Temperature alone is not enough: food-web context determines evolutionary responses to warming

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ABSTRACT

Global warming is reshaping food webs globally. Rapid evolution has been proposed as a buffer against climate change, but how simultaneous shifts in biotic and abiotic environments may influence evolution is unknown. Using experimental evolution and mathematical modeling in microbial food webs of prey algae and ciliate predators, we tested 1) how temperature affects prey evolution and 2) how the food-web context—i.e., predator identity, abundance, and competition among predators— mediates prey evolutionary dynamics. We found that temperature alone does not drive prey evolution unless predators are present, and food-web context determines ensuing evolutionary dynamics. These seemingly complex evolutionary responses are predictable from the joint effects of temperature-dependent, predator-specific predation rates, and the emergence of temperature-dependent prey plasticity. We reveal that evolutionary outcomes under warming are shaped by the broader food web context of species, suggesting that the same species may exhibit different eco-evolutionary responses in different food webs under novel climates.

SIGNIFICANCE

Predicting how species evolve under climate change is critical for understanding future changes in food webs. Evolutionary responses have long been known to be driven by environmental change—like temperature—but whether and how ecological interactions influence this process is unknown. Using experimental evolution and mathematical modeling, we show that temperature alone does not drive prey evolution. Instead, the broader food webs context—predator identity, abundance, and competition—mediates how species evolve under warming. Additionally, we demonstrate that prey evolution depends on temperature-dependent predator-specific predation rates and prey plasticity. Our findings highlight that the same species may evolve differently within different food webs, urging the need to integrate ecological interactions when forecasting evolutionary responses to climate change.

INTRODUCTION

Global warming is reshaping food webs worldwide (Barbour & Gibert 2021) through changes in population growth (Dell *et al.* 2011; Frazier *et al.* 2006; Kontopoulos *et al.* 2020) and species interactions (Blois *et al.* 2013; Dell *et al.* 2014). Additionally, increasing temperature raises metabolic costs (Clarke 2006; Clarke & Fraser 2004), forcing predators to consume more prey (Sheridan & Bickford 2011) while gaining diminishing energetic returns (Barneche *et al.* 2021). This reduced energetic intake results in the energetic choking of upper food-web trophic levels, food web rewiring (Barbour & Gibert 2021; Bartley *et al.* 2019), compositional turnover (Komatsu *et al.* 2019), and trophic collapse (Ullah *et al.* 2018; Voigt *et al.* 2003; Zarnetske *et al.* 2012).

Species evolution both influences and is influenced by novel biotic and abiotic conditions. For example, rising temperature could directly influence evolution through effects on organismal metabolism (Alton *et al.* 2024; Clarke 2003), morphology (Diamond *et al.* 2017; Yampolsky *et al.* 2014), and fitness (van Heerwaarden & Sgrò 2021; Padfield *et al.* 2016). On the other hand, predation and competition within food webs can influence evolution directly (Abrams 2000; Frickel *et al.* 2017), for example, by selecting in favor of better defended prey that are worse at competing for resources (Yoshida *et al.* 2004). In a warming world, however, changes to abiotic drivers of selection, like temperature, can influence biotic drivers of selection, like predation or competition, urging joint study of these effects.

72 Indeed, temperature influences demographic and functional traits asymmetrically among
73 interacting species (Dell *et al.* 2014; Gibert *et al.* 2022), shifting species interactions and the
74 potential for species rapid evolution under warming (Kordas *et al.* 2011; Tseng & O'Connor
75 2015). Higher temperature and predation can also trigger plastic responses that fundamentally
76 alter ecological dynamics, species interactions, and ensuing selection (Agrawal 2001). Species
77 evolutionary responses in a warming world are thus likely jointly determined by the effects that
78 rising temperature and resulting shifts in species interactions (i.e., food web context) may have
79 on phenotypic plasticity and selection. However, how these joint effects may affect evolutionary
80 responses within food webs in a warming world is not known, thus greatly hindering our ability
81 to anticipate the fate of species in new climates.
82
83 We addressed this central issue through a combination of mathematical models and microcosm
84 experiments in a model system of global distribution and importance (Bar-On *et al.* 2018; Herron
85 *et al.* 2019): the interaction between the unicellular green alga *Chlamydomonas reinhardtii* and
86 its ciliate predators, which co-occur within broader microbial food webs across ecosystems, from
87 soils to wetlands (Foissner *et al.* 2009). While evolutionary change operates in all food webs,
88 addressing how it might change in novel climates is logistically impossible to test in nature. Our
89 microbial system breaks down this intractability issue while remaining relevant to the natural
90 world through the pivotal role of microbes in all ecosystems worldwide (Foissner *et al.* 2009).
91 We thus assembled tractable microcosm food webs and studied the combined effects of
92 temperature, predation, and predator competition (as a proxy for food web context), on prey
93 evolution. We tracked population dynamics, rapid shifts in prey genetic makeup (i.e., rapid
94 evolution), and plastic phenotypic changes, to disentangle how temperature and food web

95 context co-determine eco-evolutionary outcomes within these microbial food webs across three
96 temperatures.
97
98 RESULTS
99 *Predation maintains genetic diversity and influences eco-evolutionary outcomes*
100 To make testable predictions on how predation could influence prey evolution, we used a
101 mathematical model of rapid evolution that keeps track of two genetically distinct prey strains
102 under predation (see Methods). The model suggests that different prey strains cannot coexist in
103 the absence of predators –classically, unless intra-strain competition is higher than inter-strain
104 competition– (Appendix I Equation 1-2). Predation, however, facilitates the invasion of the strain
105 that would otherwise be lost (Appendix I Equations 1,3), thus maintaining genetic diversity. We
106 tested these theoretical predictions using an experimental predator-prey microbial system where
107 the alga *Chlamydomonas reinhardtii* was preyed upon by one of three possible ciliate protist
108 species (*Glaucoma sp.*, *Tetrahymena pyriformis*, *Paramecium caudatum*, see Methods). The
109 algal prey population was composed of two genetically and phenotypically distinct strains – i.e.,
110 the fluorescently tagged wild type (WT) and untagged *vfl1-1* (*v*ariable *fl*agella) genotypes (Fig.
111 1). We kept track of prey abundances, phenotypes, and genetic frequencies over time to quantify
112 both plastic and evolutionary responses (see Methods). Our empirical results corroborated our
113 model predictions. In the absence of predators, WT consistently outcompeted *vfl1-1*; However,
114 predation either 1) facilitated the persistence of *vfl1-1* that was lost under control conditions, or
115 2) slowed down the rate of *vfl1-1* loss (Fig. 2a-d; Appendix II Figure 1-2). By altering the
116 temporal dynamics of prey genetic frequencies, predator-prey interactions can determine
117 evolutionary outcomes.

Different predators selected for WT and *vfl1-1* differently, as indicated by differences in *vfl1-1* frequencies across predator treatments (Fig. 2a-d; Appendix IV Table 1-2). Interestingly, our model can reproduce these effects and suggest that differences in predator attack rates across genotypes alone should be sufficient to explain differences in prey evolutionary outcomes across predators (Fig. 2a-b, model parameter see Appendix I Table 1). This mathematical understanding thus offers a simple explanation for otherwise seemingly complex dynamics, and shows that predator-specific differences in predation rates influence the eco-evolutionary process and prey genetic makeup.

Prey traits influence eco-evolutionary outcomes

In our experimental system, predation can be influenced by two key prey functional traits: motility and defensive clumping. Strain *vfl1-1* has impaired swimming ability compared to WT (Adams *et al.* 1985;Appendix V videos) and is predicted by theory to experience lower predation rates relative to the more motile WT, all else equal (Aljetlawi *et al.* 2004; González *et al.* 1993; Pawar *et al.* 2012; Visser 2007). Our data supports this prediction as *vfl1-1* is selected against in the absence of predators, and selected in favor in the presence of most predators (Fig. 2 c-d) owing to decreased predation through impaired motility relative to WT.

However, when predation pressure is high enough, WT can develop plastic defensive clumps (Lurling & Beekman 2006), while *vfl1-1* cannot. We quantified the onset of this clumping defensive trait as cytometric changes in algal particle size over time: a faster increase in average particle size over time should indicate a quicker onset of defensive clumping. Genotype *vfl1-1*

141 showed no significant clumping in response to predation (Fig. 2e yellow, Appendix IV Table 3)
142 while WT showed strong plastic clumping against predation (Fig. 2e green; Appendix II Fig. 3-4;
143 Appendix IV Table 4). The magnitude of the clumping, as measured by the shape of the
144 distribution of WT particle size, varied by predator (Fig. 2e, Appendix IV Table 4), indicating
145 predator-specific clumping responses likely resulting from model-predicted differences in
146 predation rates. Last, we found differences in the relative fitness of motility and defensive
147 clumping traits that are predator-specific. For example, *Glaucoma sp.* selected strongly in favor
148 of *vfl1-1* while predator *P. caudatum* drove *vfl1-1* to extinction (Fig. 2c-d). These results show
149 that food web context not only influences prey evolution, but also determines how prey traits
150 shape the evolutionary process itself.
151
152 *Temperature only affects eco-evolutionary outcomes in the presence of predation*
153 Temperature alone increased both WT and *vfl1-1* growth rates (Appendix II Fig. 5, Appendix IV
154 Table 5), but did not affect prey rapid evolution –measured as rapid shifts in genetic frequencies–
155 on its own (Fig. 3a Control panel; Appendix II Figure 2). However, temperature significantly
156 influenced prey rapid evolution in the presence of predation, indicating that the temperature
157 effects on prey evolution are mediated by predation (Fig. 3a; Appendix IV Table 6). We propose
158 two concurrent mechanisms to explain this result: first, temperature influences prey evolution
159 through differences in predator thermal performance and predation rates, leading to changes in
160 selection on prey phenotypes (Fig. 3d mechanism 1). Second, temperature can affect prey
161 evolution through its effect on the timing and magnitude of prey plastic responses induced by
162 predation (Fig. 3d mechanism 2). Importantly, in the absence of predators, neither mechanism is

163 at play, explaining why temperature does not drive prey evolution in this system on its own, and
164 how food web context can influence the evolutionary outcome.
165
166 To illustrate how these mechanisms explain the observed eco-evolutionary dynamics, we focus
167 on predation by *P. caudatum* as a case study. The first mechanism was evidenced by
168 temperature-dependent changes in the predator demographic parameters that underpin their
169 population dynamics: the intrinsic growth rate, *r* (Appendix II Fig. 6, Appendix IV Table 7), and
170 predator maximum abundances, which serve as a proxy for the carrying capacity, K (Fig. 3b,
171 Appendix II Fig. 6, Appendix IV Table 7). Both r and K are tied to predation intensity, as faster
172 population growth (r) and larger carrying capacity (K) require increased predation to fuel and
173 maintain biomass growth. Consequently, increased predation accelerated prey evolutionary
174 dynamics by exerting stronger selection on the prey, resulting in increased predation rates and
175 stronger selection against WT for its high motility in the early stage of the experiment (or,
176 conversely, in favor of *vfl1-1*, Fig. 3b data panel, bars left of dash line; Appendix IV Table 2, 6).
177 Evidence for the second mechanism comes from the differences in the timing and magnitude of
178 WT plastic responses across temperatures (Fig. 3c and Appendix II Fig. 3). With the onset of
179 plastic clumping (Fig. 3b, grey dot in conceptual panel), WT had higher relative fitness than *vfl1-*
180 *1* in the later stages (Fig. 3b conceptual panel), leading to a decline in the less-defended *vfl1-1*
181 (Fig. 3b data panel, bars right of the dash line). Importantly, temperature-dependent predation
182 rates (Fig. 3b) influence the clumping response through changes in the distribution of WT
183 particle size, including mean, median, variance, skewness, and kurtosis (Fig. 3c; Appendix IV
184 Table 8). The intensified WT plastic responses at higher temperatures (Fig. 3c) and the
185 subsequent temperature-dependent drop in *vfl1-1* frequencies (Fig. 3b) further evidenced the

186 effects of temperature on the anti-predator defense and ensuing eco-evolutionary dynamics (Fig.
187 3b).
188
189 *Predator competition modulates how predation affects prey evolution across temperatures*
190 To understand the importance of the broader food web context, we introduced a second predator
191 (i.e., a predator competitor) and assessed their effect on the observed eco-evolutionary dynamics
192 across temperatures (Fig. 4). We did so by quantifying the similarity between prey evolutionary
193 dynamics in single-predator vs predator-competition treatments using Earth Mover's Distance
194 (see Methods). First, we discovered that the directional selection applied by each predator
195 individually is attenuated when two competing predators favor distinct strains (Fig. 4).
196 Specifically, prey evolutionary dynamics of single predator treatments were least similar to one
197 another between each pair of the competing predators (e.g. prey evolutionary dynamics of
198 *Glaucoma sp.* and *P. caudatum* single predator treatments have high EMD score, or low
199 similarity; Appendix III Fig. 1). In contrast, the evolutionary dynamics of competition treatments
200 (e.g. *Glaucoma sp.* + *P. caudatum*) showed higher similarity to the evolutionary dynamics of
201 either single predator treatments (Appendix III Fig. 1).
202
203 Second, we found that predator competition mediated temperature effects on prey evolution (Fig.
204 4; Appendix IV Table 9-12) through the same mechanisms described previously (Fig. 3d).
205 Particularly, differences in predator thermal performance drive differences in their relative
206 contribution to selection on prey traits across competition treatments (Appendix II Fig. 7a),
207 leading to temperature effects on prey evolution in the presence of predation and predator
208 competition. For example, predation favored *vfl1-1* in all competition treatments where *T.*

209 *pyriformis* and *Glaucoma sp.* had their highest thermal performance in competition with
210 *P.caudatum,* (i.e. highest r and max population density at 19°C, Appendix II Fig. 6; Fig. 3d
211 mechanism 1; Fig. 4) and WT showed the slowest clumping response (Appendix II Fig. 7b),
212 resulting in strong selection against WT (Fig. 4; Appendix II Fig. 7a; Fig. 3d mechanism 2).
213 However, at warmer temperatures, *P. caudatum* became more abundant and performed better
214 (Appendix II Fig. 6), exerting stronger selection on prey than its competitors (Fig. 3d mechanism
215 1; Fig. 4). This resulted in faster and stronger plastic response by WT (Fig. 3d mechanism 2),
216 selection in favor of WT, and accelerated loss of *vfl1-1* in competition treatments at high
217 temperature (Fig. 4, 25°C; Appendix Fig. 7a). Consequently, prey evolutionary dynamics in
218 competition treatments more closely resembled those in *T. pyriformis* and *Glaucoma sp.* single
219 predator treatments at cooler temperatures, but more resembled that in *P. caudatum* treatment at
220 higher temperatures (Appendix III Fig. 1). Taken together, these results demonstrate food web
221 context influences the effects of temperature on these eco-evolutionary dynamics in a predictable
222 way through the interplay between prey plasticity, predator thermal performance, and
223 competitive ability within the broader food web. The density dynamics of both prey genotypes
224 and all predator species across all treatments can be found in Appendix II Fig. 8.
225
226 DISCUSSION
227 Our results reveal that temperature strongly but indirectly affects prey evolution within food
228 webs through differences in temperature-dependent predation rates across genotypes and prey
229 defensive plastic responses (Figs. 2-3). In the presence of multiple predators, differences in
230 predator performance across temperatures leads to predator-specific differential selection across
231 prey genotypes–which would not occur in the absence of predation and ultimately lead to

differences in prey evolutionary outcomes across food web contexts (Fig. 4). We highlight the significance of the broader food web context in shaping species evolution under global warming, and show how even identical species in different food webs may respond to warming in different ways.

Plasticity influences prey rapid evolution

Our study shows that predator differential selection on multiple prey functional traits (i.e. motility and clumping) facilitates the maintenance of additive genetic diversity (Fig. 2, Siepielski *et al.* 2020)). Moreover, we revealed the reciprocal effects of prey functional traits and species interactions, and how these underpin the dynamics of species evolution within food webs (Fig. 3). While previous studies have shown that more motile prey experience higher predation rates compared to slower prey (Andersen & Dölger 2019; González *et al.* 1993), we found that the relative fitness of the less motile *vfl1-1* strain was influenced by the onset of a plastic defensive trait in the WT strain, itself temperature-dependent (Fig. 3). The onset of this trait-mediated eco-evolutionary process sometimes led to a new equilibrium in which all genetic variants persisted, or one in which only one did (Fig. 3). Consequently, dynamic, temperature-dependent plastic responses can significantly alter the course of the evolutionary process and influence the fate of genetic variants in a warming world. However, this process is vastly underappreciated and poorly understood in food web ecology.

Predator identity and food web context affect prey plastic responses

We showed that evolutionary outcomes are strongly dependent on predator identity and the broader food web context, as the interplay between rapid plasticity and evolution can shift

255 predator preferences (Fig. 3). Additionally, multiple predators can have joint effects in shaping
256 prey plastic and evolutionary responses (Fig. 4; Appendix III Figs. 1-2), which is itself
257 contingent on the composition of the food web. Indeed, prey evolutionary dynamics under
258 selection by multiple predators reflected the combined effects of selection imposed by individual
259 predator species (Fig. 4; Appendix III Fig. 1). Phenotypic change, plastic or evolutionary, can
260 thus determine the fate of organisms within food webs: shifts in one functional trait in response
261 to one predator may affect the interaction with another predator, potentially cascading through
262 the entire community, as had been predicted by theory (Cosmo *et al.* 2023; Guimarães *et al.*
263 2017). Our study highlights the importance of achieving a mechanistic understanding of the
264 interplay between species functional trait dynamics and biotic interactions across trophic levels
265 to better understand their fate in a changing world (Henn *et al.* 2018).
266
267 *Food web context and temperature interactively drive prey evolution*
268 While temperature-mediated changes in species interactions have been hypothesized to drive
269 changes in species plastic and evolutionary functional traits in a warming world (Barbour &
270 Gibert 2021; Fischer *et al.* 2016; Fordyce 2006), empirical support for this hypothesis is lacking.
271 We show that reciprocal effects between food web context and trait dynamics together drive prey
272 evolutionary trajectories differentially across temperatures (Figs. 3-4). If food-web context and
273 temperature jointly influence rapid changes in the genetic makeup of populations, as shown here,
274 warming could severely impact species persistence across in warming food webs. For example,
275 we show that predation and predator diversity can maintain prey trait diversity (Fig. 2).
276 However, observed increasing extinction rates among predators under warming (Thunell *et al.*
277 2021; Zarnetske *et al.* 2012) may prompt a decrease in predator diversity and subsequent

reduction in prey genetic and trait diversity, in turn decreasing potential for future adaptive evolution. This loss of genetic diversity could be most pronounced when dominant predators disproportionally affect prey evolution under warming (e.g., *P. caudatum*, Fig. 4), particularly in simpler food webs at higher latitudes (Gibert 2019). Standing plasticity and the evolution of plasticity have the potential to alter these outcomes, underlining the importance of understanding their ecological consequences in a rapidly changing world. Last, while species evolution is shaped by the food web context (Figs. 2-4), the composition and network complexity of food webs are rapidly changing, which should have important but currently unknown effects on evolutionary outcomes (Barbour & Gibert 2021).

CONCLUSIONS

Our research reveals that temperature influences prey evolution exclusively in the presence of predators, highlighting the crucial role of food-web context in determining eco-evolutionary outcomes. While rapid evolution has been proposed as a potential buffer against the impacts of climate change on natural communities, our study underscores that anticipating evolutionary outcomes and species fate in novel climates demand a deeper understanding of evolutionary responses across food-web contexts and accounting for rapid shifts in these food webs as the globe warms.

METHODS

Experimental work

Unicellular microalgae can be found in diverse habitats, from soil to freshwater ecosystems worldwide (Arora & Sahoo 2015; Falkowski 1994). These organisms are at the base of all food

webs, fueling both green and brown energy pathways (Guo *et al.* 2016), and are routinely preyed upon by microbes (e.g., ciliate protists) and metazoans (e.g., rotifers, cladocerans) alike (Calatrava *et al.* 2023). Our focal prey species, the unicellular green alga *Chlamydomonas reinhardtii*, is a well-established model organism with known mutations linked to functional traits (Calatrava *et al.* 2023; Sasso *et al.* 2018; Tulin *et al.* 2024). Here we used two genetically and phenotypically distinct strains of *C. reinhardtii*: wild type (WT) and *vfl1-1* (*v*ariable *fl*agella) as our prey population.

Wild type (WT) possesses two flagella that allow the entire range of normal locomotive behaviors (Huang 1986) and can form large cell clumps, a common form of defense against predation (Herron *et al.* 2019). Strain *vfl1-1* produces individuals with variable numbers of flagella from 0 to 10 and defective swimming ability (Adams *et al.* 1985). Additionally, *vfl1-1* seems unable to clump under predation. To distinguish these two strains and keep track of their relative frequencies in mixture populations, we used mNeonGreen fluorescent protein to tag WT populations and used flow cytometry to count tagged WT vs non-tagged *vfl1-1* individuals over time (Fig. 1a). Both strains exhibit autofluorescence (Fig. 1a), but only one (WT) will show fluorescence in the near green spectrum (Fig. 1a). We used two protist predators of similar body size, *Tetrahymena pyriformis* and *Glaucoma sp.*, as the focal predators, and a larger protist, *Paramecium caudatum*, as a competitor for the focal predators (Fig. 1b). All four species are commonly found in freshwater and soil systems (Cornwallis *et al.* 2023; Foissner & Berger 1996).

323 To understand how temperature and food web context (i.e., presence of predators and predator
324 competitors) jointly influence *C. reinhardtii* evolution, we set up experimental microcosms in
325 autoclaved 250 ml borosilicate jars filled with 100 ml of 9:1 COMBO media (Kilham *et al.*
326 1998): timothy hay infusion (Brans *et al.* 2022). Each microcosm was assigned to one of three
327 possible predation treatments (no predation/Control, + *T. pyriformis*, + *Glaucoma sp.*), one of
328 two possible predator competition treatments (+ *T. pyriformis* & *P. caudatum*, + *Glaucoma sp.* &
329 *P. caudatum*), and one of three possible temperatures: 19°C, 22°C, and 25°C. The manipulations
330 produced a factorial design with 18 combinations of treatments (Fig. 1b), each replicated six
331 times, yielding a total of 108 microcosms.
332
333 Prior to experimentation, the algae strains were maintained on TAP agar (Gorman & Levine
334 1965) at room temperature. Protist cultures were maintained in bacterized timothy hay protist
335 media at 22°C and a 16:8-hour light-dark cycle. All cultures were transferred to 9:1 COMBO
336 media: timothy hay protists media and cultured under the same light and temperature regime as
337 protist stock cultures 2 weeks prior to experimental work. We carried out the experiment in two
338 blocks on two consecutive days; each block had half of the replicates in all treatments. We
339 started WT and *vfl1-1* strains at equal densities of 2000 individuals/ml in all microcosms and
340 initialized *T. pyriformis*, *Glaucoma sp.*, and *P. caudatum* populations at density of 5 ind/ml, 5
341 ind/ml, and 0.5 ind/ml, respectively. The experiment was carried out for 15 days, or ~ 30-45 *C.*
342 *reinhardtii* generations under control conditions at the three focal temperatures.
343
344 We recorded all species densities on days 0, 5, 10, and 15. We used flow cytometry (NovoCyte
345 2000R, Agilent, CA, USA) to distinguish the two genetically and phenotypically distinct strains

346 of *C. reinhardtii*: mNeonGreen tagged wild type (WT) and *vfl1-1* (*v*ariable *fl*agella). This
347 allowed us to track the abundances and frequencies of both strains over time. We used forward
348 scatter height (FSC-H) as a measure of cell/cell clump size of *C. reinhardtii* (Adan *et al.* 2017) to
349 track plastic morphological change in response to protist predation, thus providing a window into
350 both rapid plastic change (i.e., within strains), and rapid evolutionary change by clonal sorting
351 (i.e., change in genetic frequencies; Fig. 1c). We recorded the density of all protist species
352 through fluid imaging (FlowCam; Yokogawa Fluid Imaging Technologies, Portland, ME, USA)
353 at a magnification of 10x (Fig. 1c).
354
355 *Data analysis*
356 To analyze how temperature and ecological interactions influenced prey evolution, we used
357 autoregressive moving average linear mixed models (ARMA-LMMs; 'nlme' package, v. 3.1-
358 162) to examine autocorrelation in our data. After finding no significant effects of
359 autocorrelation (Appendix IV Table 1), we used classic linear mixed models (LMM) in the
360 'lme4'package (version 1.1-3 in R v. 4.3.1) to the rest of analyses. We calculated the relative
361 frequencies of *vfl1-1* in total *C. reinhardtii* population as the measure of prey evolution. To
362 better evaluate whether changes in prey evolution were affected by temperature, we also
363 calculated the relative changes in *vfl1-1* frequencies across temperatures (Δ *vfl1-1* frequency) by
364 deducting the mean *vfl1-1* frequencies at 19°C from each replicate at 22°C and 25°C in each
365 treatment.
366
367 We first performed LMM on the single predator treatments and the control (Fig. 1b) to
368 understand the individual effects of each predator on prey evolution. We analyzed the fixed

369 effects of predator species identity, temperature, and time on the prey evolution and temperature
370 effects on prey evolution, by using *vfl1-1* frequency and Δ *vfl1-1* frequency as response variables
371 respectively. We then accounted for the densities of the single predators as a fixed effect to the
372 previous model to analyze the effects of predation on prey evolution. We then performed LMM
373 on *T. pyriformis* and *Glaucoma sp.* single predator treatments and the competition treatments
374 (Fig. 1b) to test whether the presence of a second predator had joint effects with predator species
375 identity, predator density, and temperature on *vfl1-1* genetic frequency and Δ *vfl1-1* frequency
376 using previous linear mixed models.
377
378 Additionally, to quantify the relative importance of each predator on prey evolution in
379 competition treatments, we calculated Earth Mover's Distance (EMD from now on) with
380 'emdist' (R package, version 0.3-3), which quantifies the similarity of prey evolution patterns
381 over time between different predation treatments (higher EMD means less similarity).
382 Specifically, at each temperature, we calculated the EMD of *vfl1-1* frequencies 1) between the
383 single predator treatments in each competition pair and 2) between each of the single predator
384 treatments and the corresponding competition treatment.
385
386 To understand the mechanism through which predation and temperature jointly affect prey
387 evolution, we quantified demographic parameters that govern species population dynamics: 1) a
388 proxy for maximum growth rate, r, at each temperature, as $\ln(N_t)-\ln(N_0)]$/time using day 5 data
389 and their initial densities on day 0, and, 2) maximum density, N_{max}, by measuring the highest
390 daily average across replicates. We used linear models ('stats' v4.3.1) and stepwise model

selection ('stepAIC' in R package 'MASS' v7.3-60) to test the effects of temperature and competition on each of the predators.

We also used linear mixed models to understand how abiotic and biotic factors affect prey plasticity. Similar to previous LMMs, we tested how temperature, predator species, predator density, and time, affect WT and vfl1-1 particle sizes in single predator treatments. In addition to those factors, we also tested how the presence/absence of a predator competitor affects prey particle size in the competition treatment.

Mathematical modeling

To understand the processes that drive rapid evolution in the prey population in the presence/absence of a predator, we mathematically tracked the population dynamics of a system with two genetically distinct prey strains under predation by a shared predator. We assumed that WT (W) and *vfl1-1* (V) grow logistically and compete for resources at different rates using a model in differential equations. Further, we assumed that the predator (P) has a multispecies type II functional response and dies at a background mortality rate, m. Our model allows for the predator to prey on each strain at different rates. Taken together, the equations modeling the strain dynamics and predator population are:

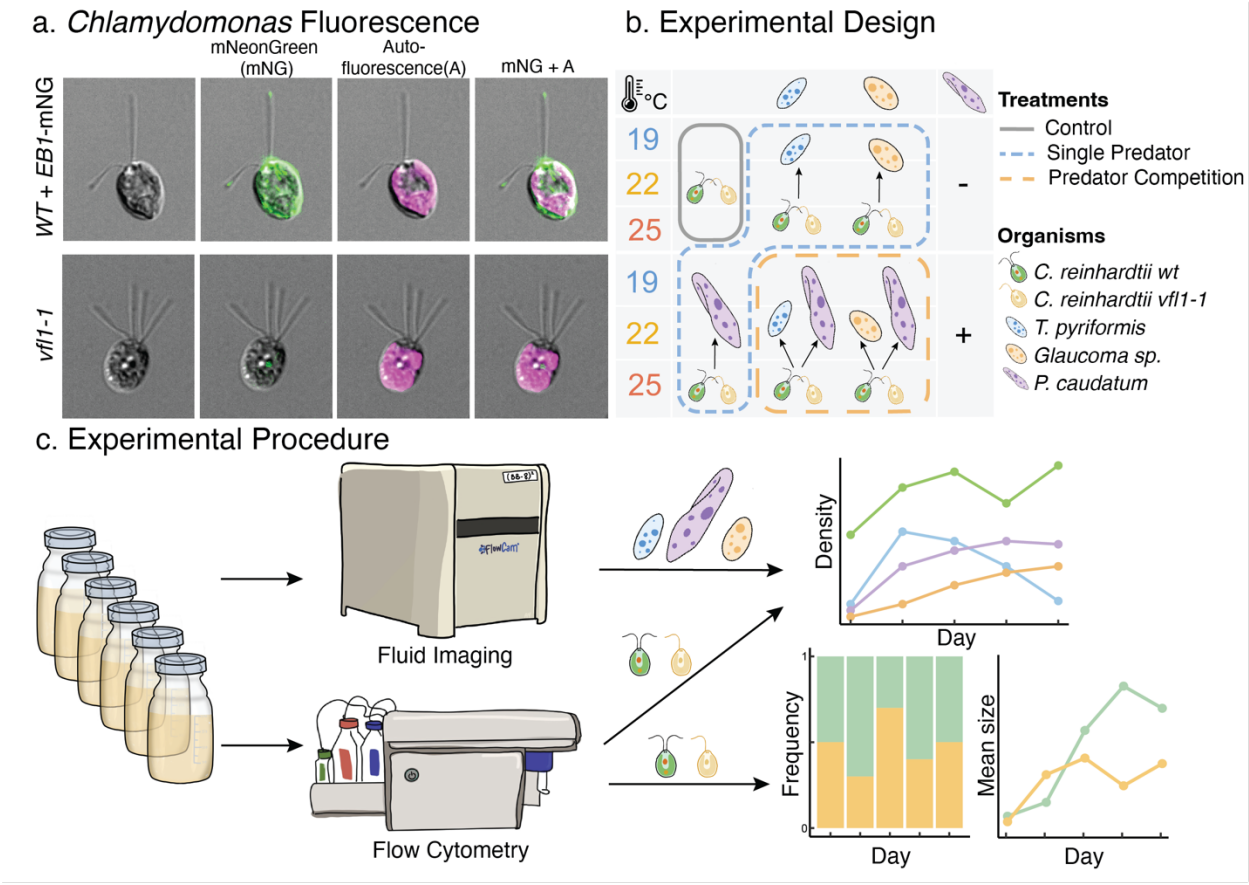
$$\frac{dW}{dt} = r_w W\left(1 - \frac{W}{K_w} - \frac{a_{wv}V}{K_w}\right) - \frac{d_{wp}WP}{1 + d_{vp}\eta_{vp}V + d_{wp}\eta_{wp}W}$$

$$\frac{dV}{dt} = r_v V\left(1 - \frac{V}{K_v} - \frac{a_{vw}W}{K_v}\right) - \frac{d_{vp}VP}{1 + d_{vp}\eta_{vp}V + d_{wp}\eta_{wp}W}$$

$$\frac{dP}{dt} = \frac{e_v d_{vp}VP}{1+d_{vp}\eta_{vp}V+d_{wp}\eta_{wp}W} + \frac{e_w d_{wp}WP}{1+d\eta_{vp}V+d_{wp}\eta_{wp}W} - mP\ ,$$

where r is intrinsic growth rate, K_i is the carrying capacity of each genotype, $a_{wv}$is the competition effect of V on W (vice versa for a_{vw}), d_{wp} and η_{wp} are the attack rate and the handling time of the predator on W, a_{vp} and η_{vp} are those of predator on V, e_w and e_v are the conversion efficiencies of W and V to predator biomass. We used the growth rate of WT and *vfl1-1* in control conditions as r_w and r_v and then explored parameter space with the remaining model parameters to find dynamics that qualitatively reproduced the observed dynamics. In the appendix we also include alternative model formulations (e.g., treating predation as a constant mortality rate, predators with a type I functional response) and provide an analytical treatment of the model and associated predictions (Appendix I).

424



425

426 Figure 1. Experimental organisms and designs. a) Images of a *C. reinhardtii* wild-type strain

427 expressing mNeonGreen (mNG)-tagged EB1 protein and a *vfl1-1* mutant strain. *vfl1-1* cells have

428 variable numbers of flagella ranging between zero to more than four. b) Factorial design of the

429 experiment, showing 3 temperatures by 3 predations by 2 competition treatments. c)

430 Experimental procedures of sampling methods.

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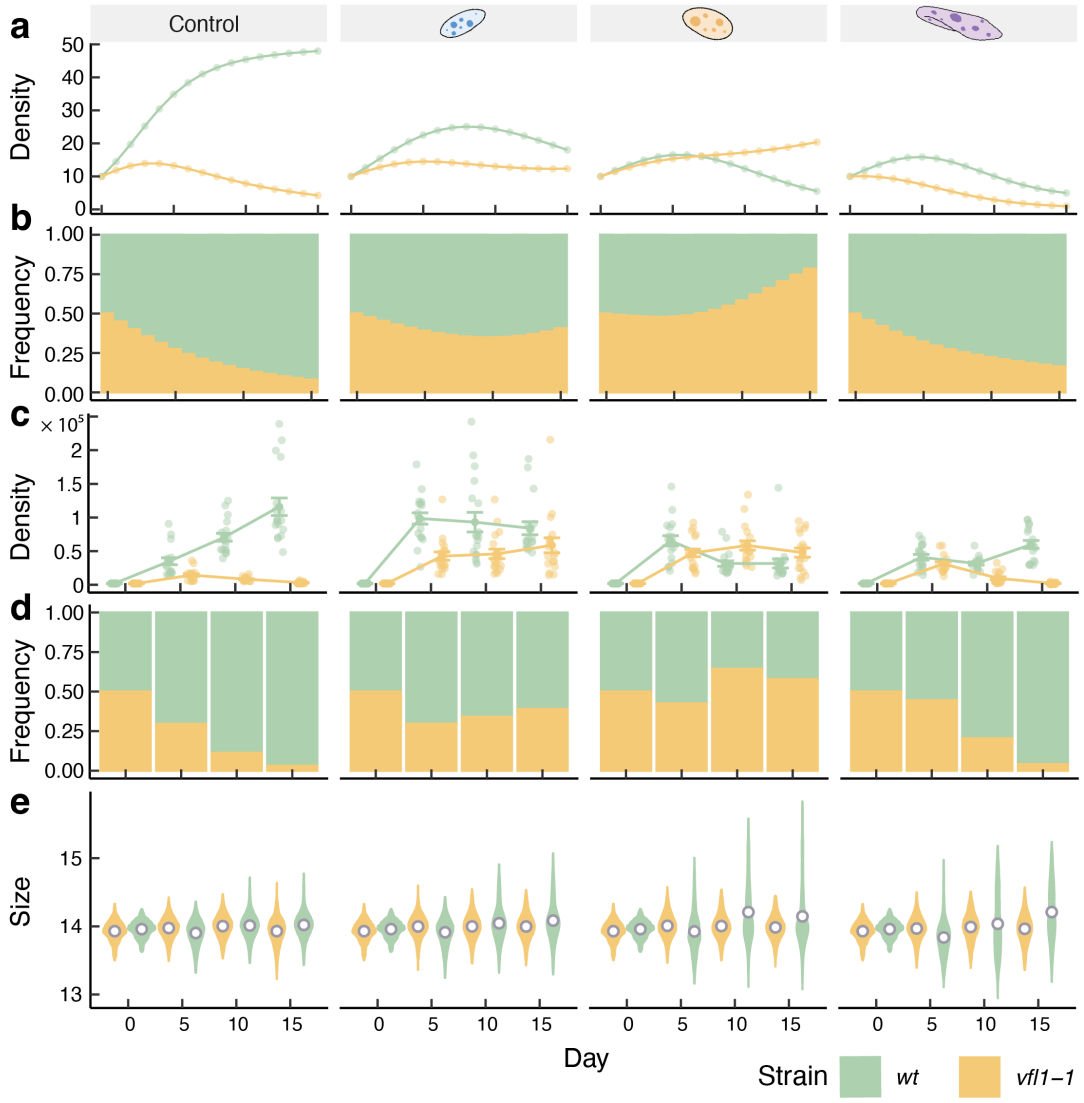

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435 Figure 2. Model prediction and empirical data of prey clonal dynamics and prey body size.

436 *C. reinhardtii* population density and genetic frequencies predicted by mathematical models (a-b;

437 Specific parameter see Appendix I Table 1) and from empirical data (c-d). Panel e shows the size

438 of *C. reinhardtii* particles over time. Yellow represents *vfl1-1* mutant strain and green represents

439 WT.

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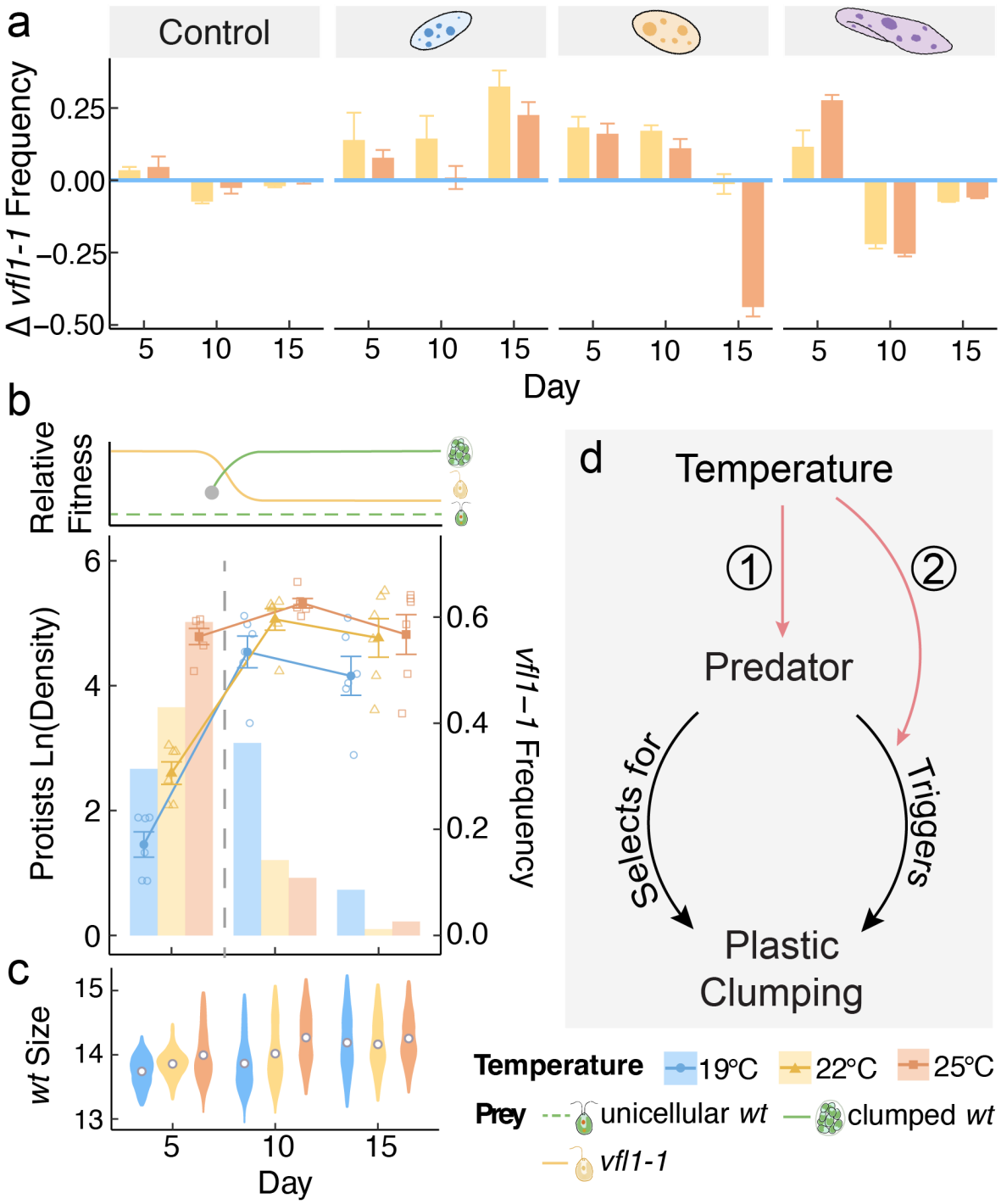

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443 Figure 3. Temperature affects prey evolution through predator thermal performance and prey

444 plasticity. a) To understand temperature effects on prey evolution, we calculated the changes in

445 *vfl1-1* frequencies across temperature in control and all single predator treatments, using *vfl1-1*
446 frequency at 19°C as the baseline (representing as blue horizontal line at 0). Blue, yellow, and
447 orange represent 19°C, 22°C, and 25°C respectively. b) shows the *P. caudatum* density (left axis,
448 solid lines) and *vfl1-1* frequency (right axis, bars) across temperature in *P. caudatum* single
449 predator treatment. Circles, triangles, and rectangles represent 19°C, 22°C, and 25°C,
450 respectively. The grey dash line represents the switch in the selection direction after plastic
451 response of WT. Top panel is a conceptual diagram indicating the decrease of relative fitness of
452 *vfl1-1* (yellow solid line) due to the onset of WT clumping (grey dot). The relative fitness of
453 unicellular WT and clumped WT are presented by green dash and green solid line, respectively.
454 c) WT particle size distribution in *P. caudatum* treatment across temperature. d) Conceptual
455 diagram of the two potential mechanisms of temperature affecting WT clumping.
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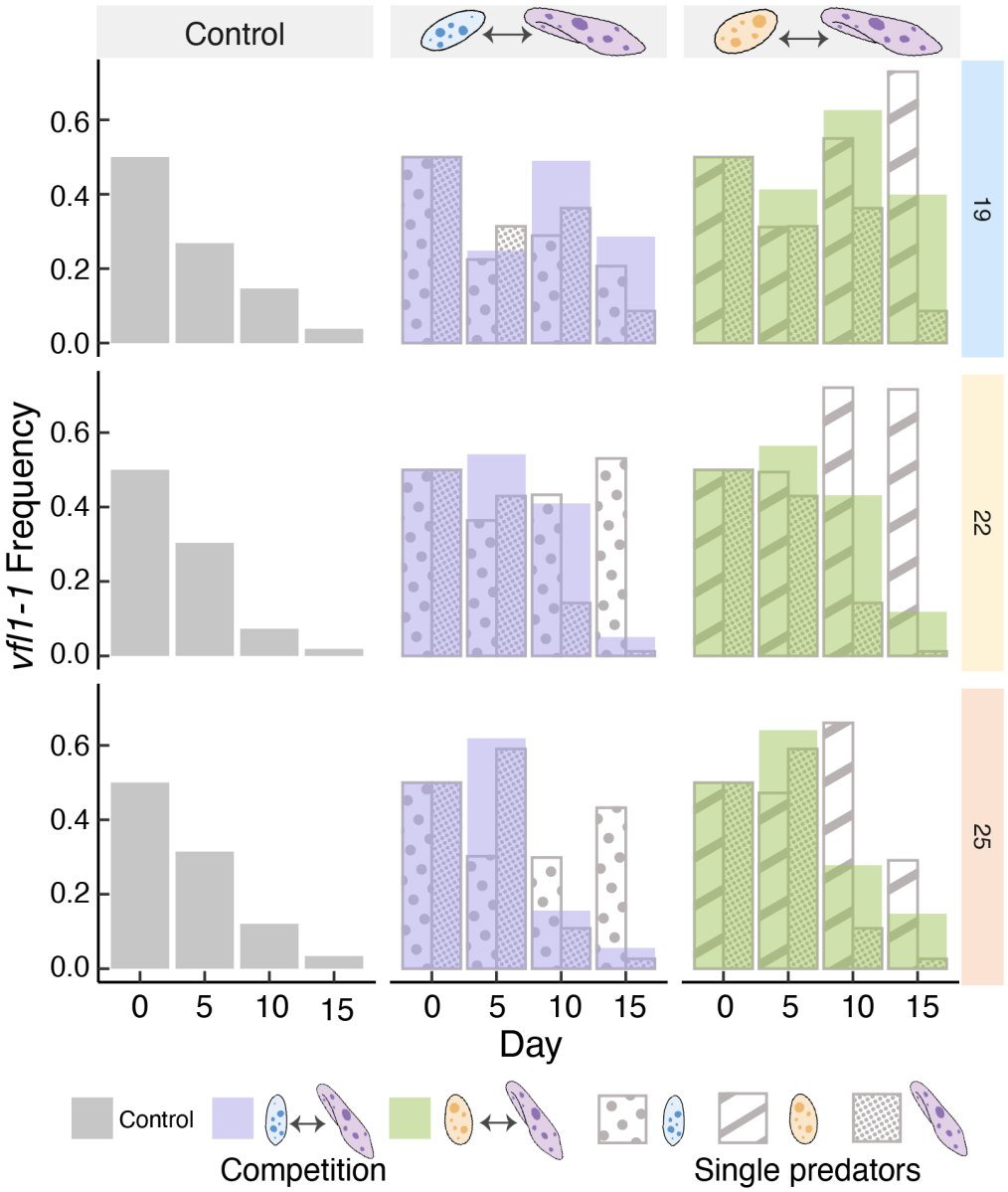

Figure 4. Prey evolution under predator competition. Frequencies of *vfl1-1* in competition treatments with two predators. Solid color bars represent the frequencies of *vfl1-1* in control and two predator-competition treatments at different temperatures. Bars with patterns represent the frequencies of *vfl1-1* in the single-predator treatments at each temperature.

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