growth rate
534 hypothesis” [33]). Consistent with expectations, competitive abilities for light were
535 negatively associated with cell size, but in contrast to expectations, N and P
536 competitive abilities were not. Furthermore, we did not find evidence for a trade-off
537 between competitive abilities for nitrogen and phosphorus.

538
539 No evidence for trade-offs in competitive abilities for different resources is in contrast
540 to observations of negative multivariate correlations observed on macroevolutionary
541 timescales [20]. This runs counter to the idea that population genetic variation occurs
542 along the same axes as variation among species - along ‘genetic lines of least
543 resistance’ [56]. There are multiple potential reasons for this lack of observed trade-offs
544 in competitive ability at this evolutionary scale. The first possibility is that essential
545 resource requirements differ from other traits because they are linked via shared
546 metabolic pathways in a metabolic network that controls the uptake, conversion and
547 allocation of energy and materials. Requirements for different resources are
548 intrinsically, metabolically linked and therefore non-independent. This suggests that
549 observed trade-offs in R^* at macroevolutionary scales are the result of major metabolic
550 innovations across clades, breaking these metabolic linkages [57]. It is also possible
551 that correlations observed at macroevolutionary scales are due to responses to local
552 selection pressures that are unrelated to resource limitation, including grazing, disease
553 and turbulent mixing [58]. A third possible explanation is that the descendant
554 populations in our experiment had not yet reached fitness or trait optima, and as such,
555 continued adaptation did not impose costs [30]. This is possible, and though we did
556 not evaluate R^* or fitness at multiple evolutionary end-points, fitness may continue
557 increasing under directional selection for tens of thousands of generations [59].
558 However, if trade-offs do not emerge within 285 generations of low-resource selection,

559 natural populations of phytoplankton evolving in response to seasonal or annual
560 variation in nutrient availability may not be expected to be optimizing along trade-off
561 axes in competitive ability for different resources. Finally, mutations affecting any
562 particular resource requirement may generally be more likely to be synergistically
563 pleiotropic than neutral or antagonistic. Given the degree of metabolic interrelatedness
564 of resource acquisition and metabolic pathways in phytoplankton, this is plausible and
565 deserves further investigation.

566

567 Patterns of genotypic variation across populations revealed negative correlations
568 between fitness at low and high resource supply for a given resource (gleaner-
569 opportunist trade-offs, Figure 1A), and positive correlations between competitive
570 abilities for different resources (Figure 3, ESM Tables 4 - 6), suggesting that the
571 evolution of competitive ability could be constrained by genetic correlations between
572 multiple resource traits under selection. The genetic correlations between different
573 resource traits could explain the positively associated trait changes (i.e. improvements
574 in multiple minimum resources requirements simultaneously). In addition, unmeasured
575 traits could be involved in the trade-off, resulting in a positive genetic covariance
576 between any two resource traits [18]. When testing for trade-offs, we accounted for
577 concurrent variation in cell size, but other fitness-correlated traits, such as resistance to
578 grazers or pathogens [60,61], may be involved in the trade-offs.

579

580 Traits relevant to competitive ability, such as cell size, are known to change as a result
581 of phenotypic plasticity and evolutionary adaptation [62]. We have demonstrated that
582 adaptation in response to resource limitation and salt stress can alter competitive traits
583 sufficiently to change the predicted outcome of competition. Adaptation to different
584 environments caused competitive traits to diverge and enable coexistence. Contrary to
585 our expectations, we found that coexistence was equally likely among two populations
586 selected in different environments as two populations selected in the same
587 environment. This may be explained by the fact that even small differences in the
588 magnitude of adaptive trait change in the same environment can be sufficient to
589 enable predicted coexistence (i.e. under P-limitation, P^* for one competitor decreases

590 slightly more than the P^* for the other competitor). The changes in resource ratios and
591 salt levels represented in our different selection environments are on the same order of
592 magnitude as gradients of resource ratios and salinity in natural environments [63]. This
593 means that predictions of the outcomes of competition should incorporate the
594 potential for evolutionary changes to influence competitive dynamics [16].

595

596 Our results are directly relevant to understanding eco-evolutionary feedbacks in
597 competitively structured communities. Theory predicts that species converge in their
598 resource-use traits when competing for essential resources [15,16]. This expectation,
599 however, depends on two critical assumptions. These assumptions are that species'
600 consumption vectors remain fixed and that competitive abilities for different limiting
601 resources trade off. While we did not grow pairs of populations together in the
602 evolution experiment, the resource limitation treatments mimicked the effects of a
603 better competitor for the limiting resource, while avoiding exclusion of the weaker
604 competitor. Our results do not provide empirical support for either of the assumptions
605 above, suggesting that theoretical predictions of evolutionary adaptation under
606 essential resource competition may need to be revised [15,16].

607

608 Understanding patterns of biodiversity and coexistence requires accounting for past
609 and current evolutionary changes in species' competitive traits. While macro-
610 evolutionary patterns show trade-offs in species' resource-use traits, we found that
611 positively correlated adaptive trait changes drive within-species responses to resource
612 limitation, altering the expected outcome of competition. Such micro-
613 evolutionary changes in species' competitive abilities should to be considered if we are
614 to improve our predictions of competitive interactions and community dynamics in a
615 changing world.

616

617

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619

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