Title: Range expansion is both slower and more variable with rapid evolution across a spatial gradient in temperature

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ABSTRACT

Rapid evolution in colonizing populations can alter our ability to predict future range expansions. Recent theory suggests that the dynamics of replicate range expansions are less variable, and hence more predictable, with increased selection at the expanding range front. Here, we test whether selection from environmental gradients across space produces more consistent range expansion speeds, using the experimental evolution of replicate duckweed populations colonizing landscapes with and without a temperature gradient. We found that range expansion across a temperature gradient was slower on average, with range-front populations displaying higher population densities, and genetic signatures and trait changes consistent with directional selection. Despite this, we found that with a spatial gradient range expansion speed became more variable and less consistent among replicates over time. Our results therefore challenge current theory, highlighting that chance can still shape the genetic response to selection to influence our ability to predict range expansion speeds.

INTRODUCTION

Predicting the movement of species ranges is a fundamental goal of ecology and is of increasing importance as biological invasions and climate change accelerate range expansion (Chen et al. 2011; Iseli et al. 2023; Nadeau & Urban 2019). Classic work on the ecological dynamics of range expansion has demonstrated that higher values of two key life-history traits – dispersal and reproductive output – increase the speed of range expansion (Kot et al. 1996; Skellam 1951). More recent work incorporating rapid evolution has shown that not only can dispersal and reproduction evolve to increase the average speed of range expansion, but that additionally,

evolution can directly influence the amount of variability in the speed of population spread across replicate, range-expanding populations (Miller et al. 2020; Ochocki & Miller 2017; Williams et al. 2016; Wiliams et al. 2019). However, when and in what direction evolution modifies the variability of, and hence our ability to precisely predict, range expansions remain unclear.

On one hand, eco-evolutionary studies of range expansion predict that evolution should increase variability in range expansion speed across replicate populations. Specifically, serial founding events during range expansion can result in the fixation of maladaptive alleles at the range front and consequent reduction in fitness (‘expansion load’) through a spatial analogue of genetic drift (‘gene surfing’) (Excoffier et al. 2009; Klopfstein et al. 2006; Perrier et al. 2020). Gene surfing in small, range-expanding populations leads to the fixation of random amounts of expansion load across replicate populations, thus generating variability in range expansion speed (Miller et al. 2020; Phillips 2015; Williams et al. 2019). The implication then, is that any single realisation of range expansion in nature is drawn from a distribution of possible outcomes, making our ability to precisely predict the dynamics of range expansion more difficult.

In contrast, some recent experimental work demonstrates that evolution can also reduce variability in range expansion speed (Miller et al. 2020; Williams et al. 2019). This is predicted to occur when directional selection (e.g., for traits conferring greater dispersal or environmental tolerance; Chuang & Peterson 2016; Freedman et al. 2020; Lancaster et al. 2015) reduces trait variance at the range front, hence increasing consistency in the speed of range expansion (Phillips 2015; Williams et al. 2019). Overall, how evolution modifies the variability in range

expansion speed may therefore depend on the relative strength of gene surfing and selection in range-front populations, and the factors that tip this balance one way or another (Miller et al. 2020; Urquhart-Cronish et al. 2022; Williams et al. 2019).

One key factor that could tip this balance between the variance-generating and variance-reducing effects of evolution is environmental variation in space. Specifically, species' ranges in nature exist across broad-scale, spatial ecological gradients. Importantly, changing abiotic or biotic conditions across space could lead to slower range expansion due to the need for populations to adapt to the underlying spatial gradient. This initial maladaptation at the range front can allow more time for individuals to arrive from the range core, consequently increasing population size and genetic variation at the range front over time (Fronhofer et al. 2017; Gilbert et al. 2017). In these 'steeper' range boundaries, directional selection favouring traits that confer tolerance to stressful ecological conditions could offset any maladaptive gene flow from the range core, and importantly, weaken gene surfing and its genetic and demographic consequences (Gilbert et al. 2017; Williams et al. 2019). Thus, selection from underlying ecological gradients in space could make the speed of range expansion among replicate populations less variable and more predictable. Although there is a need to incorporate realistic sources of environmental variation, experimentally testing how selection from ecological gradients in space modifies the predictability of replicate range expansions in nature remains difficult (Miller et al. 2020).

Experimental range expansions in "miniaturised" landscapes (Larsen & Hargreaves 2020; Lustenhower et al. 2023) can provide a powerful bridge between theory and range expansion in natural landscapes. First, these landscapes differ from traditional microcosms in that replicate

100 populations can disperse naturally across linear landscapes, such that realistic genetic and
101 demographic spatial structure arises during range expansions. Second, through replicated
102 landscapes we can track a distribution of ecological and evolutionary outcomes in colonising
103 populations. This allows experimental insights into the factors that contribute to variability in
104 range expansion that would not be possible without replication. Third, through experimental
105 evolution we can track the eco-evolutionary dynamics of range expansion unfolding over
106 multiple generations in real time, while field studies have mostly been retrospective, inferring
107 past demographic and evolutionary processes based on current spatial patterns. Despite the
108 increasing utility of these landscapes (Larsen & Hargreaves 2020), we still require experiments
109 that test the dynamics of range expansion across landscapes that incorporate selection from
110 spatial environmental variation.
111
112 Here, we use experimental populations of duckweeds in the *Lemna* species complex to provide a
113 first experimental test for how selection from spatial abiotic gradients modifies variability in the
114 rate of replicate range expansions. Using landscapes with and without a temperature gradient in
115 space, we predicted that: (i) range expansion speed should be less variable among replicate
116 populations moving across landscapes with a spatial temperature gradient than without. We
117 predicted that this would occur as: (ii) range expansion across temperature gradients would be on
118 average slower, resulting in higher population densities and steeper range boundaries.
119 Consequently, we predicted that there should be stronger signatures of selection over genetic
120 drift in populations expanding across temperature gradients. Specifically, we predicted: (iii)
121 directional selection for traits relevant to temperature stress; and (iv) less among-replicate
122 variability in genotypic composition than predicted by chance.

MATERIALS & METHODS

Lemna experimental populations

Duckweeds are small, freshwater angiosperms with a rapid generation time of 2–5 days under ideal conditions (Landolt 1986). Sexual reproduction is very rare in duckweeds, with reproduction at experimental timescales occurring exclusively through the clonal budding of daughter fronds (Docauer 1983). We sampled 20 accessions of duckweeds in the *Lemna* species complex from 20 sites around the Pacific Northwest region of North America (Table S1). Microsatellite genotyping of each accession and molecular barcoding approach using Tubulin-Based Polymorphism (TBP) fingerprinting (Braglia et al. 2021a) detected that our accessions were composed of 11 unique genotypes, potentially grouping to 6 genotypes of *L. minor* and 5 genotypes of the putative *L. minor*-*L. turionifera* hybrid *L. japonica* (Appendix S1; Tables S1-S3). Species delineation remains challenging because these two lineages are ecologically and genetically similar to one another while varying widely in ploidy levels, and different molecular markers lead to inconsistent identification at the species level (Appendix S1). In our experiment, we therefore describe evolution as occurring through the sorting of unique genotypes within part of the *Lemna* species complex.

Experimental landscapes and range expansion

We set up 50 experimental landscapes across five benches inside the UBC greenhouse, with each landscape constructed from aluminium gutters (150 cm x 12.5 cm; L x W; Fig. 1a). We set up a temperature treatment using submersible heaters (150W JBJ aquarium heaters) such that range expansion occurred across landscapes with a spatial gradient of increasing temperatures

146 ("gradient landscapes" hereafter; β = 0.027, 95% CI: 0.027 to 0.028 °C/cm) or across landscapes
147 with near-uniform temperatures ("uniform landscapes" hereafter; β = –0.001, 95% CI: –0.002 to
148 0.000 °C/cm; Fig. 1b). The position of gradient and uniform landscapes (N = 25 each) was
149 randomised within each bench. We note that because we chose to standardize mean temperatures
150 between our uniform and gradient landscapes (27.4°C; SD = 3.85°C and 26.9°C; SD = 1.53°C,
151 respectively), temperatures at the start of gradient landscapes were relatively cooler than in
152 uniform landscapes (24.3°C; SD = 2.6°C and 27.6°C; SD = 1.4°C, respectively; Fig. S1).
153 However, we note that our outcomes still reflect the presence and absence of a temperature
154 gradient across space in gradient and uniform landscapes, respectively (Appendix S2 for
155 additional experimental details).
156
157 To initiate range expansion, we established genetically diverse *Lemna* populations, with each
158 population consisting of an even density of 20 accessions (N = 20 individuals per accession for a
159 founding population size of N = 400 individuals; Appendix S2). We seeded each population at
160 one end of each landscape, allowing 2 days for plants to acclimate before range expansion. In
161 gradient landscapes, we seeded populations at the cooler end of the landscape and allowed
162 expansion into increasingly warmer temperatures (Fig. 1a). To allow all populations the
163 opportunity to colonize the full spatial extent of the landscape, the experiment ran for 112 days.
164 During our experiment, we lost one replicate due to structural damage and lost 10 replicates on
165 one bench due to a disturbance event that disrupted population spread. We also lost 6 replicates
166 due to heavy algal growth on the water surface that obstructed population spread. In total, we
167 retained 33 experimental landscapes across four benches (N = 16 gradient landscapes; N = 17
168 uniform landscapes).

169

170 **Quantifying range expansion speed**

171 We quantified the mean and variability in range expansion speed across landscapes for the initial
172 24 days (i.e., ~5–12 generations) of range expansion. This timescale represents population spread
173 during the exponential growth phase and thus is most consistent with theoretical models of range
174 expansion (Kot et al. 1996; Skellam 1951). Also, some populations had reached the range limit
175 of landscapes by day 24. These replicates would not be able to spread further and their inclusion
176 after 24 days would artificially reduce the average and among-replicate variability in range
177 expansion speed (i.e., the ceiling effect).

178

179 To quantify range expansion speed, we took aerial photographs of each landscape every 4 days
180 (Nikkon D750). From these images, we first quantified the maximum distance travelled (cm) for
181 each population by measuring the distance reached by the furthest individual every 4 days. We
182 then quantified the mean and among-replicate variability in the distance travelled at each
183 treatment level and time point. To estimate the difference in among-replicate variability of
184 distance travelled for gradient and uniform landscapes, we calculated the log coefficient of
185 variation ratio (lnCVR; Nakagawa et al. 2015) as:

186 $$lnCVR = \frac{lnCV_E}{lnCV_C} + \frac{1}{2(n_E-1)} - \frac{1}{2(n_C-1)} \qquad (1)$$

187 where subscripts *E* and *C* refer to the experimental (i.e., gradient) and control (i.e., uniform)
188 landscapes, CV is the coefficient of variation, and *n* is the sample size. We calculated lnCVR
189 such that positive values correspond to larger variability in distance travelled among gradient
190 compared to uniform landscapes. We use lnCVR because CV accounts for the linear and positive

191 mean-variance relationship on the log scale as observed in our dataset (Fig. S2) where shifts in
192 variability could be driven by corresponding shifts in the mean (Taylor 1961). In such cases, it is
193 preferable to use CV as a measure of variability to account for differences in the mean, although
194 for sensitivity we also estimated the log variation ratio (lnVR) to estimate changes in variability
195 (SD) without accounting for mean differences (Appendix S4). To report differences in CV, we
196 back-transformed estimates of lnCVR.
197
198 **Quantifying range-front population density and steepness**
199 We quantified spatiotemporal changes in population density at the range front by estimating the
200 percent cover of live (i.e., green) fronds on the water surface. We did this by colour thresholding
201 images obtained every 4 days using ImageJ (v1.53) and estimating percent cover in 2 cm x 12.5
202 cm grids at the range front (defined as the first 12 cm from the furthest travelled individual). We
203 then fit a logistic model of percent cover across space for each landscape to quantify the
204 maximum population density at the range front at each time point. We also quantified the
205 steepness of the range boundary as the rate of increase in population density across space from
206 the range front towards the range core for each landscape and time point. We did so by fitting an
207 exponential model to the first three spatial estimates of percent cover closest to the range front of
208 each landscape (i.e., percent cover at positions 0 cm, 2 cm, and 4 cm from the range front).
209 Steepness was quantified as the exponent obtained from each model (Legault et al. 2020).
210
211 **Quantifying range-front evolution**
212 We quantified evolution as the change in genotype frequencies in post-expansion populations
213 compared to the founding population. Post-experiment, we haphazardly sampled 12 individual

214 rafts of duckweeds from each replicate range front. Each sample was then genotyped using four
215 microsatellite markers in a single multiplex reaction (Appendix S1; Table S2; Hart et al. 2017).
216 We successfully obtained genotype sequences for 272 individuals (88% of all genotyped
217 samples). To balance the number of samples obtained within each replicate and treatment, we
218 rarefied samples and dropped replicates represented by less than $N = 6$ individuals due to DNA
219 extraction or genotyping failure. For all downstream analyses, we therefore obtained a total of N
220 $= 6$ individuals per replicate, for 10 and 17 replicates of gradient and uniform landscapes,
221 respectively. We note a higher extraction and genotyping failure for samples obtained from
222 gradient landscapes possibly due to greater damage to plant tissue from heat stress.
223
224 To investigate whether genotype sorting in the experiment led to trait evolution, we also
225 measured a suite of genotype-specific traits *ex situ* in incubators and across a range of
226 temperatures (Appendix S3; Fig. S5) including: maximum growth rate (r_{max}); thermal optimum
227 (T_{opt}); critical thermal maximum (CT_{max}); thermal performance breadth (i.e., the range of
228 temperatures for which growth rate is within 80% of the maximum; Padfield et al. 2021); thermal
229 tolerance breadth (i.e., the range of temperatures for which growth rate is positive); mean
230 specific-leaf area (SLA; cm^2/g); mean root-shoot ratio (cm/mg); plasticity in both SLA and root-
231 shoot ratio (i.e., CV across temperature; Valladares et al. 2006); and mean raft number of
232 duckweed fronds (i.e., the average number of fronds that stayed attached to each other during
233 clonal reproduction to form multi-frond rafts). Mean raft number was used as a proxy for
234 dispersal ability assuming plants with a smaller raft number would disperse further as fronds
235 separate and release during budding. Before analyses, we scaled and centred each trait, and

236 estimated mean trait values at the population level by weighting trait values by observed
237 genotype frequencies.
238
239 **Statistical analyses**
240 We tested for treatment differences in mean range expansion speed (prediction ii) by fitting a
241 linear mixed-effects model using the *lme4* package (Bates et al. 2015). We fit maximum distance
242 travelled (cm) as the response variable and an interaction between time (day) and temperature
243 treatment (gradient *vs* uniform) as fixed predictors, where mean range expansion speed is
244 modelled as slopes (cm/day). We also included bench ID as a fixed covariate (as there were only
245 four bench levels; Gelman & Hill 2006) and fitted a random slope (i.e., speed) for each replicate.
246 Next, we tested for treatment differences in among-replicate variability of distance travelled
247 (prediction i) by fitting a linear model with lnCVR as the response variable and time as the
248 predictor. We modelled the temporal trend in lnCVR as visual inspections of CV in distance
249 travelled showed changes over time for both gradient and uniform landscapes (Fig. 2b). For
250 sensitivity analyses, we also conducted a linear meta-regression of lnCVR to explicitly model
251 sampling variation (Appendix S5). We then tested for treatment differences in range-front
252 population density (i.e., carrying capacity) and steepness of the range boundary (i.e., exponent)
253 (prediction ii) by fitting separate linear regressions with either population density or steepness as
254 the response variable, respectively, and time, temperature treatment, and their interaction as
255 predictors. For sensitivity analyses, we also conducted a separate meta-regression of population
256 density and steepness by including the standard error around each observation using the package
257 *metafor* (Viechtbauer 2010).
258

259 We next tested for treatment differences in the evolutionary dynamics of range expansion. First,
260 we tested if evolution occurred during range expansion and if there were treatment differences in
261 the amount of genotypic or trait change (prediction iii). We estimated genotypic or trait change
262 in each landscape by quantifying the Euclidean distance between the initial and final genotype
263 frequency or trait value in multidimensional genotype or trait space (i.e., across all 11 genotypes
264 or 10 traits). We then fit separate linear regressions for genotype and trait change with Euclidean
265 distance as the response variable, temperature treatment as the predictor, and bench ID as a fixed
266 covariate. Upon observing evolution in multidimensional genotype frequencies and trait values,
267 we conducted post-hoc tests to investigate univariate changes in specific genotypes and traits.
268 For genotypic change, we tested whether certain genotypes arose to higher frequencies than
269 predicted by chance through a binomial test on the mean frequency of each genotype for each
270 temperature treatment. For trait change, we estimated the mean and 95% CI of each trait for each
271 temperature treatment and deemed traits to be significantly different from the founder population
272 if the 95% CI of the final trait value did not overlap with the initial trait value.
273
274 Second, we also tested for treatment differences in the final genotype or trait composition in
275 range-front populations (prediction iii). Here, we quantified compositional dissimilarity of
276 genotypes or traits across all pairs of replicates within each treatment using Euclidean
277 dissimilarity matrices, and then conducted separate PERMANOVAs with genotype or trait
278 dissimilarity among replicates as the response variable, temperature treatment as the predictor,
279 and bench ID as a fixed covariate. PERMANOVAs were conducted using the *adonis2* function
280 in the *vegan* package (Okansen et al. 2022). To test for treatment differences in the mean values

281 of each trait, we also conducted post-hoc *t*-tests on the final trait values observed in gradient and
282 uniform landscapes.
283
284 Third, we tested if selection led to more consistent sets of genotypes at the range front and
285 reduced among-replicate variability in genotype composition compared to a neutral model
286 (prediction iv), or, if there was still a significant role of chance in shaping genotype composition
287 at the range front. To do this, we compared the amount of observed among-replicate variability
288 in each treatment to the simulated among-replicate variability predicted by random sampling. To
289 quantify the amount of observed among-replicate variability, we averaged genotype frequencies
290 across replicates within each treatment, calculated the Euclidean distance between this mean
291 genotype composition and the observed genotype composition of each replicate (Fig. S7), and
292 then summed these Euclidean distances across all replicates within each treatment. To prevent
293 the sum value from being biased by differences in the number of replicates within each
294 treatment, for this calculation only we randomly selected 10 uniform landscapes such that the
295 number of replicates in uniform and gradient landscapes were equal ($N = 10$ for each). To
296 quantify simulated among-replicate variability predicted by random sampling, we first pooled the
297 genotype abundances across replicates within each temperature treatment (i.e., $N = 60$
298 individuals), randomly drew 6 individuals with replacement (i.e., representing the 6 genotypes in
299 our observed data) and repeated this 10 times for each treatment (i.e., representing the 10
300 replicates of gradient and uniform landscapes). We then estimated the mean genotype
301 composition for each temperature treatment, calculated the Euclidean distance between the
302 simulated mean genotype composition and the simulated genotype composition of each replicate,
303 and then finally summed these Euclidean distances for each treatment. We repeated this

304 simulation a total of 100,000 times to estimate the probability of obtaining the observed among-
305 replicate variability in genotype composition for each treatment based on random sampling.
306 Finally, we tested for treatment differences in the observed among-replicate variability through a
307 permutation test, with Euclidian distances permuted randomly across treatment 100,000 times.
308 All model-fitting was conducted in R (v4.3.0; R Core Team 2020).
309

310 **RESULTS**

311 **Effects of temperature treatment on range expansion speed**

312 On average, we found that range expansion speed across gradient landscapes was slower than
313 across uniform landscapes (time by treatment effect: $P = 0.016$; Table S4; Fig. 2a), consistent
314 with prediction (ii). Over 24 days of range expansion, the presence of a spatial temperature
315 gradient reduced the average speed of range expansion by 50.9% (95% CI: 36.0% to 53.8%)
316 compared to uniform landscapes (Table S4). We also found that the among-replicate variability
317 in distance travelled differed between gradient and uniform landscapes (Fig. 2b and 2c). At the
318 initial stages of range expansion, we found that there was less among-replicate variability (CV)
319 in distance travelled across gradient compared to uniform landscapes (e.g., a 40.3% [95% CI:
320 28.6% to 56.8%] reduction in CV across gradient compared to uniform landscapes on day 4;
321 Table S5), consistent with prediction (i). However, we found a significant increase in lnCVR
322 over time (lnCVR slope: $P = 0.002$) such that by day 24, CV in distance travelled was 78.2%
323 (95% CI: 28.6% to 151.0%) greater across gradient than uniform landscapes (Fig. 2c), contrary
324 to prediction (i). This result was consistent in the sensitivity analysis including sampling
325 variation around lnCVR in the linear model (Table S5). Sensitivity analysis of lnVR also showed

326 that the slope of lnVR over time was positive ($P = 0.002$; Fig. S3) such that SD in distance
327 travelled increased more in gradient compared to uniform landscapes over time.
328
329 **Effects of temperature treatment on range-front density and steepness**
330 We found that the population density reached at the range front differed over time and between
331 gradient and uniform landscapes (Fig. 2d-f). Although range-front densities were initially similar
332 between gradient and uniform landscapes, population density increased more rapidly over time
333 for gradient landscapes (treatment by time effect: $P = 0.002$; Fig. 2f), consistent with prediction
334 (ii). By day 24, population density at the range front was 88.1% (95% CI: 76.5% to 104.0%)
335 higher in gradient compared to uniform landscapes (Table S6). Along with increased population
336 density, we also found range boundaries to be steeper in gradient landscapes (Table S7; Fig. S4),
337 consistent with prediction (ii). These results were consistent in sensitivity models that included
338 sampling error in estimates of range-front population density and steepness (Tables S6 and S7).
339
340 **Effects of temperature treatment on evolutionary change**
341 We found that evolution in range-front populations occurred in both gradient and uniform
342 landscapes, with significant changes in both genotype frequencies (Intercept: $P < 0.001$; Table
343 S8; Fig. 3a) and trait values (Intercept: $P = 0.002$; Table S8; Fig. S6) in multidimensional
344 genotype and trait space. We found support for prediction (iii) with genetic and trait signatures
345 consistent with directional selection from a temperature gradient. First, range-front populations
346 in gradient landscapes were often dominated by one genotype (G11; Fig. 3c). Post-hoc binomial
347 tests showed that this genotype consistently increased in frequency in gradient landscapes ($P <$
348 0.001) but not in uniform landscapes ($P = 0.869$), suggesting selection for this genotype in the

former (Fig. 3e). Second, final trait values for range-front populations in gradient landscapes showed significant changes in both thermal performance breadth and thermal tolerance breadth compared to the founder population (higher and lower values, respectively; Fig. 4; Table S10), although such a change was not observed in uniform landscapes. Third, we found that genotype composition of range-front populations differed between gradient and uniform landscapes after range expansion (PERMANOVA: $P = 0.002$; Table S9; Fig. 3b). Although differences in trait composition of range-front populations between gradient and uniform landscapes were only marginally significant (PERMANOVA: $P = 0.066$; Table S9; Fig. S6), post-hoc univariate *t*-tests on final trait values found reduced plasticity in root/shoot ratio in gradient compared to uniform range-front populations ($P = 0.042$; Table S11; Fig. 4). Importantly, despite these changes suggesting selection, and contrary to prediction (iv), we found that the observed among-replicate variability in genotype composition was greater than predicted by random sampling for both gradient ($P < 0.001$) and uniform ($P < 0.001$) landscapes (Fig. 5), suggesting an additional role of chance in shaping genotype composition at the range front.

DISCUSSION

Recent studies have highlighted that rapid evolution can complicate our ability to predict the eco-evolutionary dynamics of range expansions (Miller et al. 2020; Shaw et al. 2023; Williams et al. 2019; Zilio et al. 2023). Here, we used replicate range-expanding populations to empirically test whether the presence of selection from an abiotic gradient across landscapes could modify the among-replicate variability of, and hence our ability to precisely predict, range expansion speed. Notably, we found that over time range expansion speed became more variable among landscapes with a spatial temperature gradient than without (Fig. 2c). This occurred despite

372 gradient landscapes having slower range expansions on average, greater range-front population
373 densities (Fig. 2), and genetic and trait signatures consistent with directional selection (Fig. 3;
374 Fig. 4) – conditions that would otherwise predict less gene surfing and thus more consistency in
375 the speed of range expansions across replicate populations (Miller et al. 2020; Phillips 2015;
376 Williams et al. 2019). Our results therefore challenge current theory and highlight the
377 complexities of predicting range expansions even in highly standardised, replicate mesocosms
378 with simple environmental variation.
379
380 We found that the greater variability in range expansion speed observed for gradient compared to
381 uniform landscapes over time is driven by a combination of rapidly decreasing among-replicate
382 variability (CV) in distance travelled for uniform landscapes, and slightly increasing CV for
383 gradient landscapes over time (Fig. 2b). In uniform landscapes, the decrease in CV of distance
384 travelled over time occurred as mean range expansion speed increased more quickly than the
385 variance observed across replicates (Fig. 2a). A rapid increase in mean range expansion speed
386 across uniform landscapes is consistent with previous experimental evolution work on range
387 expansions, which found that range expansion speed can increase under constant environments
388 due to spatial sorting for dispersal phenotypes and low-density selection for increased
389 reproductive output at the range front (Ochocki & Miller 2017; Wagner et al. 2017; Weiss-
390 Lehman et al. 2017). In contrast, for gradient landscapes, the slight increase in CV of distance
391 travelled over time occurred as the variance in speed among replicates increased more quickly
392 than the mean range expansion speed (Fig. 2a). This occurred as range expansion remained slow
393 for most gradient landscapes but rapidly sped up in a few replicates (Fig. 2a), consequently
394 elevating the among-replicate variability observed over time.

It is possible that the faster range expansion seen in some of these gradient landscapes was driven by selection from the temperature gradient (Moerman et al. 2022; Phillips et al. 2006; Szücs et al 2017; Williams et al. 2016). We found that gradient populations on average had higher frequencies of one genotype (G11) at the range front (Fig. 3c and 3d). In contrast, this genotype did not achieve frequencies >0.5 in any uniform landscapes (Fig. S8). We also found that gradient populations evolved higher thermal performance breadth (i.e., a broader range of temperatures across which plants can maintain near-optimal growth) but also lower thermal tolerance breadth (i.e., a narrower range of temperatures across which plants can exhibit positive growth; Fig. 4). The importance of maintaining performance across a broader breadth of temperatures has been long emphasised in studies of range expansion across latitudinal gradients in climate (Ackerly 2003; Lancaster 2016; Lancaster 2022). The concurrent evolution of lower thermal tolerance breadth is surprising and perhaps suggests a metabolic or evolutionary trade-off between maintaining high growth rates across a broader range of temperatures and the ability to withstand extreme temperature minimums or maximums, for example, if maintaining lower or upper thermal limits are costly (Barley et al. 2021). This also implies that studies on eco-evolutionary responses to temperature change need to consider evolution in the full shape of thermal performance curves to accurately predict outcomes of climate change (Malusare et al. 2022). Range-front populations in gradient landscapes also had significantly lower plasticity in root/shoot ratio compared to uniform landscapes indicating either a trade-off in responding to changes in temperature versus resource availability, or more consistent resource environments selecting for reduced plasticity, for example if there is constant competition for light and nutrients in these crowded and dense range fronts (Fig. 2d).

418

419 Despite the evidence above for selection in gradient landscapes, we also found that the among-
420 replicate variability in genetic composition was greater than predicted by random sampling (Fig.
421 5). Such a pattern could result if chance still had a large role in shaping the genetic composition
422 of range-expanding populations, for example, if individuals that initially occupy the range front
423 through chance dispersal dominate subsequent range expansion (i.e., spatial priority effects;
424 Hallatschek et al. 2007; Hewitt 2000). In combination with spatial environmental variation,
425 spatial priority effects may shape the genotypes available to respond to selection in range-front
426 populations, allowing non-optimal strategies to persist at the range front of some replicates
427 (Baym et al. 2016; Williams et al. 2016). Indeed, further tests revealed that range expansion
428 speed was significantly slower in gradient populations with no or low frequencies of the G11
429 genotype (slope = 1.832, 95% CI: 0.613 to 3.052 cm/day) compared to replicates with fixation or
430 high frequencies of this genotype (slope = 4.516, 95% CI: 1.572 to 7.460 cm/day; Fig. S10). We
431 therefore suggest that together with selection from a spatial environmental gradient, spatial
432 priority effects and its variance-generating effects at the range front could lead to range
433 expansion speeds that are slower on average, yet also increasingly different from one another
434 over time. Definitively demonstrating the effects of spatial priority during range expansion
435 would require future studies to track individual genotypes over a finer resolution in space and
436 time (Baym et al. 2016).

437

438 Our study is, to our knowledge, the first to test how selection from a spatial environmental
439 gradient alters the variability of replicate range expansions. We note that the dynamics observed
440 in our clonal system are highly relevant to biological invasions given that clonal reproduction is

441 common during range expansion and that many invasive plant species are capable of clonal
442 spread (Cadotte et al. 2006; Song et al. 2013; Wang et al. 2017). Importantly, our study suggests
443 that spatial priority effects, by blocking beneficial genotypes or mutants, could play a key role in
444 governing the expansion dynamics of asexual organisms— especially so for populations
445 undergoing unidirectional range expansions (e.g., spreading higher in elevation or latitude).
446 Although our study allows us to track a distribution of eco-evolutionary outcomes across highly
447 replicated landscapes, we note that scaling up any experimental results from any micro- or
448 mesocosm studies to natural populations requires careful consideration (Lustenhouwer et al.
449 2023). As populations traverse across environmentally variable and complex landscapes, studies
450 of natural range expansions will need to incorporate how selection from underlying
451 environmental variation in space can influence our ability to predict future range expansions.
452

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626 **Figure legends**

627 Figure 1. Experimental range expansion across landscapes with and without a spatial gradient in
628 temperature. (a) Replicate, mixed-genotype populations of duckweed (*Lemna* species complex)
629 expand their experimental ranges across increasingly warmer temperatures (blue to red) from the
630 range core to the range front in gradient landscapes, or across benign and constant temperatures
631 in uniform landscapes. (b) Observed temperatures across gradient (red) and uniform (blue)
632 landscapes during the experiment. Lines represent the slopes of linear models of temperature by
633 distance from range core. We found a significant and positive temperature slope in gradient
634 landscapes ($\beta_{gradient}$ = 0.027, 95% CI: 0.027 to 0.028 °C/cm; t = 126.49; P < 0.001) and a
635 slightly negative slope in uniform landscapes ($\beta_{uniform}$ = –0.001, 95% CI: –0.002 to
636 0.000 °C/cm; t = 68.50; P < 0.01).

637

638 Figure 2. Population dynamics of range expansion across landscapes with (red) and without
639 (blue) a temperature gradient across space. (a) Each line represents the maximum distance
640 travelled over time for a single, replicate range expansion (mean values shown by thick lines).
641 (b) Among-replicate variability (CV) in distance travelled over time for gradient and uniform
642 landscapes. (c) Slope and 95% CI of changes in the log coefficient of variation ratio (lnCVR)
643 over time, where estimates of lnCVR below zero (dashed grey line) indicate that there is less

among-replicate variability (CV) across gradient than uniform landscapes. (d-e) Population densities at the range front (i.e., the first 0 to 12 cm) during range expansion. Lines show model predictions from a logistic regression of density across space, with increasing colour intensity representing changes over time from day 4 to 24. (f) Slope and 95% CI obtained from a linear regression of range-front density over time.

Figure 3. Evolutionary dynamics of range expansion across landscapes with and without a temperature gradient across space. (a) Violin plot for the magnitude of evolution observed in gradient (red) and uniform (blue) landscapes, as quantified by the Euclidean distance of genotype frequencies between founder and evolved populations at the range front. Points show the amount of change in genotype frequencies for each replicate, while dashed lines represent the mean change. (b) Genotype composition of range-front populations in gradient (red) and uniform (blue) landscapes. The first two axes of a Principal Coordinates Analysis (PCoA) are shown, accounting for 43.7% of the total variation in genotype composition. Filled circles are treatment centroids, while clear symbols are replicate populations in each treatment. Ellipses represent one standard deviation around treatment centroids, and lines represent distance from the centroid to each replicate population. (c) Mean genotype frequencies observed across range-front populations in gradient (left) and uniform (right) landscapes, with different colours representing unique genotypes. (d) Genotype frequency for gradient (left) and uniform (right) landscapes. Coloured points and error bars represent observed genotype frequencies and their 95% CIs in evolved range-front populations. The initial genotype frequency in the founder population is denoted by ‘x’.

667 Figure 4. Evolved trait values for range-front populations in gradient and uniform landscapes.
668 Red and blue points show mean trait values averaged across gradient and uniform replicates,
669 respectively. The grey point shows mean treatment differences as estimated by gradient minus
670 uniform trait values. Error bars show 95% confidence intervals. The horizontal dotted line
671 represents the initial mean trait value of the founder population. *** Indicates traits that are
672 significantly different from the founder population (if under red bars) or between gradient and
673 uniform landscapes (if above grey bars). All trait values are weighted by genotype frequencies of
674 each range-front population, and then scaled and centred.
675
676 Figure 5. Observed versus simulated among-replicate variability in range-front genotype
677 composition in gradient (top) and uniform (bottom) landscapes. Histograms represent the
678 predicted probability distribution of among-replicate variability in genotype composition due to
679 random sampling alone, with black dotted lines representing the 95% confidence interval.
680 Vertical dashed red and blue lines show that the mean observed among-replicate variability in
681 genotype composition for gradient and uniform landscapes are similar to each other ($P = 0.741$;
682 Permutation test) but greater than predicted by random sampling ($P < 0.001$). Among-replicate
683 variability in genotype composition was quantified as the summed Euclidean distance between
684 the genotypic composition of each replicate population ($N = 10$ per treatment) and the mean
685 genotypic composition of each treatment.
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697 **Figure 1**

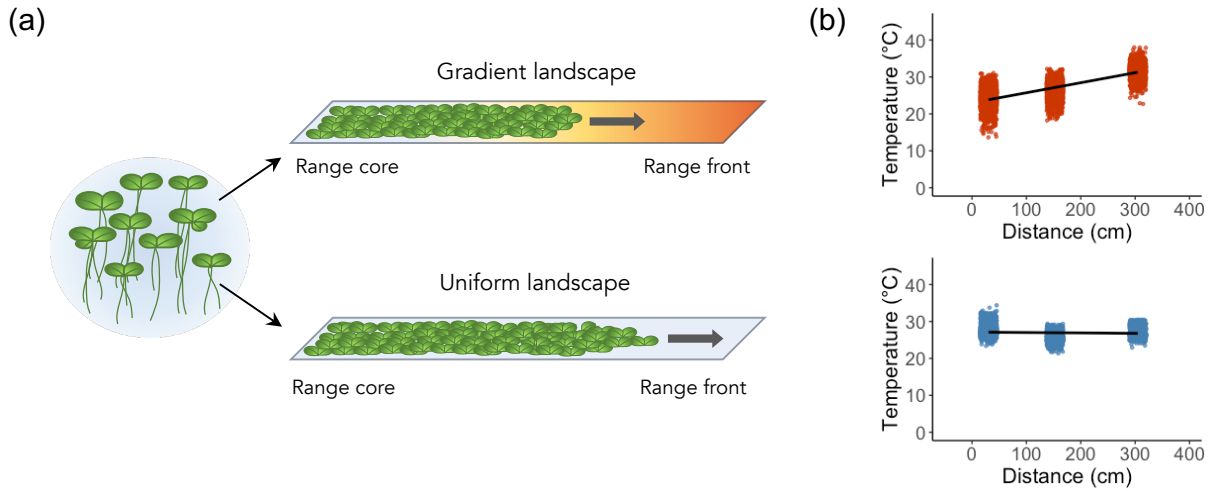

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Figure 2

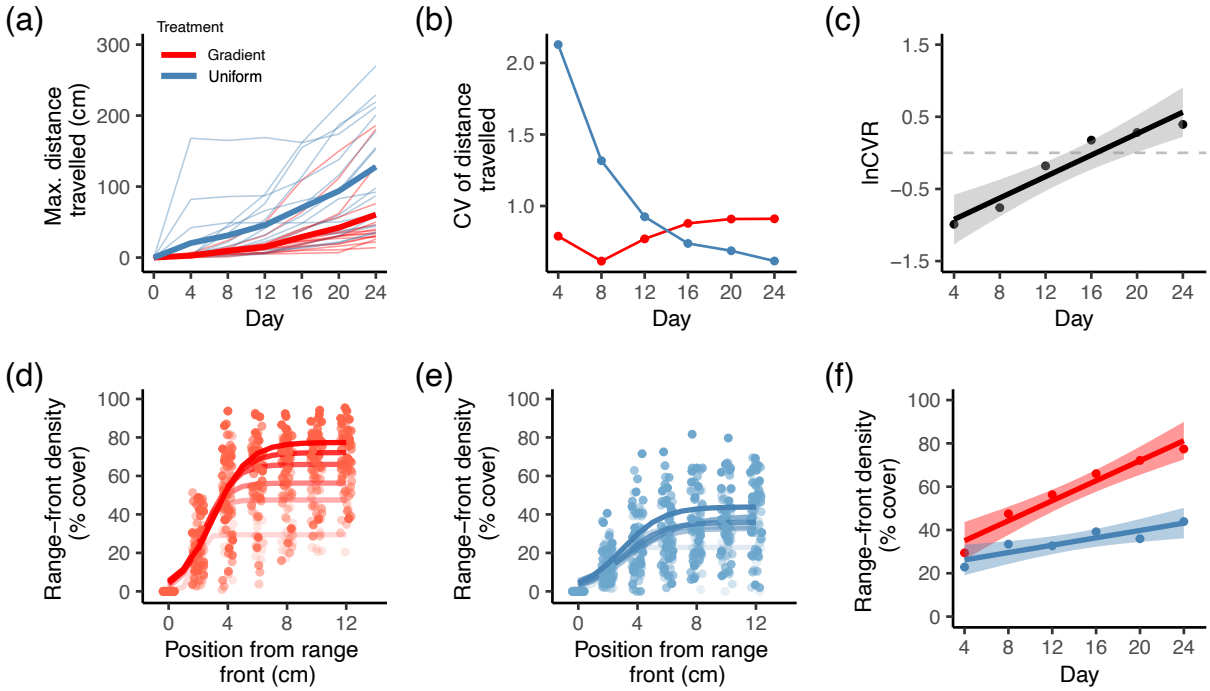
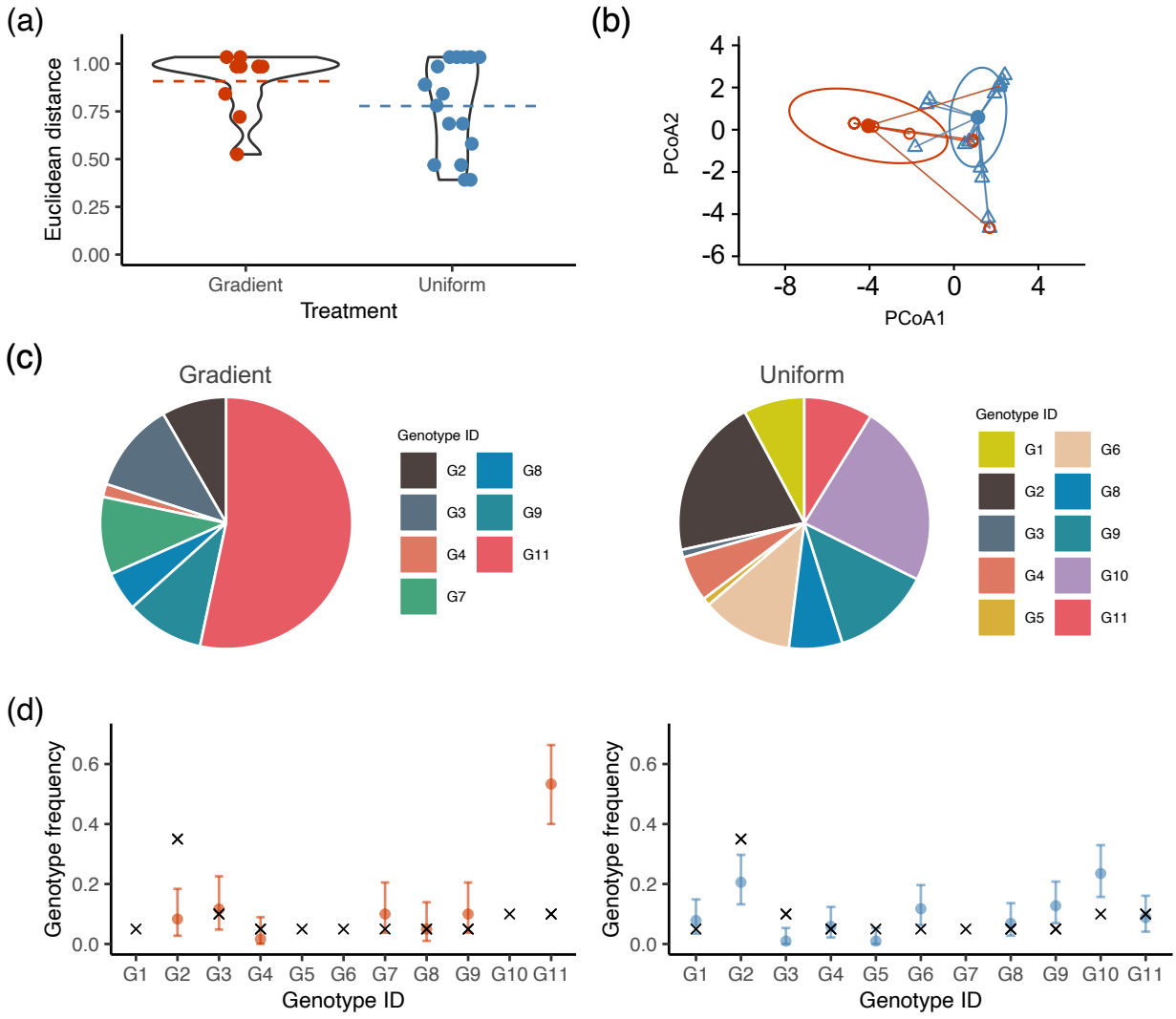
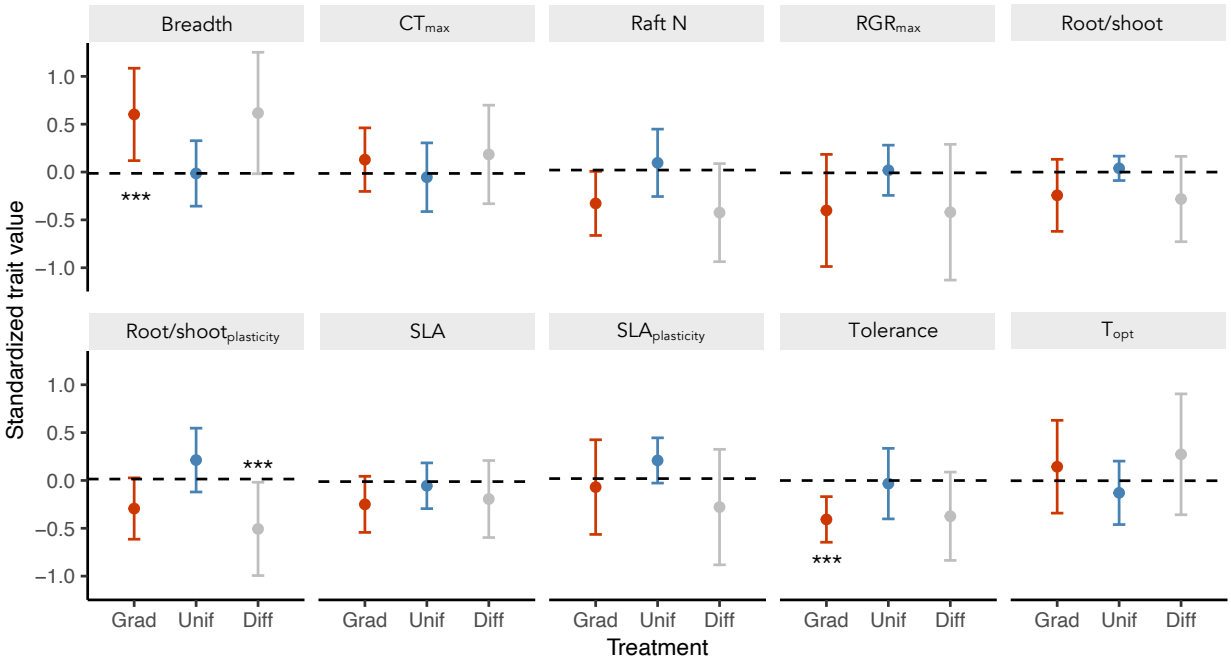

(a)
Treatment
Gradient
Uniform
Max. distance travelled (cm)
Day
(b)
CV of distance travelled
Day
(c)
lnCVR
Day
(d)
Range−front density (% cover)
Position from range front (cm)
(e)
Range−front density (% cover)
Position from range front (cm)
(f)
Range−front density (% cover)
Day

Figure 3


(a)
Euclidean distance
1.00
0.75
0.50
0.25
0.00
Gradient
Uniform
Treatment
(b)
PCoA2
4
2
0
-2
-4
-6
-8
-4
0
4
PCoA1
(c)
Gradient
Genotype ID
G2
G3
G4
G7
G8
G9
G11
Uniform
Genotype ID
G1
G2
G3
G4
G5
G6
G8
G9
G10
G11
(d)
Genotype frequency
0.6
0.4
0.2
0.0
G1 G2 G3 G4 G5 G6 G7 G8 G9 G10 G11
Genotype ID
Genotype frequency
0.6
0.4
0.2
0.0
G1 G2 G3 G4 G5 G6 G7 G8 G9 G10 G11
Genotype ID

712 **Figure 4**



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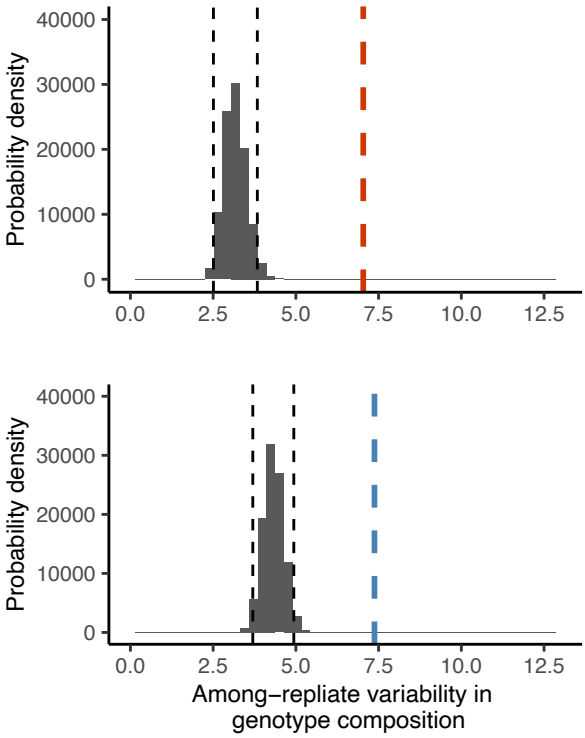
714 **Figure 5**



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