Species interactions and food-web context drive temperature-dependent prey evolution

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ABSTRACT

Understanding how global warming shapes species evolution within communities is a pressing goal of ecology. Temperature affects interacting species and can lead to changes in species interactions, but how that will alter species evolutionary trajectories within complex food webs is poorly understood. Here we address 1) whether different predators affect prey evolution differentially, 2) whether the food web context in which this happens influences prey evolution, 3) whether temperature affects prey evolution directly, and 4) whether ecological interactions mediate how temperature affects prey evolution. We use a combination of mathematical modeling and experimental evolution assays in microbial food webs composed of prey algae and their protists predators. We found that temperature alone doesn't drive prey evolution unless predators are involved. Importantly, the influence of temperature through predation is contingent on the food web structure. This leads to distinct evolutionary trajectories when prey evolves with predators alone or with a competing predator present. Our findings indicate that the species evolution to warming is likely contingent on their specific ecological contexts, suggesting that similar species across different food webs could exhibit diverse evolutionary responses to new climates.

INTRODUCTION

Global warming is reshaping natural communities worldwide (Parmesan 2006) as rising temperatures affect population growth (Dell *et al.* 2011; Frazier *et al.* 2006; Kontopoulos *et al.* 2020; Savage *et al.* 2004; Wieczynski *et al.* 2021) and species interactions (Blois *et al.* 2013; Dell *et al.* 2014; Gilman *et al.* 2010; Suttle *et al.* 2007; Tylianakis *et al.* 2008). Temperature, in particular, increases metabolic costs (Clarke 2006; Clarke & Fraser 2004), leading predators to consume more prey (Sheridan & Bickford 2011) while reaping ever decreasing energetic benefits (Barneche *et al.* 2021). This decreasing energetic intake results in the energetic choking of upper food-web trophic levels, subsequent trophic collapse (Ullah *et al.* 2018; Voigt *et al.* 2003; Zarnetske *et al.* 2012), and food web rewiring (Barbour & Gibert 2021; Bartley *et al.* 2019). This has grave implications, contributing to large changes in community composition and structure(Komatsu *et al.* 2019) as well as the marked loss of top predators throughout the Anthropocene (Estes *et al.* 2011; Voigt *et al.* 2003).

Although theory predicts that rapid prey evolution and predator-prey co-evolution may mitigate these temperature impacts, empirical evidence of rapid adaptation under global warming in a food web context remains scarce (De Meester *et al.* 2019; Tseng & O'Connor 2015). On one hand, temperature can directly influence evolution through effects on organismal metabolism (Alton *et al.* 2024; Clarke 2003; Gillooly 2000; Terblanche *et al.* 2009), morphology (Brans *et al.* 2017; Diamond *et al.* 2017; Yampolsky *et al.* 2014), and fitness (Diamond *et al.* 2018; Norin & Metcalfe 2019; Padfield *et al.* 2016). On the other hand, predation within complex food webs also drives species evolution (Abrams 2000; De Meester *et al.* 2019; Frickel *et al.* 2017; Hairston *et al.* 2005; Palkovacs & Post 2009) and often selects for more defensive but less competitive

prey (Yoshida *et al.* 2004). The link between abiotic drivers of selection, like temperature, and biotic drivers of selection, like predation, is established through the often asymmetric temperature effects on key demographic and ecological predator traits such as growth or attack rates (Gibert *et al.* 2022; Robertson & Hammill 2021; Wieczynski *et al.* 2021). This suggests that prey evolutionary responses are likely jointly influenced by temperature, as well as predation and competition among predators –i.e., the broader food web context the interactions are embedded in. But whether or how this happens in nature is poorly understood.

To disentangle this potentially complex interplay between biotic and abiotic drivers of species evolution and understand its implications within broader food webs under climate change, we focus on a model system of global distribution and relevance (Bar-On *et al.* 2018; Herron *et al.* 2019; Wieczynski *et al.* 2021): the interaction between the unicellular green algae *Chlamydomonas reinhardtii* and its ciliate predators. We assembled tractable microcosm food webs of organisms that co-occur across ecosystems (from soils to wetlands, Foissner *et al.* 2009), and studied the joint effects of temperature, predation, and predator competition (as a proxy for food web context), on prey evolution. We tracked population dynamics, phenotypic changes, and rapid shifts in prey genetic makeup (i.e., rapid evolution), to understand the eco-evolutionary dynamics that ensue within these microbial food webs across temperatures and the processes that determine them.

Specifically, we address the following questions: 1) do different predators affect prey evolution differentially? 2) Does competition between predators (i.e., food web context) influence prey evolution, and, how? 3) Does temperature affect prey evolution directly? And, 4) Do ecological

interactions mediate how temperature affects prey evolution? We show that temperature does not directly affect prey evolution in the absence of predators, but does so in their presence through direct temperature effects on the ecological interactions. Moreover, this mediation effect of temperature is determined by the food web context, such that evolution under predation with and without a predator competitor leads to different evolutionary outcomes. Our results emphasize that organismal evolutionary responses to a warming world likely are strongly dependent on the biotic context in which they are embedded, so that similar species in different food webs might show different eco-evolutionary responses to novel climates.

RESULTS

Predation maintains genetic diversity

To understand how predation influences prey evolution, we established a tractable model of rapid evolution under predation that keeps track of two genetically distinct prey strains that share a common predator (see Methods). The model suggests that two different prey strains cannot coexist in the absence of predators –classically, unless inter-strain competition is lower than intra-strain competition– (Appendix I Equation 1-2). Predation, however, facilitates the invasion of the strain that would otherwise be lost (Appendix I Equations 1,3), implying that predation can maintain genetic diversity. We tested these theoretical predictions using an experimental predator-prey microbial system where the alga *Chlamydomonas reinhardtii* is preyed upon by one of three possible ciliate protist species (*Glaucoma sp.*, *Tetrahymena pyriformis*, *Paramecium caudatum*, see Methods). The algal population is composed of two genetically and phenotypically distinct, algal prey strains – fluorescently tagged wild type (*wt*) and untagged *vfl1-1* (i.e., 'variable flagellar number1-1')– as our prey population. We kept track of their

110 abundances, phenotypes, and shifts in clonal frequencies (using their fluorescent spectrum) over
111 time as means to quantify evolution by clonal sorting (see Methods). Our empirical results
112 supported our model predictions: while *wt* consistently outcompeted *vfl1-1* in the absence of
113 predators, predation facilitated the persistence of the inferior competitor in all predation
114 treatments, regardless of the presence of the predator competitor (Fig 2a-d; Appendix II Figure
115 1-2). Interestingly, *C. reinhardtii* grew faster in all predation treatments, perhaps due to
116 increased fertilization by predator waste (Cech *et al.* 2008; McNaughton *et al.* 1997). We
117 hypothesized that predation would select for *wt* and *vfl1-1* differently (Fig 2a-d; Appendix IV
118 Table 1-2). *vfl1-1* had the highest frequency under predation from *Glaucoma sp*., but lowest
119 frequency under predation from *P. caudatum* (Fig 2d; Appendix IV Table 2).
120
121 *Prey traits determine eco-evolutionary outcomes*
122 Our model suggested that variations in ciliate predator attack rates are sufficient to explain the
123 different evolutionary outcomes across treatments (Fig 2a-b, model parameter see Appendix I
124 Table 1), thus offering a simple explanation for an otherwise seemingly complex dynamic. In our
125 experimental system, predation selects on two key functional traits of the prey: defensive
126 clumping and motility. Strain *vfl1-1* has impaired swimming ability compared to *wt*, due to
127 pleiotropic effects of mutations on *VFL1* gene, (Adams *et al.* 1985; Silflow *et al.* 2001)
128 Appendix IV videos) and is therefore predicted to experience lower predation rates relative to the
129 more motile *wt*, all else being equal (Aljetlawi *et al.* 2004; González *et al.* 1993; Pawar *et al.*
130 2012; Visser 2007). Our data supports this prediction as *vfl1-1* is selected against in the absence
131 of predators (Fig 2 c-d), and selected for in the presence of most predators, with frequencies
132 higher relative to control, likely owing to decreased predation through impaired motility relative

133 to *wt*. However, in addition to increased mobility, *wt* can form large defensive clumps (Lurling &
134 Beekman 2006), while *vfl1-1* shows impaired clumping ability under predation in lab. Through
135 cytometry, we measured the particle size of the algal population as a proxy for the onset of
136 clumping, with larger average particle size and maximum particle size indicating clumping (see
137 Methods). Although, *vfl1-1* showed very small size changes in response to predation (Fig 2e,
138 Appendix IV Table 3), *wt* showed strong plastic clumping defense against predation with an
139 increase in both average and maximum particle size (Fig 2e green; Appendix II Fig 3-4;
140 Appendix IV Table 4). The distribution of *wt* particle size additionally vary by predators (Fig 2e,
141 Appendix IV Table 4), suggesting species-specific predation rates, in support of our model
142 prediction. Finally, the relative fitness of motility and clumping traits is predator-dependent:
143 *Glaucoma sp.* selected for *vfl1-1* while *P. caudatum* drove *vfl1-1* to extinction (Fig 2c-d;
144 Appendix IV Table 2).
145
146 *Temperature directly affects ecological dynamics and prey plasticity, but indirectly affects prey*
147 *evolution*
148 Although temperature influenced the growth rate of *wt* and *vfl1-1* (Appendix II Fig 6),
149 temperature alone did not affect prey genetic frequencies, and hence, prey evolution (Fig 3a
150 Control; Appendix II Figure 2). Only in the presence of predation did temperature significantly
151 affect prey evolution, suggesting that temperature effects on prey evolution are mediated by
152 predation. This likely happened through a combination of differential predator thermal
153 performance and prey plastic responses (Fig 3; Appendix IV Table 2,5). Indeed, temperature
154 significantly affected the demographic parameters that underpin the ecological dynamics of each
155 predator: the intrinsic growth rate, *r*, and their maximum abundance (a measure of their carrying

156 capacity, K; Fig 3c, Appendix II Fig 7, Appendix IV Table 6-7). These temperature-dependence
157 of key predator demographic parameters likely determined predation intensity, leading to
158 significant differences in the mean, median, variance, skewness and kurtosis of *wt* particle size
159 (Fig 3b; Appendix IV Table 8). As temperature indirectly affects *wt* plastic response through the
160 predator-prey interaction, predator strain-specific attack rate on *wt* and *vfl1-1* will also likely
161 change over time depending on predator preference.
162
163 Interestingly, the fitness of *vfl1-1* and *wt* under predation by *P. caudatum* shifted dynamically
164 over time, and these shifts were most pronounced the higher temperature treatment (Fig 3c).
165 Indeed, *vfl1-1* frequency increased with *P. caudatum* density by day 5 compare to control, but
166 dropped to near extinction by day 15, and this happened more rapidly at higher temperatures (Fig
167 3c). This indicates that the low motility of *vfl1-1* was favored at first through differential
168 predation (rapid fast evolutionary response, Fig 3c, left) while the plastic clumping defense of *wt*
169 was favored later on (Fig 3c, right). In other words, intermittent rapid evolutionary and plastic
170 responses ultimately determined the eco-evolutionary response of the prey. Additionally, the
171 boom-bust change in *vfl1-1* frequency, with initial increase that followed by a steep drop, at
172 higher temperatures (Fig 3c) likely indicates that the faster increase in *P. caudatum* density at
173 higher temperature (Fig 3c) led to a faster onset of defensive the clumping response by *wt* (Fig
174 3b). Therefore, direct temperature effects on predator ecological dynamics and prey plastic
175 responses sped up changes in strain relative fitness that resulted in changes in genetic
176 frequencies and prey evolution (Fig 3c). Altogether, our results reveal the mechanisms by which
177 temperature indirectly affect prey evolution through its direct and differential effects on predator

178 ecological dynamics and prey plastic responses, which ultimately shape prey functional traits and
179 evol-evolutionary dynamics.
180
181 *Temperature-mediated predator competition (i.e., food web context) affects prey evolution*
182 The presence of a second predator independently and interactively affected prey evolution and
183 prey plastic clumping with temperature, predator species, and time (Fig 4; Appendix IV Table 9-
184 12). Specifically, we observed a combined effect from both predators on prey evolution in
185 competition treatments (Fig 4a; Appendix III Fig 1). Indeed, when we analyzed the evolutionary
186 dynamics of the *C. reinharditt* (i.e. genetic frequencies of *vfl1-1* over time) using Earth Mover's
187 Distance approach (see Methods), we found that the similarity of *vfl1-1* genetic frequencies over
188 time between competition treatments and single predator treatments (for example, between
189 *Glaucoma sp* + *P. caudatum* and *P. caudatum* or *Glaucoma sp*) were higher than the similarity
190 between those single predator treatments (for example, between *Glaucoma sp.* and *P. caudatum*;
191 Appendix III Fig 1).
192
193 Meanwhile, temperature also indirectly affected the relative contribution of each predator on
194 prey evolution within predator competition treatments (Fig 4a) through its effect on predator
195 performance. *P. caudatum* had increased initial population growth rate, *r*, and maximum
196 population density in higher temperature while those of *T. pyriformis* and *Glaucoma sp.*
197 decreased in higher temperature (Fig 4b; Append II Fig 7), and *vfl1-1* genetic frequencies over
198 time in competition treatments that include *P. caudatum* resemble most to those in which *P.*
199 *caudatum* is the only predator at high temperature (lowest EMD; Appendix III Fig 1).
200 Additionally, the size distribution of *wt* in competition treatments also showed combined effects

of both predators and temperature mediated the predator-specific effects on *wt* plastic responses (Fig 4c; Appendix III Fig 2; Appendix IV Table 11). Altogether, our result show how temperature mediates the effect of food web contexton these eco-evolutionary dynamics, through differences in predator thermal performance, predator competition, and prey plasticity.

DISCUSSION

Our results show that temperature strongly but indirectly affects prey evolution in the presence of predation through its direct effects on predator population dynamics and species interactions. Predator-specific selection on prey traits and predator thermal performance together determine prey plastic responses, which in return led to changes in fitness of prey functional traits overtime as ecological dynamics unfold (Fig 2-3). Moreover, multiple predators can concurrently respond to temperature and jointly affect the prey phenotypic responses that underpin prey evolution (Fig 4), thus highlighting the importance of the food web context in indirectly shaping species evolution under global warming.

Prey motility, defensive clumping, and predator identity, jointly determine prey rapid evolution

Our study shows that predator differential selection on multiple prey functional traits (i.e. motility and clumping) allow the less dominant strain to persist (Siepielski *et al.* 2020), thus maintaining higher additive genetic diversity (Fig 2). More so, we revealed the reciprocal nature of the effects of species functional traits and species interactions, and how these underpin the dynamics of species evolution within food webs (Fig 3).While previous studies showed that more motile prey experience higher predation rates than slow or immotile prey (Andersen & Dölger 2019; Brodin & Johansson 2004; González *et al.* 1993; Visser 2007), the relative fitness

224 of low motility of *vfl1-1* under predation is ultimately controlled by the onset of *wt* plastic
225 clumping and the predator response to *wt* clumping defense. In other words, dynamic plasticity
226 in one strain alters the course of the evolutionary process within food webs by influencing
227 relative fitness across strains.
228
229 *Predator identity affects prey plastic responses*
230 *Chlamydomonas reinhardtii* is known to develop clumping in response to abiotic and biotic
231 stressors (Fischer *et al.* 2016; Herron *et al.* 2019). However, our study showed predator
232 preferential selection led to a novel predator-specificity in plastic clumping distribution of *C.*
233 *reinhardtii* (Fig 3). Moreover, multiple predators can have coordinated effects in shaping prey
234 plastic traits distribution in predictable ways (Fig 4; Appendix III Fig 2). Prey evolutionary
235 dynamics under selection by multiple predators reflected the combined effects of selection
236 imposed by individual predator species (Fig 4; Appendix III Fig 1). These results emphasize the
237 interesting but unexplored consequences of species phenotypic plasticity: shifts in one functional
238 trait in response to one predator may affect the interaction with another predator, which could
239 cascade down through the entire complex community, as previously suggested theoretically
240 (Cosmo *et al.* 2023; Guimarães *et al.* 2017). Our study thus highlights the importance of a
241 mechanistic understanding of the interplay between species functional trait dynamics and biotic
242 interactions across trophic levels in complex communities, not just pairwise interactions (Henn *et*
243 *al.* 2018).
244
245 *Food web context and temperature interactively drive prey evolution*

Our study shows the underlying mechanisms through which reciprocal effects between food web context and trait dynamics together drive prey evolutionary trajectories across temperatures (Fig 3-4). We observed that asymmetric warming effects on species at different trophic levels influence predator-prey interactions (Gibert *et al.* 2022; Kordas *et al.* 2011; Lindmark *et al.* 2019), which had been previously suggested to be an important aspect of food web dynamical responses to rising temperatures (Gibert *et al.* 2022; Parain *et al.* 2016; Thakur *et al.* 2018; Vasseur & McCann 2005). We also show that different predator thermal responses mediate competition among predators at the same trophic level, allowing the more dominant predators to shape prey traits and evolutionary dynamics more strongly (Fig 4; Appendix III Fig 1-2).

Temperature-mediated changes in species interactions drive changes in species plastic and evolutionary functional traits (Barbour & Gibert 2021; Fischer *et al.* 2016; Fordyce 2006), which in turn can reshape food web dynamics, structure, and function (Bartley *et al.* 2019; DeLong *et al.* 2023; Gibert 2019; Han *et al.* 2023; Lister & Garcia 2018; Petchey *et al.* 1999; Thakur *et al.* 2018). As warming increases extinction rates among predators (Thunell *et al.* 2021; Voigt *et al.* 2003; Zarnetske *et al.* 2012), a decrease in predator diversity might also lead to decreases in prey trait diversity that may result in disproportionate effects from more dominant predators and increased loss of genetic diversity within prey populations. Our results have a major implication: species evolution in novel climates will be shaped by the food web context within which they are embedded, such that both the composition of the food web, but also their network complexity, likely will determine the evolutionary outcome, and hence the fate of these species (Barbour & Gibert 2021).

Concluding remarks

Overall, we showed that biotic interactions mediate temperature effects on species rapid thermal adaptation. We showed that predator-specific selection can change over time depending on the onset of plastic responses in other traits, which determines the evolutionary outcome. Additionally, predation triggers prey thermal adaptation, and this is in turn co-determined by predator thermal performance, prey plasticity, and temperature effects on competition among predators (food web context). Our results emphasize that, by directly affecting biotic interactions and species plastic traits, temperature can play an indirect –but key– role, in determining species evolution in a warming globe.

METHODS

Experimental work

Unicellular microalgae can be found in diverse habitats, from soil to freshwater ecosystems across the world (Arora & Sahoo 2015; Falkowski 1994). These organisms are at the base of all food webs, fueling both green and brown food webs (Brito *et al.* 2006; Descroix *et al.* 2010; 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 algae *Chlamydomonas reinhardtii*, is a well-established model organism with known mutations linked to functional traits (Sasso *et al.* 2018; Calatrava *et al.* 2023). Here we used two genetically and phenotypically distinct strains of *C. reinhardtii*: wild type (*wt*) and *vfl1-1* (i.e., 'variable flagellar number1-1') as our prey population, and kept track of their abundances, phenotypes, and shifts in genetic frequencies over time as means to quantify evolution by clonal sorting.

291 Wild type (*wt*) possesses two flagella that allow the entire range of normal locomotive behaviors
292 (Huang 1986) and can form large cell clumps, a common form of defense against predation
293 (Herron *et al.* 2019; Lurling & Beekman 2006). Strain *vfl1-1* produces individuals with variable
294 numbers of flagella from 0 to 10 and defective swimming ability (Adams *et al.* 1985; Silflow *et*
295 *al.* 2001). Additionally, *vfl1-1* seems to be unable to clump under predation. To distinguish these
296 two strains and keep track of their relative frequencies in mixture populations, we used
297 mNeonGreen fluorescent protein tagged *wt* populations and used flow cytometry to count tagged
298 *wt* vs non-tagged *vfl1-1* individuals over time (Fig 1a). Both strains can autfluoresce (Fig 1a) and
299 be detected by the cytometer, but only one (*wt*) will show fluorescence in the near green spectrum
300 (Fig 1a). We used two protist predators of similar body size, *Tetrahymena pyriformis* and
301 *Glaucoma sp.*, as the focal predators, and a larger protist *Paramecium caudatum*, as a competitor
302 for the predators (Fig 1b). All four species are commonly found in freshwater and soil systems
303 (Cornwallis *et al.* 2023; Foissner & Berger 1996).
304
305 To understand how temperature and ecological interactions (predation and competition among
306 predators, as a proxy for food web context) jointly influence *C. reinhardtii* evolution, we set up
307 experimental microcosms in autoclaved 250ml borosilicate jars filled with 100ml of 9:1
308 COMBO media:timothy hay infusion and one wheat seed as a carbon source. Each microcosm
309 was assigned to one of three possible predation treatments (No predation/Control, + *T.*
310 *pyriformis*, + *Glaucoma sp.*), one of two possible predator competition treatments (No
311 competition/Control, + *P. caudatum*), and one of three possible temperatures (19°C, 22°C, and
312 25°C). The manipulations produced a factorial design with 18 combinations of treatments (Fig
313 1b), each replicated six times, yielding a total of 108 microcosms.

314

315 Prior to experimentation, the algae strains were maintained in TAP agar (Rajam & Kumar 2007)
316 at room temperature. Protist cultures were maintained in bacterized timothy hay protist media at
317 22°C and a 16:8-hour light-dark cycle. All cultures were transferred to 9:1 COMBO media:
318 timothy hay protists media (Brans *et al.* 2022) and cultured under the same light and temperature
319 regime as protist stock cultures 2 weeks prior to experimental work. We carried out the
320 experiment in two blocks on two consecutive days, each block had half of the replicates in all
321 treatments. We started *wt* and *vfl1-1* strains at equal densities of 2000 individuals/ml in all
322 microcosms and initialized *T. pyriformis*, *Glaucoma sp.*, and *P. caudatum* populations at density
323 of 5 ind/ml, 5 ind/ml, and 0.5 ind/ml, respectively. The experiment was carried out for 15 days,
324 or ~ 30-45 *C. reinhardtii* generations.

325

326 We recorded all species densities on days 5, 10, and 15. We used flow cytometry (NovoCyte
327 2000R, Agilent, CA, USA) to distinguish mNeonGreen tagged *wt* individuals from untagged
328 *vfl1-1* individuals. This allowed us to track the abundance and frequencies of both strains over
329 time. We used forward scatter height (FSC-H) as a measure of cell/cell clump size of *C.*
330 *reinhardtii* (Adan *et al.* 2017) to track plastic morphological change in response to protist
331 predation, thus providing a window into both rapid plastic change (i.e., within strains), and rapid
332 evolutionary change (i.e., change in genetic frequencies; Fig 1c). We recorded the density of all
333 protist species through fluid imaging (FlowCam; Yokogawa Fluid Imaging Technologies,
334 Portland, ME, USA) at a magnification of 10x (Fig 1c).

335

336 We hypothesized that 1) different predators select for different traits of the prey, 2) competition
337 between predators might weaken directional selection on prey, and 3) temperature affects species
338 performance differently and may lead to changes in the strength of predator-prey interactions,
339 which can mediate prey evolution.
340
341 *Data analysis*
342 To analyze how temperature and ecological interactions influenced prey evolution, we used a
343 combination of autoregressive moving average linear mixed models (ARMA-LMMs; 'nlme'
344 package, v. 3.1-162) and classic linear mixed models (LMM) using the 'lme4'package (version
345 1.1-3 in R v. 4.3.1). We calculated the relative frequencies of *vfl1-1* in total *C. reinhardtii*
346 population as the measure of prey evolution. To better evaluate changes in prey evolution
347 affected by temperature, we also calculated the changes in *vfl1-1* frequencies across temperatures
348 (Δ *vfl1-1* frequency) by deducting the mean *vfl1-1* frequencies at 19°C from each replicate at
349 22°C and 25°C in each treatment.
350
351 We first performed LMM on the single predator treatments and the control (Fig 1b) to
352 understand the individual effects of each predator on prey evolution. We analyzed the fixed
353 effects of predator species identity, temperature, and time on the prey evolution and temperature
354 effects on prey evolution, by using *vfl1-1* frequency and Δ *vfl1-1* frequency as response variables
355 respectively. We then added in the densities of the single predators as a fixed effect to the
356 previous model to analyze the effects of predation pressure of prey evolution. We then
357 performed LMM on *T. pyriformis* and *Glaucoma sp.* single predator treatments and the
358 competition treatments (Fig 1b) to test whether the presence of a second predator had joint

effects with predator species identity, predator density, and temperature, on *vfl1-1* genetic frequency and Δ *vfl1-1* frequency using previous linear mixed models.

Additionally, to quantify the relative importance of each predator on prey evolution in competition treatments, we calculated Earth Mover's Distance (EMD from now on) with 'emdist' (R package, version 0.3-3), which quantifies the similarity of prey evolution patterns over time between different predation treatments (higher EMD means less similarity). Specifically, within each temperature, we calculated the EMD of *vfl1-1* frequencies 1) between the single predator treatments in each competition pair and 2) between each of the single predator treatments and the corresponding competition treatment. Similarly, to quantify the relative effects of each predator on prey plasticity, we also calculated the EMD of *wt* particle size distribution between: 1) the single predator treatments in each competition pair, and 2) each of the single predator treatments and the corresponding competition treatment.

To understand the mechanism through which predation and temperature jointly affect prey evolution, we additionally quantified demographic parameter that govern species ecological dynamics. Specifically, we calculated 1) the initial growth rate, r, at each temperature, as $\ln(N_t)-\ln(N_0)]$/time using day 5 data and their initial densities on day 0, and, 2) maximum density, N_max, by measuring the highest 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 across temperatures and in the presence/absence of a predator, we mathematically kept track 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 classic Lotka-Volterra competition framework. 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:

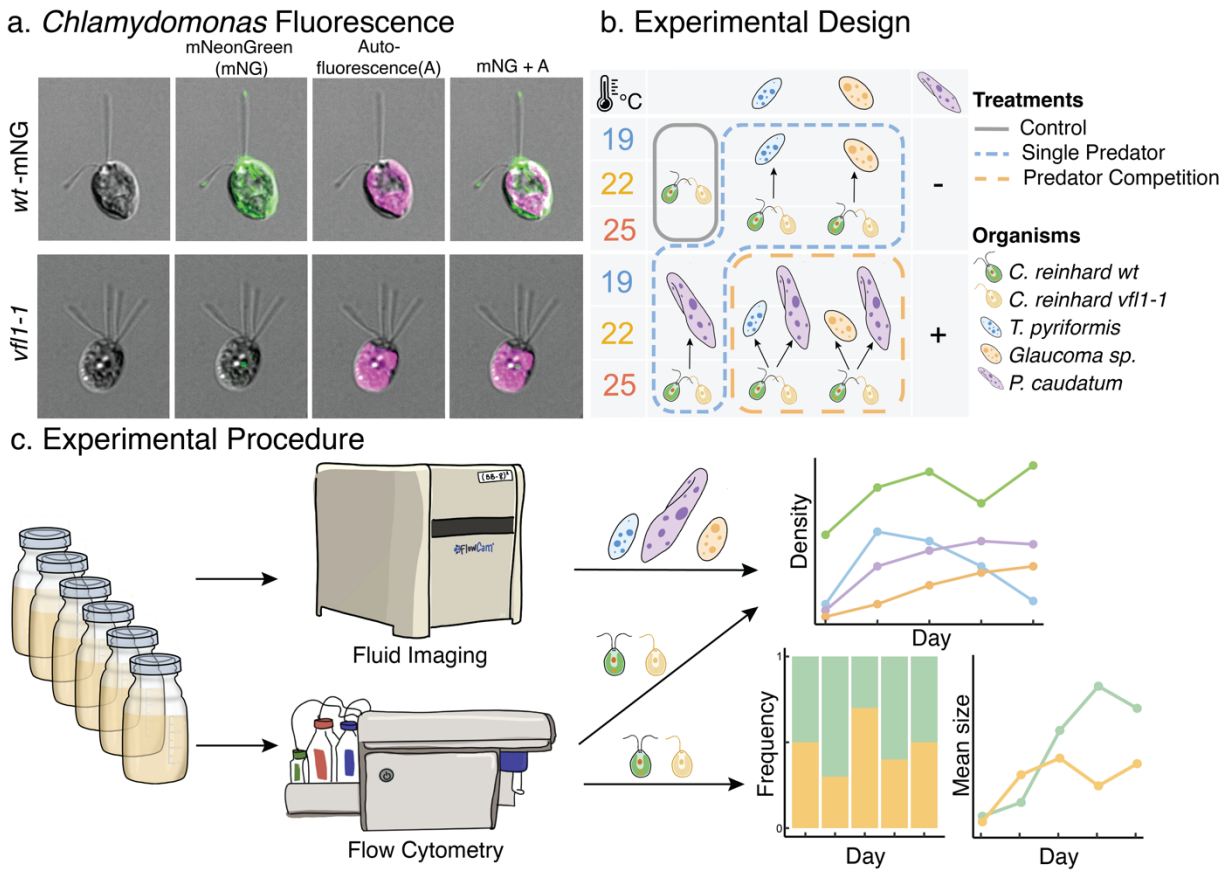
$$\frac{dW}{dt} = r_w W\left(1 - \frac{W}{K_w} - \frac{a_{wv}V}{K_w}\right) - \frac{d_{wp}WP}{1+d_{vp}\eta_{vp}V+d_{wp}\eta_{wp}W}$$

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

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

where r is intrinsic growth rate, K is the carrying capacity, $a_{wv}$is the competition coefficient *vfl1-1* has on *wt* (vice versa for a_{vw}), d_{wp} and η_{wp} are the attack rate and the handling time of predator on *wt*, a_{vp} and η_{vp} are those of predator on *vfl1-1*, e_w and e_v are the conversion efficiencies of *wt* and *vfl1-1* to predator. We used the growth rate of *wt* and *vfl1-1* in control 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).



411

412

413 Figure 1. Experimental organisms and designs. a) Images of *Chlamydomonas reinhardtii* wild

414 type with mNeonGreen tagged on EB1 protein and *vfl1-1* mutant strain. b) Factorial design of the

415 experiment, showing 3 temperatures by 3 predations by 2 competition treatments. C)

416 Experimental procedures of sampling methods.

417

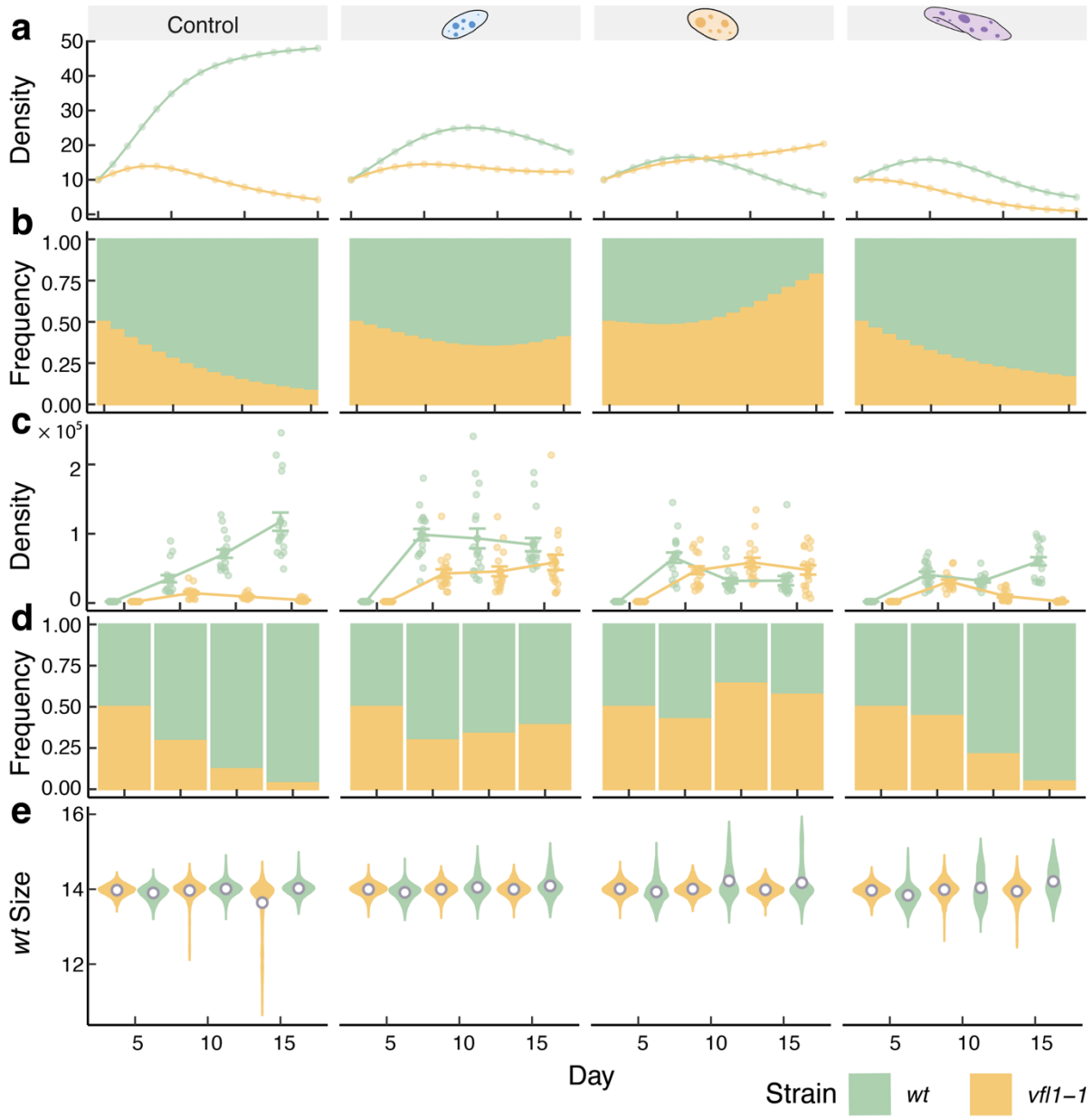


Figure 2. Model prediction and empirical data of prey clonal dynamics and prey body size. *Chlamydomonas reinhardtii* population density and genetic frequencies predicted by mathematical models (a-b) and from empirical data (c-d; Specific parameter see Appendix I XX). Panel e shows the partical sizes of *C. reinhardtii* over time. Yellow represents *vfl1-1* mutant strain and green represents *wt*.

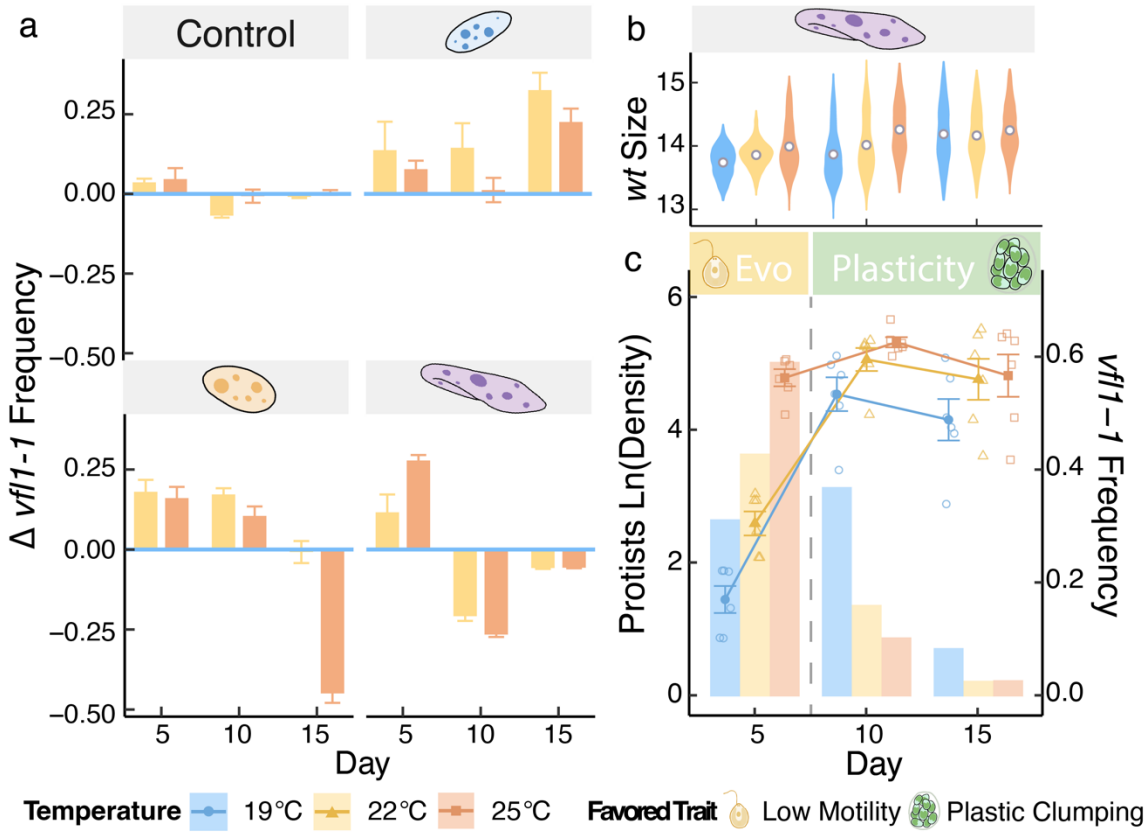


Figure 3. Temperature affects prey evolution through predator thermal performance and prey pasticity. a) To understand temperature effects on prey evolution, we calculated the changes in *vfl1-1* across temperature in control and all single predator treatments, using *vfl1-1* frequency at 19°C as the baseline (representing as blue horizontal line at 0). b) *wt* particle size distribution in *P. caudatum* treatment across temperature. c) shows the *P. caudatum* density (left axis) and *vfl1-1* frequency (right axis) across temperature in *P. caudatum* single predator treatment. Circles, triangles, and rectangles represent 19°C, 22°C, and 25°C respectively. Yellow and green bands on top indicate the favored functional trait at different time while the grey dash line represents the switch in selection direction after strong plastic response of *wt*.

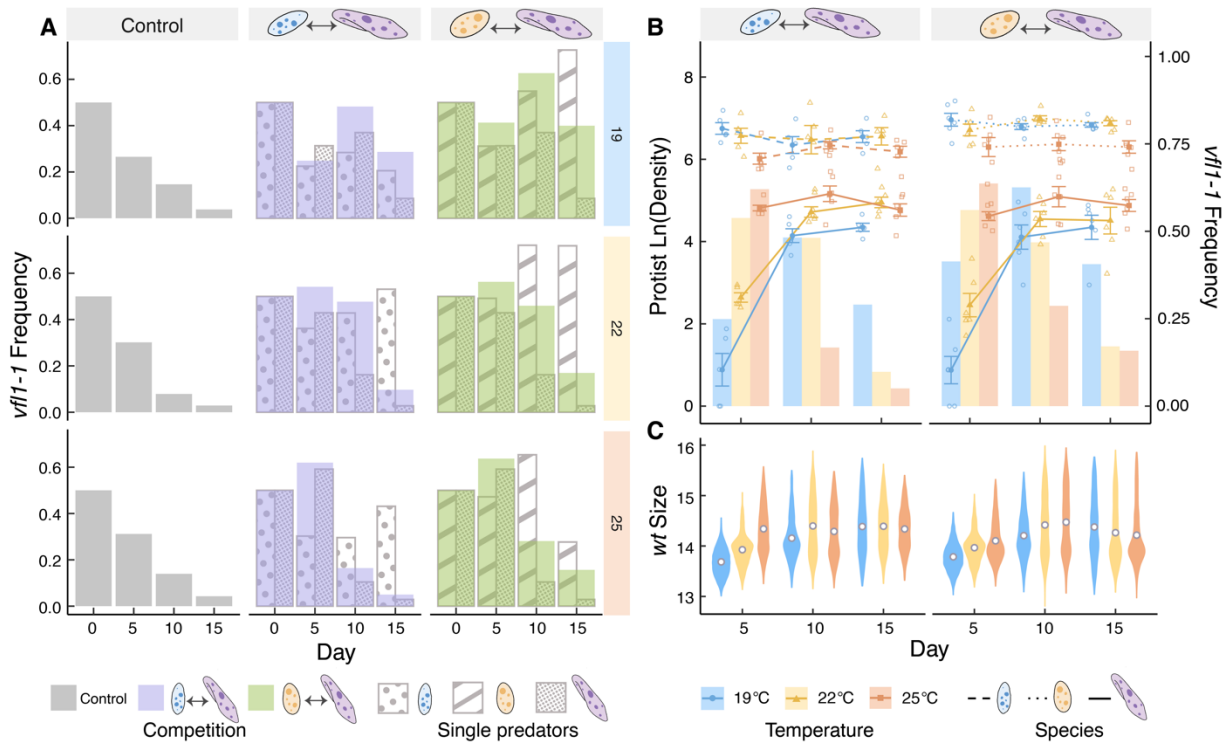


Figure 4. Prey evolution under predator competition. a) Frequencies of *vfl1-1* in competition treatments with two predators. Solid color bars represent the frequencies of *vfl1-1* in control and two predator-competition treatments at different temperature. Bars with patterns represents the frequencies of *vfl1-1* in the single predator treatments at each temperature. b) *vfl1-1* frequencies and all predator log density with error bars at different temperature. Triangles, squares, and circles represent *T. pyriformis*, *Glaucoma sp.*, and *P. caudatum* respectively. Open shapes represent each replicate while the solid shapes represent the mean within each treatment. c) Wild type size distribution measured as log FSC-H at different temperature over time. White triangles and grey open circles represent the mean and the median.

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