Received: 25 April 2022 Accepted: 29 September 2022

DOI: 10.1111/1365-2435.14214

Functional Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Temperature-mediated transgenerational plasticity influences movement behaviour in the green algae *Chlamydomonas reinhardtii*

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Funding information
National Science Foun Award Number: DEB1856 Stafford Summer Resea Fund

Handling Editor: Michael Pfren

Abstract

1. A prerequisite for the survival and repro navigate thermal environmental conditions While effective movement behaviour has be nism by which organisms and populations m directional environmental warming, it rem ity may mediate such effects, plasticity can l generations.
2. Here, we examine the capacity for transgen ment behaviour of *Chlamydomonas reinhardtii* algae to changes in thermal conditions.
3. We first acclimated *C. reinhardtii* populations to thermal en (25°C), below (12.5°C) or above (37.5°C) t population growth rate. Subsequently, we m ment behaviour of these populations in th across a period of 2 weeks in each respe evaluating the influence of thermal histo thermal environment.
4. Our results indicate that thermal history of *C. reinhardtii* individuals for up to 10 generati which phenotypes converge on their acclim ear. Subsequently, we demonstrated that assay i environment at first thermal acclimation history ca patterns at ecologically relevant scales.
5. Collectively these findings indicate the ticity to modify behavioural responses over multige mated states via nonlinear trajectories. U for navigating novel thermal environments

amidst environmental warming, may thus re... sent environmental conditions.

KEYWORDS
behavioural plasticity, climate change, movement variability, transgenerational plasticity

1 | INTRODUCTION

Organisms persist in a mosaic of conditions that must be successfully navigated to survive and reproduce. Maximizing fitness within this mosaic involves effectively processing and responding to information across timescales, such as predation risk, or the distribution of food resources (... 1999; Hermes ... 2006; ... Tarie ... 2020). Yet, biological responses to environmental cues occur at varying rates; ranging from near-instantaneous (within seconds to minutes) such as ... 2020) and behavioural responses (Tuomainen & Candolin, 2011) to those that occur over longer time-scales (e.g. developmental or morphological ... 2019). When the rates of such phenotypic adjustments do not perfectly track the environment (i.e. gradual plasticity), they can mean ... processes (... 2010; Fey et al., 2021; Kremer et al., 2018; ... 2022; ... 2020). As such, determining the trajectories of phenotypic change in response to environmental variation remains critical for understanding ...

Phenotypic plasticity—the ability of genetically identical individuals to express systematically different phenotypes depending on environmental conditions (... 2013)—is ... (Badyaev, 2005; Price et al., 2003) when it allows organisms to integrate information from their environment to express phenotypes on appropriate ... For example, ... size decrease observed in *Amblyrhynchus cristatus* during El Niño events may be ... efficiency (Wikelski & Thom, 2000) ... over multiple ... exhibit varying ... from short-term, reversible changes (i.e. acclimation), to those that remain fixed for an organism's lifetime (... 2015; ... 2019; ... Stamps, 2016), as well as processes ... conditions (e.g. maternal effects ... 2012). This ... of transgenerational plasticity (TGP) ... conditions of previous generations ... populations. Previous research ... TGP can confer a physiological benefit ... metabolic rate in response to ... temperature over adjacent generations (... 2018). However, the extent to which TGP is ... is, the coordinated responses of organisms to internal or external ...

responses (... 2009) remains comparatively ... ied (e.g. ... 2013; ... 2022; Crill et al., 1996; ... 2017; ... 2018; ... 2014; ... 2013). ... *Drosophila melanogaster* ... 1996) ... *Poecilia reticulata* ... 2017) ... 2014) ... (... 2021; ... 2015) ... 2019) ... *Chlamydomonas reinhardtii* ...

of population growth rate was demonstrated in *C. reinhardtii* populations (Fey et al., 2021). Together, our findings suggest that (1) the thermal history of a population can have long-lasting consequences for the ecological performance of individuals, and (2) the temperature at which phytoplankton communities converge on acclimated states would be a key trait mediating the ecological consequences of rapid environmental change. We subsequently evaluated the potential consequences of the observed changes in movement behaviour for the dispersal ability of phytoplankton.

2 | MATERIALS AND METHODS

2.1 | Microscale movement behaviour

2.1.1 | Initial assay conditions

Replicate populations of *Chlamydomonas reinhardtii* (wild-type strain 21gr; Chlamydomonas Resource Center, University of Minnesota, USA) were isolated from a single clone (from E. Litchman, Michigan State University, USA) and maintained in batch culture for a month prior to being inoculated into experimental cultures (Kremer et al., 2018). These samples were then transferred into 50 ml Duran glass beakers and placed into their respective thermal environments (either 12.5, 25 or 37.5°C) for a period of 2 weeks (Kremer et al., 2018). We considered an adequate *C. reinhardtii* (Kremer et al., 2018). This species was selected because previous studies on *C. reinhardtii* show a thermal dependence (Fey et al., 2021), it is easy to culture (Fey et al., 2021) and it also exhibits some genetic variation (Fey et al., 2021). Although *C. reinhardtii* demonstrates thermal plasticity (Fey et al., 2021), its behaviour was recorded in response that enables organisms to move away from stressful environments into more optimal ones (Sauvage et al., 2018). Cultures were periodically diluted during the exponential growth phase to ensure that cultures in the exponential growth phase were used throughout the experiment. The thermal environments were chosen to cover the temperature range that a freshwater phytoplankton community may experience (Yvon-Durocher et al., 2012), where 12.5°C was below, near or above the thermal optimum for population growth (Fey et al., 2021; Fey et al., 2021). Although 37.5°C approaching the upper critical temperature (mean ± 95% CI; Fey et al., 2021), it was still within the range of temperatures that were recorded in freshwater habitats (Yvon-Durocher et al., 2012). Over the course of the experiment, 37.5°C water temperatures are rare (Yvon-Durocher et al., 2012), they do occur (Yvon-Durocher et al., 2012) and they represent a physiologically stressful, yet manageable (Sauvage et al., 2018), challenge for the samples experiencing them. Light was provided at 80 μmol photons/m²/s and was converted into lux (Fey et al., 2021).

Following the acclimation period, the experimental cultures were transferred to one of three acute thermal environments (12.5, 25 or 37.5°C, identical to acclimation temperatures), filtered through sterile 0.2 μm Nalgene filters into 100 ml glass beakers (Sauvage et al., 2018) and left to acclimate for 30 min (Fey et al., 2021). Cultures were then inoculated into the acute environments (3 acclimation temperatures × 3 acute environments × 3 replicates) containing the same filtered medium (Fey et al., 2021) that had been previously heated in the water bath (Fey et al., 2021). Cultures were then inoculated into the acute environments (3 acclimation temperatures × 3 acute environments × 3 replicates) containing the same filtered medium (Fey et al., 2021) that had been previously heated in the water bath (Fey et al., 2021).

We monitored *C. reinhardtii* movement behaviour for the first 30 min of the acute exposure period that immediately followed the acclimation treatments. Movement behaviour data were recorded on days 0 (the first day of exposure to populations transferred into a novel thermal environment) and on days 1, 2, 5, 6 and 9 of the experiment in order to observe movement behaviour responses to acute temperature change (day 0; no acute temperature change) and to observe movement behaviour responses to acute temperature change after the cultures had been exposed to the acute thermal environments for 1, 2, 5, 6 and 9 days (Fig. 1b). Movement behaviour was recorded using a Leica DMi8 inverted microscope (Leica Microsystems, Wetzlar, Germany) with a 4× objective (Leica HC PLAN APO 4×/0.10 PH1) and a Leica DFC3000 G camera (Leica Microsystems, Wetzlar, Germany). Videos were recorded at 20 frames per second (fps) for 30 s (600 frames) at a resolution of 1,024 × 1,024 pixels (Fig. 1c). Videos were recorded as AVI files (Fig. 1c) and were then converted into .mp4 files using the software VLC media player (version 3.0.16; VideoLAN, 2021) for storage purposes. Videos were then converted into .mp4 files using the software VLC media player (version 3.0.16; VideoLAN, 2021) for storage purposes.

2.1.2 | Image analysis

Videos were analysed using the software TrackMate (version 6.0.1; Ershov et al., 2021) in Fiji (ImageJ version 2.1.0/1.53c; Schindelin et al., 2012). TrackMate is a versatile and extensible particle tracking software that is open source and scriptable (Ershov et al., 2021). We used the Laplacian of Gaussian detector with sub-pixel localization and a median filter. Cells were detected with an estimated blob diameter of 10 μm and a quality threshold of 0.5. Tracks were constructed using the simple LAP tracker with a maximum linking distance of 15 μm and a maximum gap-closing distance of 15 μm. Tracks shorter than 5 s were discarded. From the resulting tracks, we extracted the mean speed, the straight-line speed (net displacement divided by track duration) and the mean squared displacement (MSD) as a function of time lag. The MSD was fitted with the model $MSD = 4Dt + v^2t^2$, where D is the diffusion coefficient and v is the swimming speed, using nonlinear least squares. Only the first 10 s of each track were used for the MSD fit to avoid the curvature that appears at longer lags due to confinement by the finite field of view.

2.1.3 | Statistical analysis

All statistical analyses were performed in R (version 4.1.2; R Core Team, 2021). Linear mixed-effects models were fitted with the package lme4 (Bates et al., 2015), and p-values for fixed effects were obtained by likelihood ratio tests against reduced models. Acclimation temperature, acute temperature and their interaction were treated as fixed factors, and biological replicate was included as a random intercept. Mean speed was log-transformed before analysis to meet the assumptions of normality and homoscedasticity of residuals, which were checked visually with diagnostic plots. Post hoc pairwise comparisons among the nine treatment combinations were made with estimated marginal means and Tukey-adjusted contrasts using the package emmeans (Lenth, 2021). The diffusion coefficient was analysed in the same way. Because the MSD fit is sensitive to the number of tracks, we weighted each observation by the number of tracks contributing to it. Differences were considered significant at $\alpha = 0.05$, and all reported values are means ± standard error unless otherwise stated.

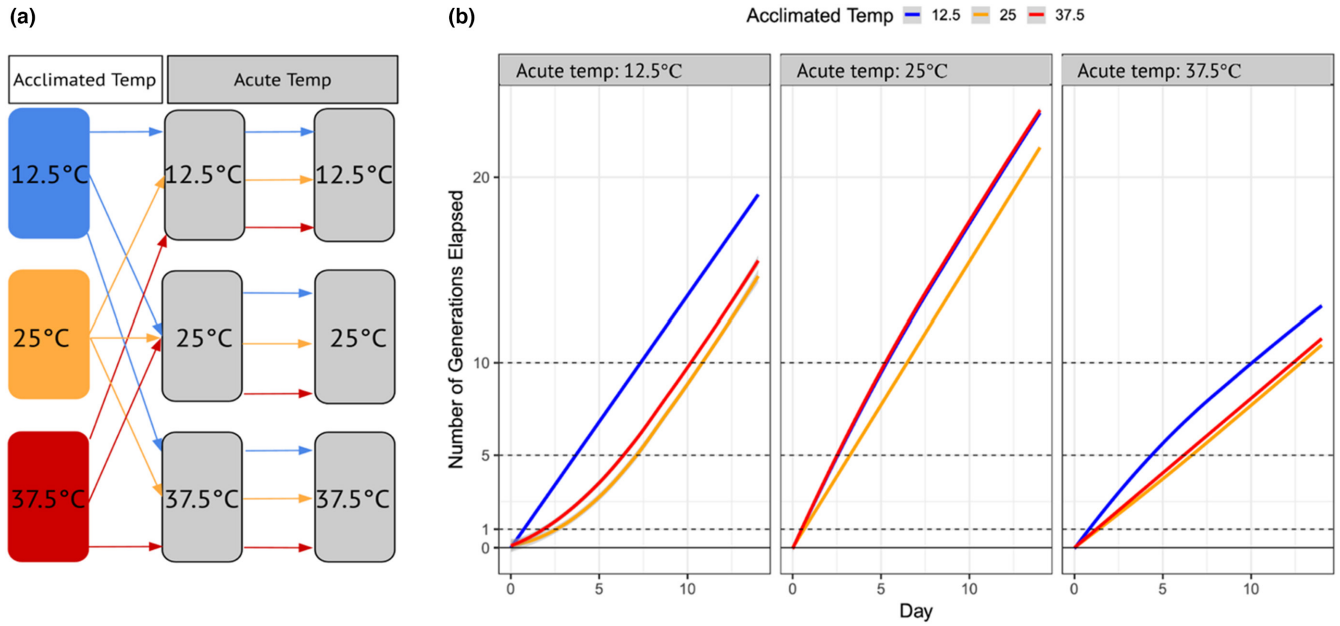

FIGURE (a) Conceptual representation of the experimental design. Arrow colour among acclimation and acute environments, cor=12.5°C, yellow=25°C, to the initi red=37.5°C) and box colour (thermal environments identical to arrow co environment. (b) Estimated number of generations elapsed across the acclimation environments, colours identical to (a)

package (Wickham, 2019). The TrackMate output provided by FIJI contains the spatial (x, y) and (time coordinates of each grid vertex on the arrival to the course of the sample, as well as the mean 1D acceleration of the individual. These metrics were summarized for each of the daily replicate and used to estimate the mean (if spread) (median for a given track) and the proportion of the sampled (2) in comparison with the entire population. The moveHMM package was used to estimate, a priori, absolute turning angle of particles behaving in a way that, lower absolute turning angle reflected directed movement (Figure S2).

2.1.3 | Statistical analysis

We examined the potential for the Mean Squared Displacement (MSD) thermal conditions to modify phytoplankton movement. We did this using two approaches. First, to determine whether the rate of MSD initially modified the different movement patterns observed in the immediate and transgenerational impacts of the behavioural traits examined, we fitted linear models to the data of an initial acute exposure (AcV1), for all response variables, first checking for homogeneity of variance. We additionally applied Holm–Bonferroni correction (Holm, 1979) to multiple statistical tests made on the same response variable to avoid inflating Type I error. To determine whether the observed changes in behaviour were sustained over enough generations that we can distinguish the behavioural observation error. Quantitatively, we compared the 95% confidence intervals satisfied these criteria a

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we decided that the corresponding cell that sent those of a cell that was observed to move with a meaningful displacement. This approach excluded periods of time.

Both of these features (S3 and S4) could contribute to the inclusion of erroneous cell tracks in these data together across hundreds of observed cell tracks sample reveals additional challenges to the correlation among movement metrics, we also used large number of cells moving in some experimental namics, disturbance speeds) obtained from the analysis by the mechanisms described above.

Having determined which observations contained cells that were moving consistently, we computed rics characterizing the movement properties of whether these properties varied in systematic ing ways across our treatments. We focused proportion of observation intervals in which cells velocities of moving cells and the directionality periods when they were actively moving. To value summarizing an entire population, we actually measured at the level of individual and produce a distribution of values for each and time point. We then characterized the distributions by estimating the median (a measure tendency that is more resilient to outliers) of ulation of moving cells. Similarly, we modelled the turning behaviour of cells. Differencing each the turning angle of a cell can be computed in radians from −π to π (−180° to 180°). To cause it is immaterial to us whether a cell turned (negative) or the other (positive), we took the turning angle, ans called the 'absolute angle'. The result is a distribution of values between 0 (no turning) and 1 (a cell that ran back along the same line). To tions are reasonably well characterized by a we fit to each unique replicate, we again used extract the mean of the fit beta distribution (the sure of the overall turning behaviour of cells. Note that the underlying distribution is many cells hardly move at all, many very small This property is well captured by the beta intuitively reflected in their mean and variance, roughly interpretable as the average angle vals in which moving cells turned (consistency).

Finally, because our analysis aimed to haviour of entire populations of cells (rather estimates), it is important to consider the populations. To this end, we used a bootstrapping approach to calculate 95% confidence metrics of interest.

To test whether the mean cell speed and cell speed varied with acclimation temperature, generalized additive models (GAMs) via the for noise in the data we used a bootstrapping based on resampling individual cells from each sample 1,000 times, and then computed 95% confidence intervals of the fitted GAM. We used a linear model with acclimation temperature as a fixed effect and day as a random effect (Zuur et al., 2009). Specifically, we used the following model structure:

$$\text{Response} \sim s(\text{day}, \text{by} = \text{Acclimated\_Temp}, k = 6) + \text{Acclimated\_Temp}.$$

We assessed whether the rate of cell speed vary by acclimation history by comparing

involving a single smooth term ignoring acclimation history.

$$\text{Response} \sim s(\text{day}, k = 6) + \text{Acclimated\_Temp}$$

This reduced model was compared comparison (not shown). In the model performed substantially the full models but note the was favoured.

While the above descriptions all movement metrics, several analyses. For median speed, log link function to describe is necessarily >= 0. Finally, mean scaled absolute turning a logit link function, which tween 0 and 1.

Finally, to observe the combined treatment, we performed a on how all combinations of influenced *C. reinhardtii* movement et al., 2019). This analysis median speed, proportion solute turning angle combination of acclimation time point. This analysis metrics. Using this matrix, analysis (PCoA) and with a different acclimation two axes retrieved by ment (Legendre, 2018). each treatment indicates of the given acute environment. total distance travelled acute environment in order acclimated versus change in behavioural were calculated to evaluate acclimation histories

2.2 | Dispersal movement behaviour assays

2.2.1 | Experimental set-up

To determine movement behaviour an assay designed and 25°C acclimated

retlocatdinsgpeourse reaswpaynsfeoromaaiavbalren teonvtiirmoe climated populations. We first-t creatial gradient using thre86, cuPetromiva Scientific, Inc.), positio5 5W. We adjac ets ot bhieishetbtalenowpiegha tuusrieng grAaldCicent 31 fmraigmnoirhidtyteosft ctaosecsoltdhees trepobusciedtion) w 8 0 mol W/m^2/psr.e sTehnets et hteemrpeesrualtusr eosf were e the sittuoatciroenast ewhaenr ee stthaeb lriesdhuecde dtemmopdeerlatur optimal temperature known to *C. eli reinhardtii* (Steokioguur2018). Metamaldy,des of 6- allow all mbovement metrics, several key to heatbumesvediamfoegedinbeabvaeem rs, we pos analyses. For median speed, we upsleedxiagalgaasmsmatudbiesstraidbuotsisoniwciuthataor log link function to describe vaGiraitpipcenr, amCoantgeyt)reoantmtehmetse,ndasdtspeeedt is necessarily >= 0. Finally, for both prpposirtiionedhosvuiolgtalmalt faor25% of the t mean scaled absolute turning angleex,tewrenaulseidncaubeatarsdisandb50% oofwitthe a logit link functionr.iaEachfotruvealhuedsfthvaet lsocaledsb tween 0 and 1. eter, the holes were spread out a or

Finally, to observe the combinedalpiavaly ofhraughttaheitsesapercotsiveaimu treatment, we performed a communaintdy1t.6a0jenecttoreys)anaalysiesma(CTEA) uncap on how all combinations of acclSmaantpilcens avredeacotfeecetempefaomreach of influenced *C. reinhardtii* movement traits acrosexpteirmne(nDe CSáudbeet)leesogtavpsen(3a8jacent et 2019). This analysis consideredwtehrse rseusrpnroousmedevdarbiyabaldedsitofontahle PVC median speed, proportion of p-arttihcleesxtmeorvirionrg,ofantdhethiencsubaalteodrsabtom solute turning angle averaged actesmpereapturieatedsweinsthuireeachinuenairquhe combination of acclimation histoTrybeasdwaceteesubsempeueandtdyeCfMaBlCeadanwhit time point. This analyCsuirstibsegdainstsbiymeidqaurinigbBarbey—to set temperatures ity index to compute the distancachmsaaytrix among these behaviour metrics. Using this matrix, we pePrfiormetdlo adipsCpiremindsrpibiala lpaospsuadyast,iin-antse we analysis (PCoA) and projected thcelitmaatjeelctionrythoefeexapcohnepnotpiualtagiroonwth with a different acclimation his1t2o.r5y°Cin,2a5 °bCipalmodt3f7o.r5m°eCdfboyrtahepefrirosc two axes retrieved by the PCo-A fgolrasesacbheaakceurtse.tFhoelrlmcawlinegnvtihrionsn acl ment (Legendre, 2018). Lelgnentdhries, CTA, tphoeputlraatjieocntsorfyrofmoreach Ma=e2 ltiomaali)nwe each treatment indicates the -patahsoavyeerdtfiomrtbhye wahbiiclhitythetomdvieslperve ment metrics of each acclimatiogradeantmebtaseidnvergteheoraftohensmentioneof the given acute environment. Mbeolsouwbisnegqutehnetleyxpcoosnupruteeodf pbopeulatiotal distance travelled for eacahmdac4cbliamyast.iTmehedlaysrwyeweitsheinadeteach acute environment in order to deotfemmisicnaedewhriecshpocnosmebsinaantdiotnosopfan acclimated versus nonacclimated spiogpnuilfaitciamnts exchaibniteted dhiefmoencesin change in behavioural Kendalls tau rafiallendtoestoMabnen-observed. Because only were calculated to evaluate whetpheerrdtahye,thaejecdterieswhicalmyactowloimat acclimation histories exhibit-ed deteidmenced orfandcoomvleyr.gence (con sistent with monotonic decreases in distance) in the same acute environment.

2.2.2 | Data collection

Duriecurryabsesaryes each assay, the tem points along a randomly selected tu (Thermoworks, American Fork, UT) to trials, and *C. reinhardtii* cackegrmsouinndiesweremo

C. reinhardtii cells were manually and continuously pipetted (Trilogy, Turner D density), using a compatible R^2 > 0.99 (Fey Cells were inoculated into a turbidostat

three acclimation temperatures were cultured to ~1.5 million cells/ml. Each C. reinhardtii [illegible] hardtii at the hottest position, each tube was sampled three times (each at [illegible] tube) from each of the five horizontal positions from the hottest to the coldest [illegible] formed for each acclimation history (immediately), and after 1 (24 H) [illegible] 37.5°C environment.

2.2.3 | Statistical analyses

We calculated C. reinhardtii density within [illegible] as the sum of cell densities across [illegible] tions and calculated the relative [illegible] at each position (corresponding to [illegible] from the incubation site). We subsequently calculated [illegible] persal of each C. reinhardtii population as the [illegible] C. reinhardtii individual across the five [illegible] We then used ANOVAs (following [illegible] of normality and homogeneity of variance [illegible] each assay day to determine whether [illegible] a significant effect on the mean [illegible]

3 | RESULTS

3.1 | Microscale movement behaviour assays

Acclimation to historical thermal environments [illegible] observed movement by C. reinhardtii [illegible] exhibited significant initial differences [illegible] scaled absolute angle using ANOVAs [illegible]

[illegible] ($F_{2,24} = 10.22$ [illegible]) [illegible] ($F_{2,24} = 14.84$, $p = 0.0013$; Figure 2). While [illegible] not [illegible] After [illegible] 30 [illegible] observed week-evide[illegible] [illegible] ($F_{2,24} = 3.223$, $p = 0.0576$; Figure 2). The [illegible] Each assay [illegible] 37.5°C [illegible] 12.5°C [illegible] 25°C [illegible] treatments initially had the lowest [illegible] cells moving (16.48%) and [illegible] (0.45 average scaled absolute angle) [illegible] the acclimation histories (Figure 2) [illegible] Additionally, we observed no evide[illegible] [illegible] the replicate [illegible] ANOVAs: (proportion [illegible] $F_{2,24} = 0.314$, $p = 0.58$; speed $F_{2,24} = 0.003$, $p = 0.96$ [illegible] $F_{2,24} = 0.068$, $p = 0.80$) [illegible] of the total cell densi[illegible] [illegible] population [illegible] assayed in 12.5°C enviro[illegible] [illegible] observed [illegible] (Figure 3) [illegible] C. reinhardtii populations acclimated t[illegible] [illegible] during the assay. The 25[illegible] similarly on average across the du[illegible] ence in Figure 3). While the 37.5°C mov[illegible] on average than the others (as seen [illegible] interim (Figure 3). Our most apparent instances of a[illegible] a sustained impact on C. reinhardtii behaviour [illegible] 25°C [illegible] acclimated population exhibited beh[illegible] 25°C [illegible] (Figure 3) [illegible] (Figure 1). (During this interim, [illegible]

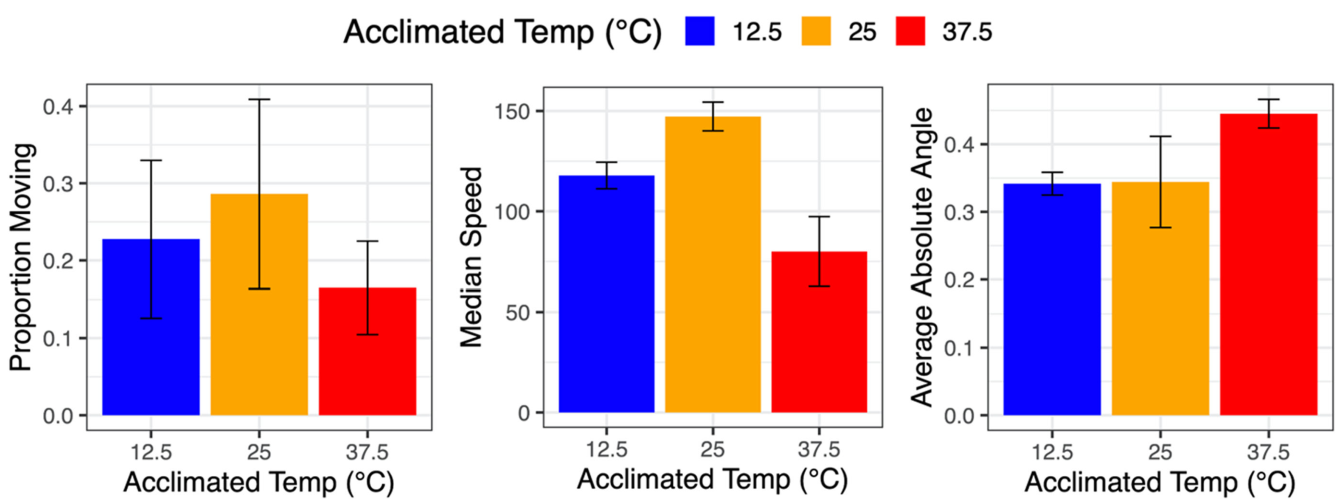

FIGURE 2 The behavioural responses for populations on experiment day 0, [illegible] (centre) median speed (microns/sec) and (right) average scaled absol[illegible] (blue = 12.5°C, yellow = 25°C, red = 37.5°C) and error bars +/− 1 standard error

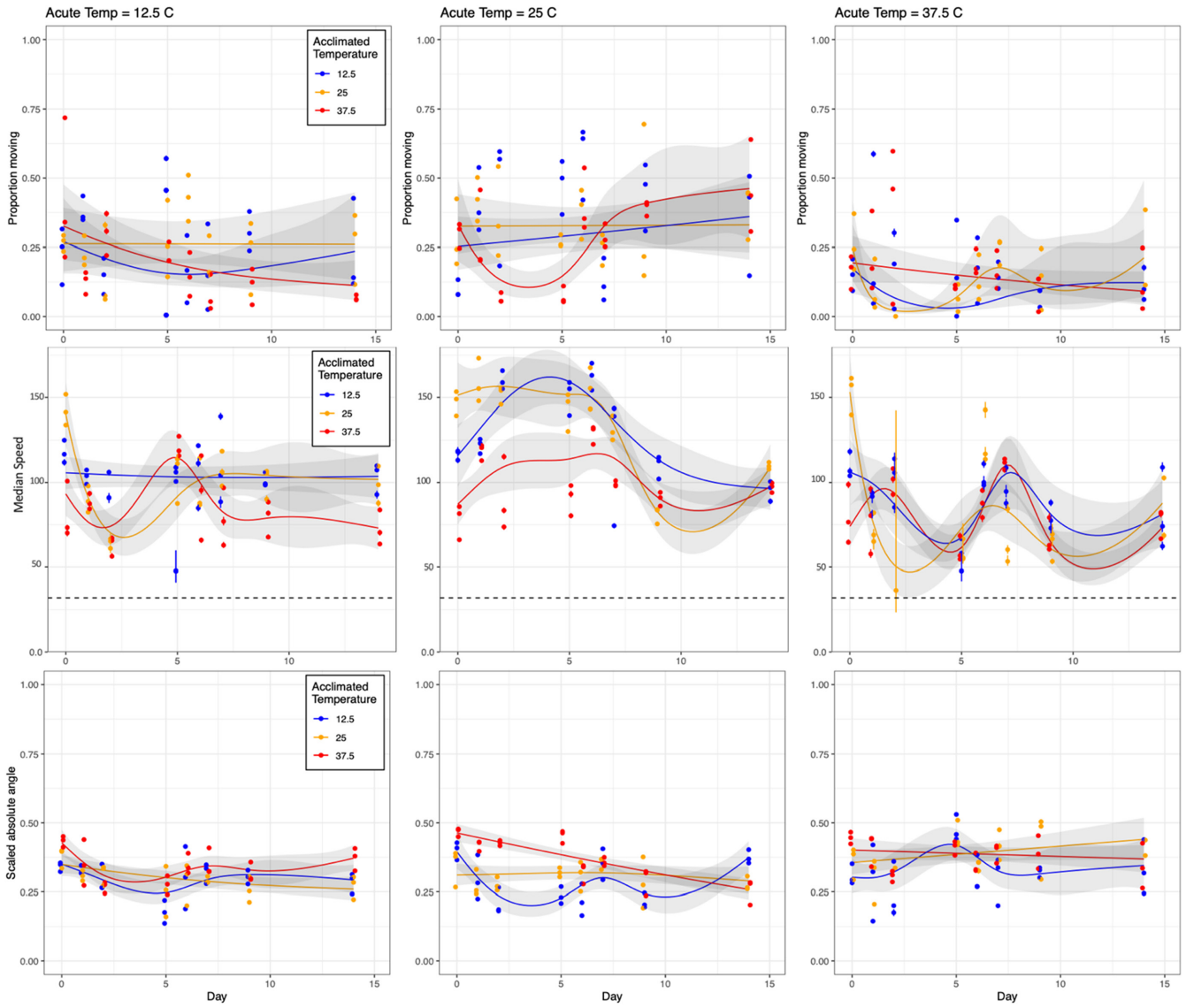

FIGURE 3 Trajectories of movement behaviours based on acclimation and acute temperature. (top) proportion of moving particles, (middle) median speed (microns/sec), (bottom) scaled absolute angle (radians/sec). Points represent the mean ± standard error bars (SE) for each day), columns indicate the acute temperature and colours represent the different acclimation environments (consistent with Figure 2). Lines represent the regression fits and shading represents 95% confidence bands

populations exhibited relatively consistent proportions of moving particles across the 5 days of the experiment. However, the 12.5°C acclimated and the 25°C acclimated populations exhibited significantly lower scaled absolute angles (i.e. straighter swimming paths) relative to the 37.5°C acclimated populations (Figure 3, Table S6). Interestingly, the 12.5°C acclimated populations exhibited increased proportion moving and increased median speeds relative to the 25°C acclimated populations (Figure 3, Table S6). Together, these results indicate that the 12.5°C acclimated populations exhibited more active swimming behaviours relative to the 25°C acclimated populations, but that the straightness of their swimming paths did not differ significantly from the 25°C acclimated populations.

Acclimation rate varied depending on the extent of the initial temperature change, as the 12.5°C acclimated populations exhibited significantly greater changes in behaviour relative to the 25°C acclimated populations (Figure 3, Table S6). Specifically, the 12.5°C acclimated populations exhibited significantly greater increases in proportion moving and median speed relative to the 25°C acclimated populations (Figure 3, Table S6). Together, these results indicate that the 12.5°C acclimated populations exhibited more active swimming behaviours relative to the 25°C acclimated populations, but that the straightness of their swimming paths did not differ significantly from the 25°C acclimated populations.

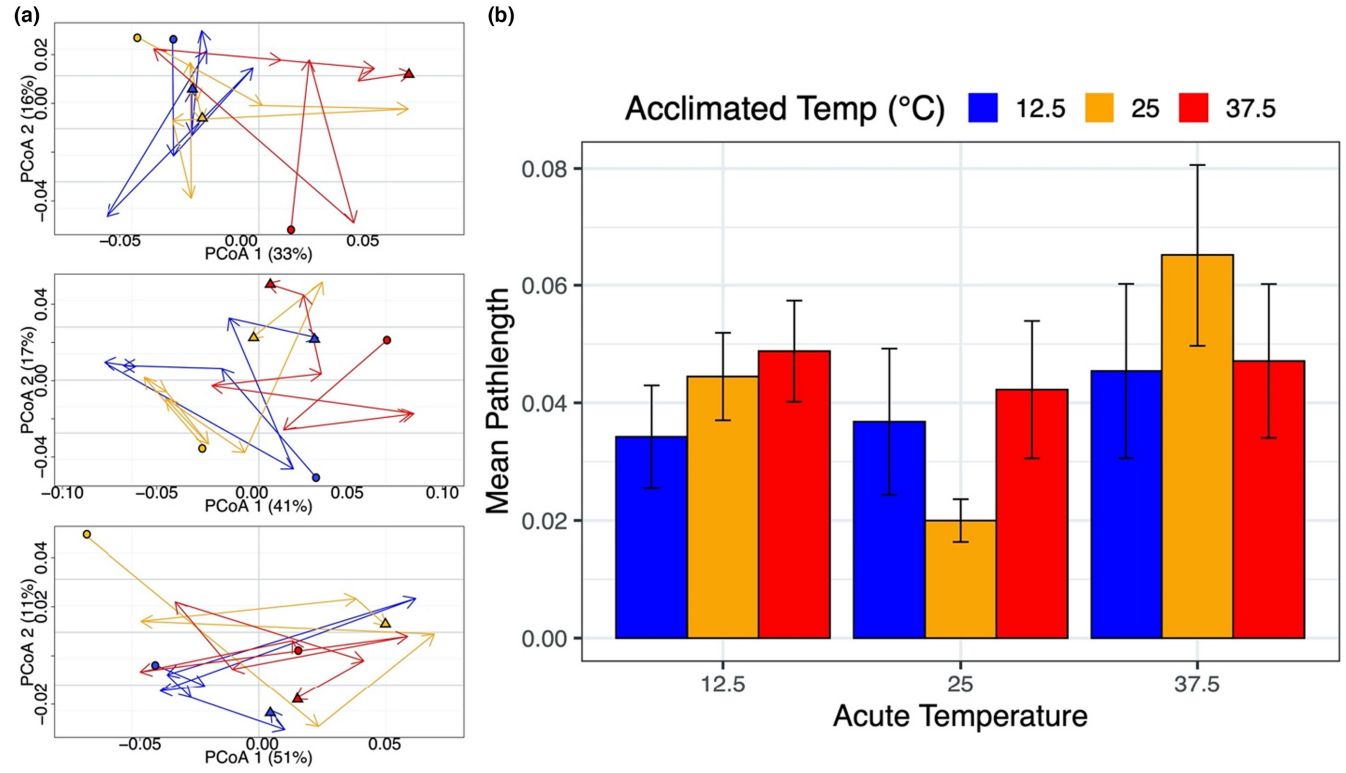

FIGURE 4 Experimental community trajectory analysis (CTA) in 12.5°C environments. Panels are faceted by acute environment to visualize w near each other) once populations experience a common *Chlamydomonas* envi *reinhardtii* behavioural phenotypes, averaged within treatments, from day 0 1, 2, 5, 6, 7, 9, and 14, arrows indicate the direction of movemen a from Figure 2 and 3). Note the axis labels. (b) Mean pathlength corresponding to th Colours represent acclimated temperature and error bars represent

trajectories (Figure 4b; $p = 0.044$), environment, we observed litt the 37.5°C acclimated populations differences in $F_{2,12} = 2.49$, $p = 0.$ the acclimated populations relative to the 12.5°C acclimated p when transitioned into these novel thermal environments, populations on averag

3.2 Dispersal movement behaviour

Based on the aforementioned results, we anticipated that populations acclimated to 25°C would demonstrate the greatest dispersal while populations acclimated to 37.5°C would disperse the least dispersal. Thermal acclimation differences in shorter-term behavioural responses (Figure 5; one-way ANOVA: day 0, $F_{2,12} = 6.53$, $p = 0.012$; day 1, $F_{2,12} = 3.97$, $p = 0.048$). The differences on day 1 resulted from 37.5°C acclimated populations dispersing significantly less than 25°C acclimated populations (Tukey's HSD: $p = 0.012$) and while not significantly different, this trend was also observed between 37.5°C and 12.5°C acclimated populations ($p = 0.051$). The low dispersal observed in 37.5°C acclimated populations may be explained by the fact that warm-acclimated samples represent 18% and 14% reductions in swimming speed and activity, respectively, relative to cool-acclimated samples (Table 1). Similarly, differences on day 1 resulted from 37.5°C acclimated populations dispersing less than 25°C acclimated populations (Lewington-Pearce et al., 2023), a trend also observed in *Tribolium* beetles (Weaver et al., 2014) and

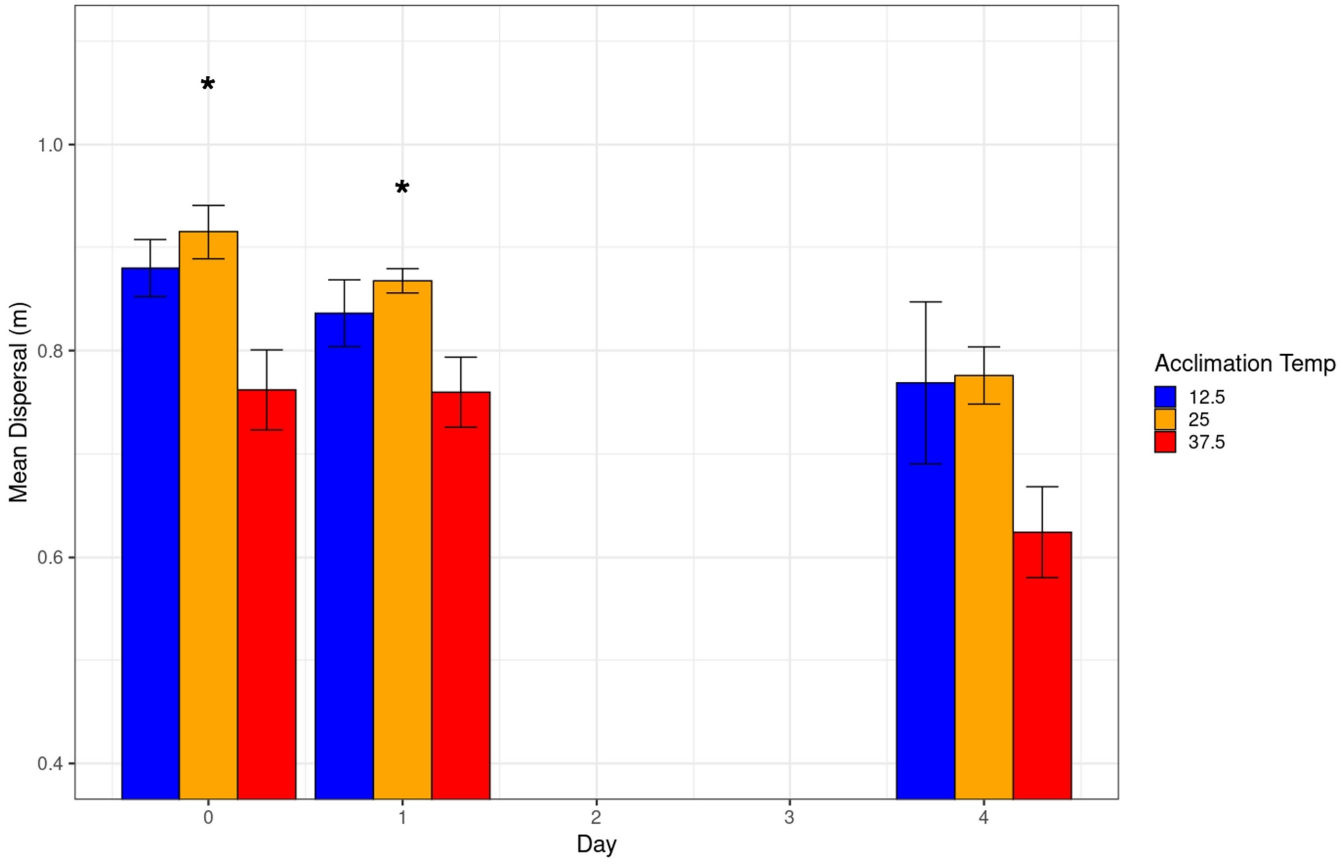

FIGURE Mean dispersal (±95% CI) of *Chlamydomonas reinhardtii* populations in a spatially heterogeneous environment where a mean dispersal of 0 indicates populations that remained in the centre of the habitat. Acclimation temperature, and days indicates days after being transitioned to a 37.5°C environment. Asterisks indicate significant differences among mean dispersal distances

to *Acanthochromis polyacanthus* (Welch et al., 2014). However, progression to a target behaviour for extent to which TGP can modify behaviour. For example, while *C. reinhardtii* populations acclimated remains comparatively underexplored. Currently, studies that have documented changes in behaviour across multiple generations, and/or the persistence of any observed effects, all report effects from days 2 to 5 on swimming speed and *Tetrahymena thermophila* photokinetic response, which persisted for up to 35 days (Ashe et al., 2021; Fig. 3). This is a phenomenon often referred to as the persistent effects we observed here (i.e. 5 days at 37.5°C) estimated populations assayed in novel environments (25°C) with ments are among the most sustained changes in behaviour during TGP recorded to date. Continuing to emphasize the importance of behavioural phenotypic plasticity will address whether these effects are rare because the experiments have not historically been designed for and duration.

Beyond emphasizing the importance of behaviour, our results suggest a further neglected dimension of phenotypic plasticity: trajectory. For certain combinations of environmental (i.e. acclimation to acute environmental stress), the resulting phenotypes expressed were only transient

et al., 2009; Sih et al., 2011; Sunday et al., 2014). Overall, data from the literature suggest that the individuals that could promote range expansion into cooler environments (those experiencing lower temperatures) least possess the *C. reinhardtii* strains tested in this study. Individuals from populations acclimated to 25°C were observed to exhibit the greatest dispersal capacity, even when faced with a 37.5°C environment. Body size and shape are morphological traits that covary across data agreed that the 37.5°C acclimated populations exhibited the poorest dispersal abilities as a result of their relatively high (though absolute angle) directional movement even when faced with speeds. Such patterns may occur when the relative importance dependence of performance, similar to that observed in (Fig. 1) also declines at such temperatures (Cavanaugh, 2014), a observation in relying on behavioural strategies may at times be more reliance on physiologically based *C. reinhardtii* populations at stress environments and signal across cellular machinery such as heat (Schulz et al., 2007). Similarly, the relationship between the proportion of moving population growth and investment in reproduction observed in response to *Escherichia coli* (Arnoldini et al., 2010).

We posit that the behavioural strategies in environments that undergo periodic these exact consequences remain unexplored. Heatwaves have been recognized as a threat to freshwater ecosystems (Woolway et al., 2021), and such events will likely increase in freshwater environments from climate change, increasingly warm and thermally stressful periods of time. During such exposures, the relationship between dispersal capacity for extended periods of time may be advantageous. We observed the susceptibility of 12.5 and 25°C acclimated populations to exposure at least, thus the gradual nature of these changes may be beneficial for *C. reinhardtii* individuals transitioning from environments. While behavioural strategies at which maximal dispersal capabilities are achieved (e.g. ing thermal performance curves) may lead to speciation and evolution to behavulations have an extended transient phase of movement (e.g. moved to colder environments), the *C. reinhardtii* may not have an equally negative fitness impact in hot environments. However, our data do not suggest that lake heatwaves exert on phytoplankton evolution. What life history values. What the overall adaptive value of behaviour typical conditions and the role that behavioural strategies across more complex patterns of environmental variability posed in this present study (e.g. remains an open area of inquiry.

In addition to the idealized temperature experiments this experiment, the experimental arena was from the lake and pond ecosystems

accurately predicting how organisms respond to changing environmental conditions may require an enhanced integration of how past and current environmental regimes jointly influence ecological processes.

AUTHOR CONTRIBUTIONS

Samuel B. Fey, Tamara J. Layden and Hannah S. Meier designed the experiments; Tamara J. Layden and Isaac J. Schuman conducted the experiments; Colin T. Kremer, Hannah S. Meier, Anna Ritz and Samuel B. Fey analysed the data; Hannah S. Meier, Samuel B. Fey and Colin T. Kremer wrote the initial draft of the manuscript, with all authors contributing substantially to revisions.

ACKNOWLEDGEMENTS

This research was made possible by NSF grant DEB1856415 to SBF and the Helen Stafford Summer Research Fellowship Fund at Reed College. Thanks to G. Glover, J. Ewing, A. Klinger and the Kremer Lab for logistical support, and to T. Michelot, for analytical support.

CONFLICT OF INTEREST

At the time of authorship we identify no immediate conflict of interest.

DATA AVAILABILITY STATEMENT

All data that support the findings of this study are available on Dryad Digital Repository at https://doi.org/10.5061/dryad.v6wwpzh0c (Meier et al., 2022).

All code is available on Zenodo: https://doi.org/10.5281/zenodo.7150637 and will be appropriately archived following the publication of this manuscript.

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How to cite this article: Meier, H. S., Schuman, I. J., Ritz, A., Kremer, C. T., & Fey, S. B. (2021). Predator-mediated transgenerational plasticity in behaviour in *Chlamydomonas reinhardtii* algae. *Functional Ecology*, *36*, 2969–2982. https://doi.org/10.1111/1365-2435.14214