The evolution of competitive ability for essential resources

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Abstract

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

Introduction

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

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

population-level R^* for the shared limiting resource [14,15]. However, adaptation may be constrained by physiological limits, genetic correlations between multiple traits [16], or lack of genetic variation in resource traits [17]. These constraints may be particularly strong in the case of adaptation to essential resource limitation because there are few opportunities for divergence in adaptive strategies.

Trade-offs among species in competitive ability have been observed at large evolutionary scales (i.e. across clades) [18,19]. Turnover in species abundances across gradients of resource ratios suggests that these trade-offs structure species distributions and patterns of biodiversity [1,20]. These trade-offs may arise due to differences in the local conditions in which the traits evolved, or from biophysical or genetic constraints that prevent individuals from optimizing several resource-use traits simultaneously. There are at least two types of trade-offs which can govern resource competition: gleaner-opportunist trade-offs, (Electronic Supplementary Material (ESM) Figure S1) [21,22], and trade-offs in the ability to acquire different essential limiting resources (e.g. light versus nitrogen or nitrogen versus phosphorus) [18,19,23,24]. Although the existence of trade-offs in resource-use traits has been demonstrated on a macroevolutionary scale spanning large swaths of evolutionary time, the microevolutionary processes by which they may arise and the mechanisms that maintain them are still poorly understood.

To understand how essential resource competition traits evolve and how adaptation is constrained, we used experimental evolution with a model organism, *Chlamydomonas reinhardtii*. Experimental evolution allowed us to control the ecological conditions of selection in chemostat, to isolate the effect of single limiting resources, and to minimize confounding selective factors across treatments and replicates. We created seven distinct selection environments in chemostats that varied either in the supply of essential resources or salt concentration and quantified how populations' resource-competition traits and salt tolerances evolved. We replicated the evolutionary treatments across five ancestral populations in order to quantify heterogeneity in the responses to selection, and the repeatability of evolutionary outcomes [25]. Using

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

We tested three predictions:

1) When populations are exposed to limitation of essential resources, selection on resource-use traits should reduce R^*, the minimum resource requirement. Additionally, evolutionary changes in R^* should be larger in the genotypically diverse population relative to the isoclonal populations [26] because adaptation from standing genetic variation can occur more rapidly [27] than adaptation acting on novel mutations [28]. Lastly, we predicted that salt stress, in addition to resource limitation would lead to greater adaptive trait change, particularly because stress can increase rates of mutation [29].

2) Adaptive trait change is subject to trade-offs. Trade-offs between competitive abilities for different resources, gleaner-opportunist trade-offs, or trade-offs between resisting salt stress and having a high growth rate or low R^* may constrain or structure adaptive change in resource traits [15,19] and potentially cause adaptation in one environment to come at a cost to performance in another environment [30]. Alternatively, positively correlated competitive traits may cause selection for a lower R^* for one resource to reduce R^* for another (pleiotropic or correlated fitness benefits in low-resource environments) [30–32].

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

Methods

Evolution experiment

We obtained a strain of *Chlamydomonas reinhardtii* (cc1690 wild type mt+) from the Chlamydomonas Center (chlamycollection.org). We selected four random colonies derived from single cells (hereafter referred to as Anc 2, Anc 3, Anc 4 and Anc 5), and inoculated them into liquid COMBO freshwater medium [33]. We randomly assigned seven chemostats to each of the four isoclonal ancestral populations (Anc 2-5) and the genotypically diverse population, cc1690. The seven chemostats assigned to each of the ancestral populations were then randomly assigned to one of seven treatments which we maintained for 285 days: COMBO (hereafter referred to as 'C'), nitrogen limitation ('N'), phosphorus limitation ('P'), light limitation ('L'), salt stress ('S'), biotically-depleted medium (i.e. medium previously used to grow seven other species of phytoplankton) ('B'), and a combination of salt stress and biotically-depleted medium ('BS'). Here we used the term 'population' to refer to Anc 2, Anc 3, Anc 4, Anc 5, cc1690 (the 'ancestors') as well as all of their descendant populations ('descendants'). In total there were five ancestral populations, and 32 descendant populations because three were lost to contamination. Detailed information on experimental evolution methods is available in the Supplementary Methods in the ESM (Appendix A).

Determination of R^* and salt tolerance

We determined the minimum resource requirements for positive population growth (R^*) for each population [1] via batch culture experiments. We defined N^* as the minimum nitrogen concentration and P^* as the minimum phosphorus concentration for positive population growth. We define I^* as the minimum light level required for positive population growth (similar to I_c in [34]). We estimated R^* by measuring population growth rates at ten resource levels for each of nitrogen, phosphorus and light for three days (see ESM Appendix A: Supplementary Methods: *'Competitive trait assays'* for more details on the resource levels, acclimation and measurements). We estimated 'consumption vectors' ([1]) for N and P via stoichiometry of exponentially growing populations [3], and cell size by measuring single cell lengths using a high throughput

imager (Biotek®Cytation 5), and calculating cell biovolume assuming cells were spheres using 4/3*pi*radius^3.

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

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

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

$$r(R) = \mu max\left(\frac{R}{k_s + R}\right) \tag{1b}$$

using nonlinear least-squares regression with the *nls.LM* function in the *minpack.LM* package [36] in R. Population growth rate, *r*, is a function of *μmax*, the maximum population growth rate, *R*, the resource concentration, and k_s, the half-saturation constant for population growth.

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

$$R^* = \frac{m\, k_s}{\mu max - m} \tag{2}$$

where *m* is the mortality rate, which we set to be 0.56/day to reflect the mortality caused by dilution in chemostat experiments. To simplify our analyses, we used Equations 1 and 2 to estimate minimum light requirements (I^*), where *R* = irradiance. We also included ESM Figure S2 with parameters estimated from an Eilers-Peeters curve [37] for light.

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

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

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

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

Quantifying trait change and testing for trade-offs

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

We tested for trade-offs between:

1. growth rates at high vs low supply of a given resource (ie. μmax vs R^*, or a gleaner-opportunist trade-off),

2. competitive abilities for different resources, or competitive ability and cell size, and

3. changes in traits in multiple dimensions.

We tested for trade-offs using multiple linear regressions. We quantified competitive ability for a given resource as $1/R^*$ [19], and tested for trade-offs among competitive abilities for different resources (trade-off 2). In order to assess trade-offs among multiple traits and cell size, we centered and scaled the variables using the *scale* function in R (mean=0, standard deviation=1) so all variables could be compared on the same scale. In all cases of multiple regression, we included ancestor ID as a fixed effect to account for relationships among ancestors and descendants.

We tested for differences in multivariate trait change as a function of selection treatment and ancestor using redundancy analysis (RDA) with the *capscale* function in the R package *vegan* [39], version 2.5-4. Here we included all of the traits we measured: R^*s, cell biovolume, consumption vectors (i.e. stoichiometry), and salt tolerances. We used permutation tests (*anova.cca* in *vegan*) to test the statistical null hypothesis that selection treatment and ancestor ID had no significant impact on any independently varying linear combination of traits. We used the same approach to test the effects of treatment on trait variation along the individual axes. We assessed which descendant populations had diverged from their ancestors in different environments using post-hoc Tukey tests using the *TukeyHSD* function in R.

Quantifying genetic changes associated with selection environments

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

Testing the potential for altered predicted outcomes of competition

We used resource competition theory [1] to predict the outcome of pairwise competition for two resources: nitrogen and phosphorus. RCT predicts that two

279 populations can coexist stably if they meet three conditions: 1) their zero net growth
280 isoclines (ZNGIs) cross (i.e. populations differ in the identity of the resource that most
281 limits their growth), 2), they each consume more of the resource which most limits their
282 growth (i.e. each population has a steeper consumption vector for the resource which
283 is most limiting to it) and 3), the supply point of resources in the environment falls
284 above their ZNGIs and between the consumption vectors of the two populations. If the
285 pair of populations meets criterion 1 and 3 but not 2, theory predicts unstable
286 coexistence or priority effects. If the pair of populations meets one or none of these
287 criteria, theory predicts competitive exclusion. We compared all possible combinations
288 of the five populations in their ancestral state and after selection in the different
289 resource environments. We then assessed the proportion of these pairwise interactions
290 that would be expected to lead to unstable coexistence, stable coexistence or
291 competitive exclusion.
292

293 Results

294

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

296 Relative to their ancestors, P^* declined in all five populations exposed to P limitation (P,
297 Figure 1A). Declines in P^* ranged from 43% to 85% across the replicate populations. In
298 response to N limitation, N^* declined in two populations (14%, 34% decline), did not
299 change in two populations and increased in one population (47% increase) (N, Figure
300 1B). I^* increased in two populations exposed to low light (L, 12%; 28% increase), and
301 did not change in the remaining three populations exposed to low light (Figure 1C).
302 Salt tolerance increased in all populations exposed to high salt (93% - 369% S and BS,
303 Figure 1D). Consumption vectors, quantified as the P:N molar ratio in the biomass of
304 populations growing exponentially, decreased in all of the populations subjected to
305 nitrogen limitation, and increased in four of the five populations exposed to
306 phosphorus limitation (ESM Figure S4, see also Figures S5-6). This suggests that
307 populations selected under nitrogen limitation contained more nitrogen relative to
308 phosphorus whereas populations selected under low phosphorus contained more
309 phosphorus relative to nitrogen. Contrary to our predictions, the descendants of the

genotypically diverse cc1690 population did not show more trait change than any of the isoclonal populations (triangles vs small dots in Figure 1). However, the genotypically diverse cc1690 population did match our predictions in terms of the direction of adaptive trait change in all selection environments: P^* decreased under P-limitation, N^* decreased under N-limitation, salt tolerance increased in the high salt environment, and I^* decreased under low light, though the change in I^* was not statistically significant.

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

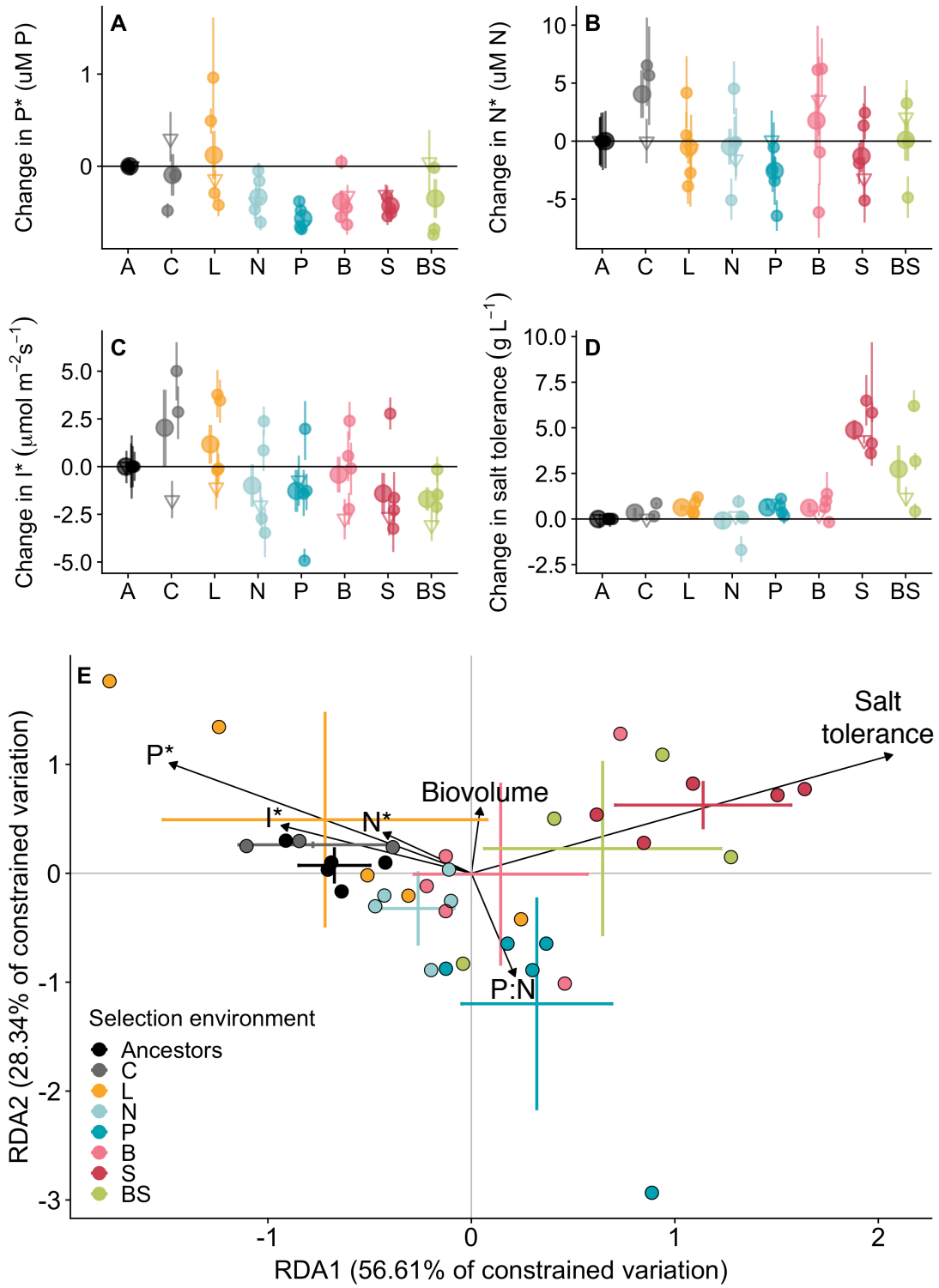


Figure 1. Changes in resource competition traits relative to ancestors in seven different selection environments (A: ancestors, C: COMBO, L: light-limited, P: P-limited, N: N-limited, B: biotically depleted media, S: high salt, BS: biotically depleted and high salt).

340 Small points correspond to individual populations and large points correspond to the
341 average change (error bars are ± 1 SE) of all populations in a given environment.
342 Populations represented with a triangle are the genotypically diverse populations
343 (cc1690), circles are isoclonal. Error bars on small points in A-E correspond to 95% CI
344 from non-parametric bootstrapping. E) Redundancy Analysis of N^*, I^*, P^*, salt tolerance,
345 consumption vectors and cell biovolume across selection environments.
346
347 ***The structure of trait variance: observed trade-offs***
348 Maximum growth rate across populations increased with minimum resource
349 requirements (R^*) for light (OLS slope = 0.021, 95% CI: 0.0027, 0.039, Adjusted R^2 =
350 0.11), for nitrogen (OLS slope = 0.019, 95% CI: 0.0078, 0.031, Adjusted R^2 = 0.30), and
351 for phosphorus (OLS slope = 0.12, 95% CI: 0.0094, 0.23, Adjusted R^2 = 0.13),
352 indicating a trade-off between growth at high and low resource supplies (because a
353 lower R^* indicates faster growth at minimum resource levels) (Figure 2, A-C). Across
354 populations, competitive abilities for N and P (CN and CP) were positively associated
355 (ESM Tables 4-6, Figure 2D, ESM Figures S13-15). After accounting for covariance with
356 competitive abilities for other resources and ancestor ID, competitive abilities for light
357 were negatively associated with cell biovolume, while and N and P competitive abilities
358 were not related to cell size (ESM Tables 4-6, ESM Figures S13-15). Principal
359 components analysis of cell biovolume, competitive abilities for light, nitrogen and
360 phosphorus showed that 74% of the variation in cell volume and competitive abilities is
361 explained by the first two PC axes. The first two PC axes demonstrate a positive
362 association between competitive abilities for N and P, and a possible trade-off
363 between biovolume and competitive ability for light (Figure 2D).
364

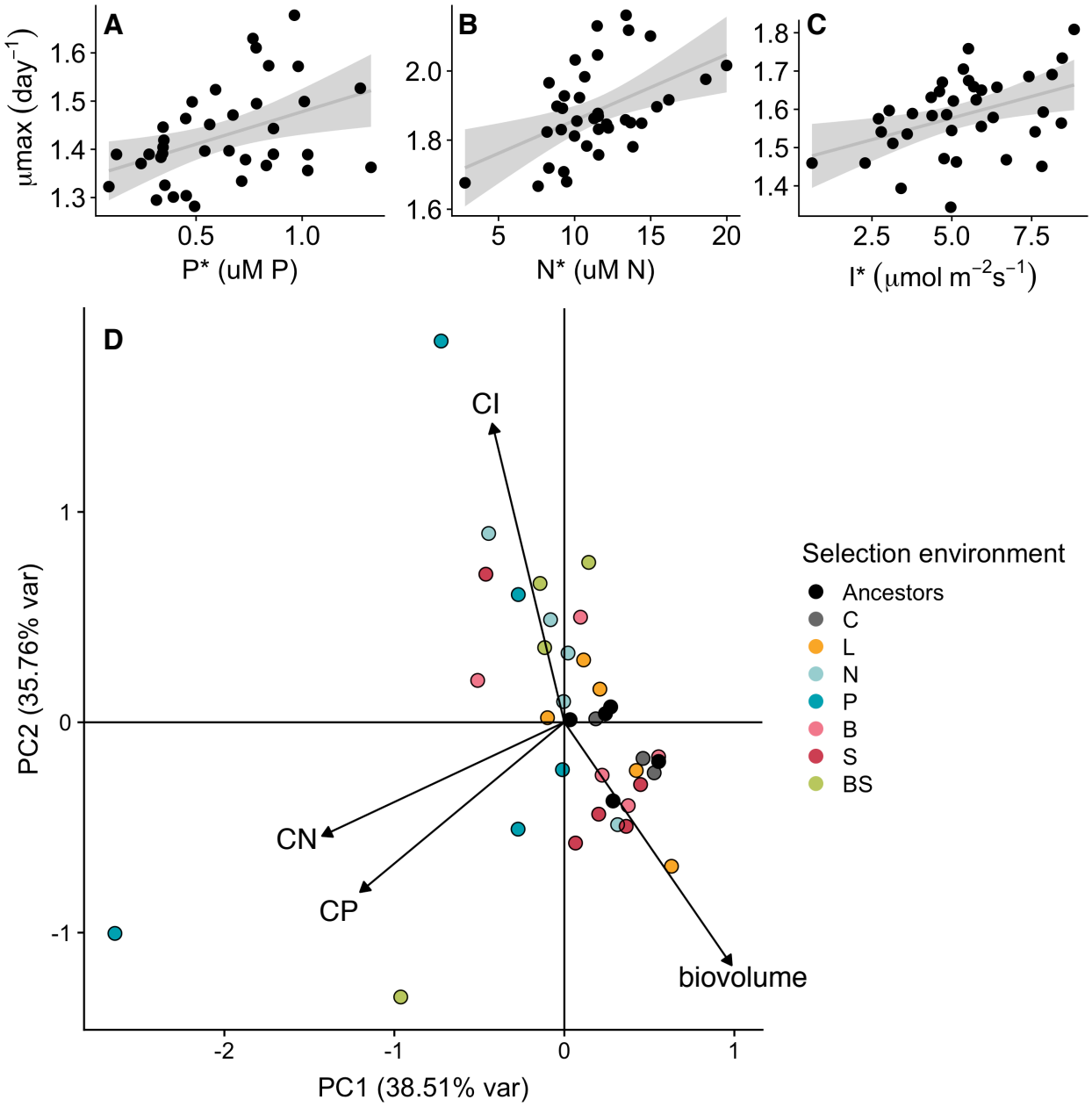


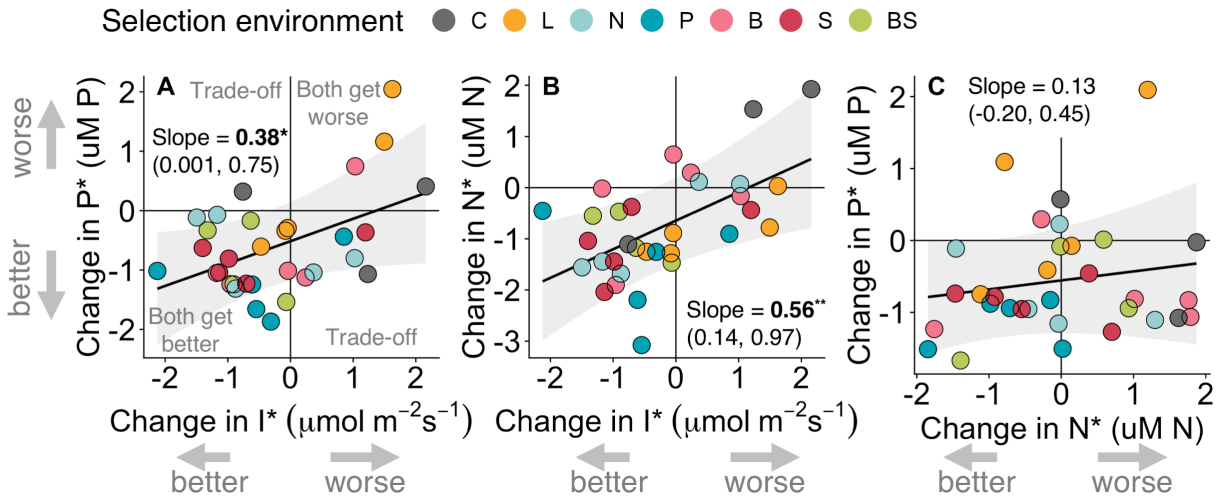
Figure 2. High maximum population growth rates (μmax) are positively associated with high minimum resource requirements for phosphorus (A), nitrogen (B) and light (C). Estimates of μmax and R* in A-C are from Monod curves generated over independent gradients of phosphorus, nitrogen and light. D) Loadings on the first two PC axes of a PCA using competitive abilities for phosphorus, nitrogen and light (CP = 1/P*, CN = 1/N*, CI = 1/I*) and cell biovolume. 74.3% of the variation in resource use traits and cell size is explained by the first two PC axes.

373

374 ***Correlations in changes across traits***

375 Though theory often assumes that competitive abilities for different resources are
376 negatively related [15,40], our results did not support this finding either when
377 considering absolute variation in competitive abilities (ESM Tables 4-6) or variation in
378 the change in R* relative to the ancestral populations (Figure 3; ESM Tables 1-3, ESM
379 Figure S10). The changes in R* for different resources never showed evidence of any
380 trade-offs, and instead were either positively associated (Figure 3A, B) or showed no
381 significant relationship (Figure 3C, ESM Figure S10).

382



383

384 **Figure 3.** Partial regression plots showing how changes in descendants relative to
385 ancestors in two traits are related to each other, while holding all factors in the
386 statistical model that are not being displayed constant (complete model results in ESM
387 Tables 1-3). Positive slopes indicate positively associated trait changes. Grey arrows
388 indicate the directions in which descendant populations' traits got better or worse
389 relative to ancestors. Panel A describes the four quadrants delineating areas where
390 both traits get better, both get worse, or one gets better while the other gets worse
391 (trade-off).

392

393 ***Genetic changes after selection***

394 Genetic differences between the ancestral and descendant populations were identified
395 by whole genome re-sequencing. The presence or absence of single nucleotide
396 polymorphisms (SNPs) identified within the populations were compared between the
397 ancestors and descendants for each selection treatment. The number of variable SNPs
398 ranged from 396 to 582 (ESM Table 7), roughly corresponding to mutation fixation
399 rates of 1.25e-8 to 1.83e-8 mutations/[sites × generations]. Contrary to our
400 expectations, salt stress did not increase the number of fixed mutations. Selection
401 treatment had no significant effect on the total number of fixed mutations (ESM Figure
402 S11 A; ANOVA $p = 0.788$), but the effect of the ancestor was highly significant (ESM
403 Figure S11 B; ANOVA $p < 1e-7$).
404
405 ***Evolutionary adaptation altered the predicted outcomes of competition***
406 Divergence in minimum resource requirements and consumption vectors among
407 populations of the same ancestor selected in different environments (Figure 1E) was
408 sufficient in some cases to lead to predicted coexistence. One such example is
409 illustrated in Figure 4, where descendants of Ancestor 3 selected in low light and high
410 salt environments have diverged sufficiently in their P^* and N^* such that they could
411 possibly coexist. While neither descendant population could coexist with Ancestor 3,
412 the two descendant populations could coexist with one another at a supply point
413 illustrated as a yellow dot. In their ancestral state, out of all pairwise combinations of
414 our five ancestor populations, resource competition theory [1] predicts unstable
415 coexistence in three of 10 cases and competitive exclusion in seven of 10 cases. After
416 selection across the range of environments in our study, resource ratio theory predicts
417 unstable coexistence in 19% of all possible pairwise interactions (698 total), stable
418 coexistence in 28% of all possible pairwise interactions and competitive exclusion in
419 53% of all possible interactions (ESM Figure S12). Among populations selected in the
420 same environment (59 total), resource ratio theory predicts competitive exclusion in
421 49%, stable coexistence in 32%, unstable coexistence in 19%.
422
423

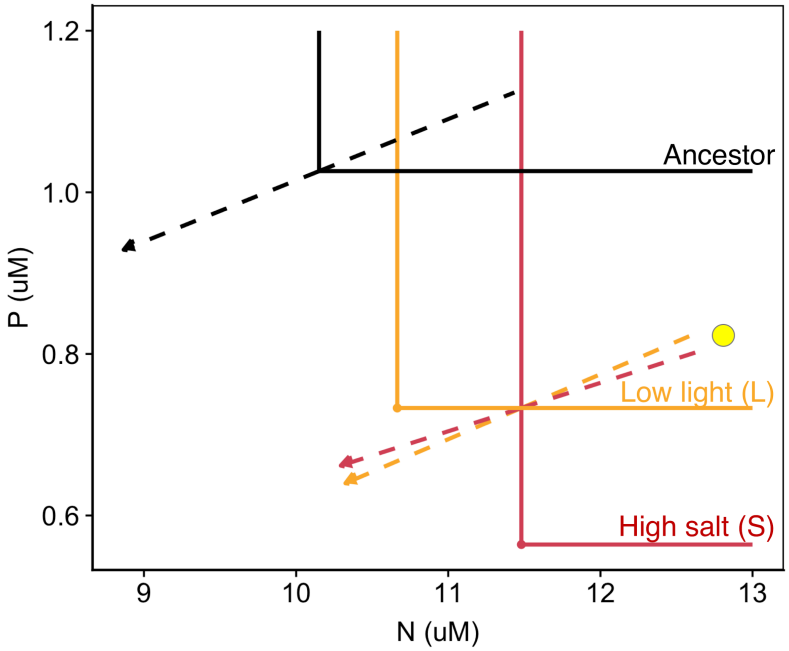


Figure 4. Descendants of Ancestor 3 evolved in high salt (red) and low light (orange) environments have diverged in their P^* and N^* such that they can co-exist. Neither descendant could co-exist with the ancestor. Zero net growth isoclines (solid lines) and consumption vectors (dashed lines) for Ancestor 3 (black) and the descendant of Ancestor 3 selected in low light (orange) and high salt (red).

Discussion

Resource competition is among the most important processes structuring ecological communities [41], but competition theory often assumes that traits underlying competitive abilities remain fixed over ecological time scales [1,42]. Here we found that the traits that underlie competitive abilities for essential resources can adapt rapidly in new resource-limited environments. Populations often adapted to resource limitation by reducing their minimum resource requirements. When exposed to high salt, populations evolved higher salt tolerances. Not only could populations respond adaptively to new environments, but they could also adapt within approximately 285 generations. While we observed gleaner-opportunist trade-offs, we did not find evidence for trade-offs in competitive abilities for different resources. Instead, adaptive

443 changes in competitive ability for one resource were often positively associated with
444 improvements in competitive ability for another. Since the ancestral and descendant
445 populations were maintained under identical conditions when estimating their traits,
446 the changes we observed were heritable.
447
448 Trade-offs in resource-use traits have been invoked to explain changes in dominance
449 across supply ratio gradients and the coexistence of as many species as resources
450 [1,18]. Trade-offs in competitive abilities for nitrogen and phosphorus [19], iron and
451 light [23], and light and nitrogen [24] have been documented among and within
452 species of phytoplankton. These trade-offs may arise due to local adaptation, or due to
453 biophysical constraints on the acquisition or metabolism of different resources [18,40].
454 Individuals may invest resources into two main types of cellular machinery: uptake or
455 assembly machinery. Uptake machinery is composed of nutrient uptake proteins and
456 chloroplasts, which are both relatively nitrogen-rich, and both of which may scale with
457 cell size because uptake and photosynthesis must take place at the cell surface.
458 Assembly machinery, primarily composed of ribosomes, is relative phosphorus-rich and
459 may also depend on cell size, as smaller cells tend to grow faster ("growth rate
460 hypothesis" [43]). Consistent with expectations, competitive abilities for light were
461 negatively associated with cell size, but in contrast to expectations, N and P
462 competitive abilities were not. Furthermore, we did not find evidence for a trade-off
463 between competitive abilities for nitrogen and phosphorus.
464
465 Trade-offs between competitive abilities for different resources may not arise if the
466 same resource is required for multiple metabolic processes. For example, positive
467 correlations in changes in competitive ability, such as we observed, may arise because
468 the ability to grow in one resource-limited environments results from multiple
469 components of metabolism becoming more efficient simultaneously [44–46]. For
470 example, selection under low light could cause individuals to increase the efficiency
471 with which nitrogen-intensive chloroplasts harvest light, thereby simultaneously leading
472 to reduced nitrogen requirements.
473

474 Patterns of genotypic variation across populations revealed negative correlations
475 between fitness at low and high resource supply for a given resource (gleaner-
476 opportunist trade-offs, Figure 2), and positive correlations between competitive
477 abilities for different resources (Figure 1E, ESM Tables 4 - 6), suggesting that the
478 evolution of competitive ability could be constrained by genetic correlations between
479 multiple resource traits under selection. The genetic correlations between different
480 resource traits could explain the positively associated trait changes (i.e. improvements
481 in multiple minimum resources requirements simultaneously). In addition, it is possible
482 that unmeasured traits can be involved in the trade-off, resulting in a positive genetic
483 covariance between any two resource traits [17]. When testing for trade-offs, we
484 accounted for concurrent variation in cell size, but other fitness-correlated traits that we
485 did not measure, such as resistance to grazers or pathogens [47,48], may be involved in
486 the trade-offs.
487
488 Traits relevant to competitive ability, such as cell size, are known to change as a result
489 of phenotypic plasticity and evolutionary adaptation [49]. We have demonstrated that
490 adaptation in response to resource limitation and salt stress can alter competitive traits
491 sufficiently to change the predicted outcome of competition. Adaptation to different
492 environments caused competitive traits to diverge and enable coexistence. Contrary to
493 our expectations, we did not find that coexistence would be more likely among two
494 populations selected in different environments than two populations selected in the
495 same environment. This may be explained by the fact that even small differences in the
496 magnitude of adaptive trait change in the same environment can be sufficient to
497 enable predicted coexistence (i.e. under P-limitation, P^* for one competitor decreases
498 slightly more than the P^* for the other competitor). The changes in resource ratios and
499 salt levels represented in our different selection environments are on the same order of
500 magnitude as gradients of resource ratios and salinity in natural environments [50]. This
501 means that predictions of the outcomes of competition should incorporate the
502 potential for evolutionary changes to influence competitive dynamics [15].
503

Our results are directly relevant to understanding eco-evolutionary feedbacks in competitively structured communities. Theory predicts that species will converge in their resource-use traits when competing for essential resources [14,15]. This expectation, however, depends on two critical assumptions. These assumptions are that species' consumption vectors remain fixed and that competitive abilities for different limiting resources trade off. While we did not grow pairs of populations together in the evolution experiment, the resource limitation treatments mimicked the effects of a better competitor for the limiting resource, while avoiding exclusion of the poorer competitor. Our results do not provide empirical support for either of the assumptions above, suggesting that theoretical predictions of evolutionary adaptation under essential resource competition may need to be revised [14,15].

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

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