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1

2 # Evolution of phenotypic plasticity during environmental fluctuations

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4 **Running head: Evolution of phenotypic plasticity**

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32 **Author contributions**

33 ZS, MIL, IIR and EB designed the experiment, ZS, ER and MT-B performed the phenotypic

34 assays with the aid of MIL. EIFF and ZS analysed the data, with the aid of EB. ZS, MIL and

35 EIFF drafted the manuscript. All authors contributed to the revision of the manuscript.

36

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41

42 **Conflict of Interest statement**

43 The authors report no conflict of interest.

44

45 **Data Availability Statement**

46 Upon acceptance, the data will be archived at *Figshare*

47

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48 **Abstract**

49

50 Evolution in fluctuating environments is predicted to disfavor specialization and instead
51 select for alternative strategies, such as phenotypic plasticity or possibly bet-hedging,
52 depending on the accuracy of environmental cues and type of fluctuations. While these two
53 alternatives are often contrasted in theoretical studies, their evolution are seldom studied
54 together in empirical work.

55 We used experimental evolution in the nematode worm *Caenorhabditis*
56 *remanei* to simultaneously study the evolution of plasticity and bet-hedging in environments
57 differing only in their temperature variability. We exposed worms for 30 generations to either
58 fluctuating or slowly increasing temperature, these two environments had the same average
59 temperature over evolutionary time. After experimental evolution, we scored size at sexual
60 maturity and fitness in full siblings reared in two different temperatures, optimal 20°C and
61 mildly stressful 25°C.

62 Experimental evolution in the fluctuating environment resulted in the evolution of
63 increased body size plasticity but not increased bet-hedging, compared to evolution in the
64 slowly changing environment. Plasticity followed the temperature size rule as size decreased
65 with increasing temperature and this plastic response was adaptive. In addition, we
66 documented substantial standing genetic variation in body size, which represents a potential
67 for further evolutionary change.

68

69 **Key words**

70 Adaptation, Bet hedging, *Caenorhabditis remanei*, Experimental evolution, Phenotypic
71 plasticity, Temperature

72

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73 **Introduction**

74

75 Natural environments are generally not stable, but fluctuate on both long and short
76 timescales, and a developing organism needs to take this environmental variation into
77 account. While the parental environment can be a reliable cue if the environmental
78 fluctuations are slow and predictable (Lachmann & Jablonka, 1996; Kuijper & Hoyle, 2015;
79 Leimar & McNamara, 2015; Uller *et al.*, 2015) and can result in the evolution of anticipatory
80 parental effects (Dey *et al.*, 2016; Lind *et al.*, 2020), faster and random fluctuations select
81 against inheritance of the parental phenotype. Instead, phenotypic plasticity or bet-hedging is
82 predicted to be adaptive (Moran, 1992; Simons, 2011). If the developmental environment
83 provides reliable cues for the optimal offspring phenotype, theory predict the evolution of
84 adaptive phenotypic plasticity (Moran, 1992; Gavrilets & Scheiner, 1993; Simons, 2011)
85 which is the ability of a genotype to produce different phenotypes depending on
86 environmental conditions (West-Eberhard, 2003). If plasticity is present, individuals will
87 canalize fitness between environments by adjusting their phenotype according to the
88 environmental conditions.

89 Alternatively, individuals may express bet-hedging, which acts to reduce variation in
90 fitness (especially to avoid very low fitness values in certain environmental states) at the cost
91 of lowered arithmetic mean fitness, often by producing offspring with a range of phenotypes
92 (diversified bet-hedging), where some of the offspring always matches the environment and
93 is successful (Philippi & Seger, 1989). In contrast to phenotypic plasticity, bet-hedging is
94 generally seen as favored when environmental cues have low predictability (Cohen, 1966;
95 Slatkin, 1974; Meyers & Bull, 2002; Kussell & Leibler, 2005; Wolf *et al.*, 2005), or when
96 there is low correlation between the environment of development and selection (Tufto, 2015).

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97 While both phenotypic plasticity and bet-hedging can be adaptive in variable
98 environments (Simons, 2011; Furness *et al.*, 2015), most empirical studies of evolution in
99 variable environments focus on the evolution of plasticity. While phenotypic plasticity is
100 common and well documented (DeWitt & Scheiner, 2004), very few studies have
101 investigated the evolution of plasticity. These studies generally follow two lines, either they
102 investigate whether increased plasticity evolves in more variable environments (Moran, 1992;
103 Tufto, 2015), or whether increased plasticity evolves as a (possibly transient) step in
104 adaptation to a novel but stable environment (Lande, 2009; Chevin *et al.*, 2010; Levis &
105 Pfennig, 2020). Studies focusing on the role of environmental heterogeneity have found
106 stronger phenotypic plasticity in natural populations (Lind & Johansson, 2007; Lind *et al.*,
107 2011) or species (Hollander, 2008) originating from more variable environments. Moreover,
108 two recent experimental evolution studies in microalgae have shown that variable
109 environments can select for increased phenotypic plasticity (Schaum *et al.*, 2022), but that
110 very unpredictable environments also can select against plasticity (Leung *et al.*, 2020). One
111 possible explanation for selection against plasticity during rapid fluctuations is that it may
112 take time for the plastic phenotype to be induced (Burton *et al.*, 2022; Dupont *et al.*, 2024).
113 Studies that instead focus on the evolution of plasticity during adaptation to new stable
114 environments have found that plasticity can rapidly evolve in a new environment (Sikkink *et*
115 *al.*, 2014b; Corl *et al.*, 2018), but also that maladaptive plasticity can play a major role
116 (Ghalambor *et al.*, 2015; Campbell-Staton *et al.*, 2021).
117 In contrast to phenotypic plasticity, empirical studies documenting bet-hedging are
118 rare (Simons, 2011), and the few examples suggest that it is more likely to evolve if the
119 environments differ dramatically in fitness (Bull, 1987), such as delayed germination in
120 desert plants (Philippi, 1993; Clauss & Venable, 2000; Venable, 2007), diapause in killifish
121 (Furness *et al.*, 2015) or experimental evolution in fluctuating environments with large fitness

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122 differences (Beaumont *et al.*, 2009; Graham *et al.*, 2014). However, bet-hedging has been
123 suggested as a mechanism explaining the strain-specific variance in reproduction between
124 natural isolates of the nematode *Caenorhabditis elegans* (Diaz & Viney, 2014).
125 One environmental factor that is known to result in evolutionary adaptations
126 (Berteaux *et al.*, 2004; Geerts *et al.*, 2015), but also alternative strategies, is temperature. Not
127 only is temperature gradually increasing due to the ongoing climate change (Berteaux *et al.*,
128 2004), but climate change also results in increased temperature variability (Easterling *et al.*,
129 2000; Palmer & Räisänen, 2002; Van Aalst, 2006) potentially favoring evolution of increased
130 plasticity or possibly bet-hedging. Indeed, most documented responses to climate change in
131 natural populations are caused by pre-existing plasticity, while genetic adaptation seems rare
132 (Merilä & Hendry, 2014).
133 Among traits showing plastic responses to temperature, body size is of immense
134 importance to reproductive fitness. For females, a large body generally translates into
135 increased egg production, and also males often benefit from large size (Hedrick & Temeles,
136 1989; Andersson, 1994). Therefore, perhaps surprisingly, in warm environments organisms
137 generally increase growth rate, accelerate maturation but mature at a smaller size, which is
138 called the temperature-size rule (Ray, 1960; Atkinson, 1994; Verberk *et al.*, 2021). This has
139 sometimes been argued to be a passive by-product of other temperature-dependent processes
140 (Atkinson, 1994; van der Have & de Jong, 1996; Forster *et al.*, 2011). However, small size
141 may actually be actively regulated and beneficial in warm environments (Fryxell *et al.*, 2020;
142 Verberk *et al.*, 2021) for example by being advantageous for thermoregulation (Partridge &
143 Coyne, 1997), or allowing better regulation of oxygen demand and supply ratio (Walczyńska
144 *et al.*, 2015; Verberk *et al.*, 2021).
145 We set out to investigate whether exposure to fluctuating temperature results in
146 evolution of increased phenotypic plasticity compared to evolution in an environment with

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147 slowly increased temperature and whether any plasticity in body size is adaptive, using
148 experimental evolution in the nematode *Caenorhabditis remanei*. *C. remanei* has a fast
149 generation time and, since it is dioecious, harbors substantial standing genetic variation
150 which makes it ideal for experimental evolution studies (Teotónio *et al.*, 2017). In addition,
151 *C. remanei* has been shown to respond to manipulations in temperature (Sikkink *et al.*,
152 2014a; Lind *et al.*, 2020), has the ability to respond plastically to new environmental
153 conditions (Lind *et al.*, 2020), and the plasticity to withstand heat-shock can itself evolve
154 (Sikkink *et al.*, 2014b). Body size of *C. remanei* is under directional selection towards an
155 optimum larger than the mean size under standard temperature conditions (Stångberg *et al.*,
156 2020), and pharmacologically lowered body size results in lower female reproduction (Lind
157 *et al.*, 2016). It's close relative *C. elegans* also follow the temperature-size rule (Kammenga
158 *et al.*, 2007). As an alternative to phenotypic plasticity, we also investigated if populations
159 evolving in variable environments had evolved increased diversifying bet-hedging.
160 We used previously established experimental evolution populations of *C. remanei*
161 (described in Lind *et al.*, 2020). During experimental evolution, replicate populations were
162 exposed to 30 generations in one of two regimes; *Fast temperature cycles* or *Increased*
163 *warming*. The *Fast temperature cycles* regime were switched between two temperatures
164 (20°C, 25°C) every second generation, and although the fluctuations are deterministic, it
165 represents an uncorrelated (and therefore unpredictable) fluctuating environment each
166 generation, as the next generation will either be in the same or in a different temperature. The
167 evolution in this environment was compared to the *Increased warming* regime, where worms
168 were exposed to experimental evolution in a gradually increasing temperature which slowly
169 raised from 20°C to 25°C over 30 generations, and which served as a control. Importantly,
170 these two regimes had the same average temperature (22.5°C) over evolutionary time, and
171 only differed in the rate and predictability of environmental change. While no theoretical

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172 model to our knowledge have investigated evolution of plasticity in fluctuating versus slowly
173 changing environments, it corresponds to different degrees of fluctuation, which is well
174 explored theoretically (Moran, 1992; Gavrilets & Scheiner, 1993; Tufto, 2015). After the 30
175 generations of experimental evolution, we reared full-siblings in either standard 20°C, or
176 warm 25°C, and scored them for reproduction and body size.

177 We predict that worms evolving in fluctuating temperature every 2nd generation
178 would evolve increased phenotypic plasticity (relative to the *Increased warming* regime),
179 since the timescale of this environmental variation is well within the parameter space where
180 plasticity (and bet-hedging) is favored (Tufto, 2015). We predict that evolution of phenotypic
181 plasticity is more likely than the evolution of bet hedging, since the differences in fitness
182 between the two temperatures is likely to be relatively small (Lind *et al.*, 2020). If increased
183 phenotypic plasticity has evolved, we predict that the *Fast temperature cycle* populations
184 would show increased size difference between temperatures, but not increased phenotypic
185 variance within one temperature. If instead increased diversifying bet-hedging had evolved,
186 we predict that the *Fast temperature cycle* populations will show (1) increased within-family
187 variance within each temperature, and (2) decreased heritability of size, because of an
188 increased environmental component of phenotypic variance (Tufto, 2015). We also predict
189 that any plasticity in body size will follow the temperature-size rule, and that this plasticity is
190 adaptive.
191
192
193

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194 **Methods**

195 *Experimental evolution*

196 For the experimental evolution we used *C. remanei* nematode worms, strain SP8 which has
197 been lab adapted for 15 generations at 20°C and subsequently exposed to 30 generations of
198 experimental evolution in two regimes (*Increased warming* and *Fast temperature cycles*).
199 The experimental evolution has been previously described in detail in Lind *et al.*, (2020b).
200 Briefly, in the *Increased warming* experimental evolution regime, the temperature gradually
201 raised from 20°C to 25°C, which is a novel and mildly stressful temperature. This gradual
202 change over 30 generations represent an increase of 0.1°C every 2.13 days and results in a
203 correlated parental and offspring environment. In the second regime, *Fast temperature*
204 *cycles*, the temperature fluctuated every second generation between 20°C and 25°C, resulting
205 in 14 temperature shifts but no exposure to the intermediate temperatures. The environmental
206 change is deterministic (every second generation) but since parents and offspring would end
207 up in either the same or in different temperature, it represents uncorrelated parental and
208 offspring environment. The generation time in *C. remanei* is temperature dependent; 4 days
209 long in 20°C and 3.4 days long in 25°C. Despite these differences, the average temperature
210 and the total chronological time of experimental evolution were identical for both regimes, at
211 22.5°C and 110 days respectively.

212 Each evolutionary regime consisted of six replicate populations. The populations were
213 maintained on 92 mm Petri dishes poured with NGM agar in climate chambers set to 60%
214 relative humidity. In order to prevent bacterial and fungal contamination, the agar and
215 bacterial LB contained the antibiotics streptomycin and kanamycin and the fungicide
216 nystatin. The plates were seeded with 2 ml of an antibiotic-resistant OP50-1 (pUC4K) strain
217 of *E. coli* (Stiernagle, 2006) that served as a source of food. Every 1-2 days, a piece of agar
218 containing approximately 150 worms of mixed ages was cut and transferred to a new plate

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219 containing fresh bacteria. This resulted in populations with overlapping generations that were
220 maintained in a constant exponential growth phase. After the experimental evolution,
221 populations were expanded for two generations and frozen in -80°C for later revival and
222 subsequent phenotypic assays.
223

224 ***Experimental set-up***

225 Each replicate population of each of the two selection regimes was run in a separate block
226 resulting in 12 experimental blocks in total. For logistic reasons we focus on females, since
227 they are responsible for population growth rate and their fitness is straightforward to
228 measure.
229 Briefly, populations were revived from freezing and exposed to 25°C for 3
230 generations, to avoid any maternal effects associated with freezing. The third generation of
231 worms were split into eight families, each family consisting of one male and one female
232 worm. From each family, we randomly picked eight offspring females (full siblings) and
233 placed four females in 20°C and four in 25°C. Since our focus was evolution in females, their
234 fitness was assessed by mating them with standardized males from the ancestral line. Females
235 were kept together with two males (in case one of the males would be infertile/escape from
236 the plate). The ancestral line was, in contrast to selection regimes, maintained for three
237 generations in 20°C and subsequently split into 20°C and 25°C together with studied females.
238 For the detailed description of the experimental set up, see supplementary figure 1. In total,
239 we used 48 families per evolutionary regimes, and for each family we set up 4 offspring in
240 each temperature.
241

242 ***Phenotypic assays***

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243 *Daily reproduction*

244 Female and male worms were transferred to a new plate every 24 hours. The old plate was
245 kept for two days to allow eggs to hatch and afterwards the number of viable offspring was
246 counted to determine age-specific reproduction and calculate individual fitness. In the case of
247 the males dying or escaping from the plate, they were replaced by a new male of the same
248 age from the ancestral line. The female worm was discarded after dying, or after three
249 consecutive days of zero reproduction.

250

251 *Body size*

252 The body size of the worm changes with time, reaching a peak before it declines (Lind *et al.*,
253 2016), and the age at maximum body size is temperature dependent. Worms in 20°C reach
254 their peak size at day 4 of adulthood (Lind *et al.*, 2016). The peak size in 25°C is on day 2 of
255 adulthood, which was determined during pilot assays. Photographs of worms were taken
256 during their peak size using a Lumenera Infinity2-5C digital microscope camera mounted on
257 a Leica M165C stereomicroscope. Body size was measured from the photographs using
258 *ImageJ 1.46r* (https://imagej.nih.gov/ij/) as total cross-section area.

259

260 ***Statistical analyses***

261 All statistical analyses were conducted in R 3.6.1 (R Core Team, 2015).

262

263 *Individual fitness*

264 We used the age-specific reproduction data to calculate rate-sensitive individual fitness λ_{ind}
265 for each individual (Brommer *et al.*, 2002), which is analogous to the intrinsic rate of
266 population growth (Stearns, 1992). Individual fitness was calculated by constructing a
267 population projection matrix for each individual, and then calculating the dominant

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268 eigenvalue of this matrix, following McGraw & Caswell, (1996). Since we kept the
269 population size and age structure constant during experimental evolution, individual fitness is
270 maximized during evolution and is therefore the most appropriate fitness measure for this
271 study (Mylius & Diekmann, 1995).
272
273 *Thermal reaction norms*
274 To test whether the degree of phenotypic plasticity has evolved, we used linear mixed-effect
275 models to separately estimate the thermal reaction norms of body size and individual fitness,
276 using the package *lme4* (Bates *et al.*, 2015) in R. The models included either body size (area)
277 or individual fitness (λ_{ind}) as response variables. The full model included three fixed effects:
278 mean-standardized temperature as a covariate, the experimental evolution regime as a
279 categorical factor, and an interaction between temperature and evolution regime. We expect
280 this interaction to be significant if the degree of plasticity has evolved during experimental
281 evolution. Experimental line and dam identity were included as random effects. Significance
282 of the fixed effects was evaluated using Wald χ^2 tests. Pseudo-R^2 values were calculated as
283 the squared correlation coefficient between fitted values from the model and observed values.
284
285 *Selection*
286 To test if temperature responses in size are adaptive, we estimated the selection on body size
287 and compared it to the observed temperature response. Selection on body size (area) was
288 estimated using mixed-effect models in R with the package *lme4* and individual fitness (λ_{ind})
289 as the response variable. The full model included the following fixed effects: area, area^2,
290 temperature (as a categorical factor with 2 levels), experimental evolution regime and all
291 interactions between these variables except for interactions involving both area and area^2
292 together. Experimental line was included as random effect, assumed to only affect the

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293 variance of the intercept. Significance of fixed effects was evaluated using Wald χ^2 tests.
294 Pseudo-R^2 values were calculated as the squared correlation coefficient between fitted values
295 from the model and observed values. The optimal size (i.e. the area that maximizes fitness)
296 was calculated as: $-b/(2\times c)$, where b = the slope of the regression (i.e. the linear selection
297 gradient) and c = the squared term of the regression (i.e. the quadratic selection gradient).
298 Confidence intervals of the temperature-specific optimal sizes were generated by
299 bootstrapping, implemented in the *boot* package using 10 000 bootstrap replicates.
300
301
302 *Within family coefficient of variance*
303 To test whether the degree of diversifying bet-hedging has evolved, we tested whether the
304 experimental evolution regimes differed in the mean within family variance within
305 temperatures. For each family, we used the trait values of the offspring (within a temperature)
306 to calculate the within family variance. To account for differences in the means of the traits,
307 we used within family means (i.e. the mean trait value of the family's offspring) to mean-
308 standardize the variance by calculating the within family coefficient of variance (CV): CV =
309 σ/μ, where σ = within family standard deviation, and μ = within family mean. An ANOVA
310 was used within each temperature to test if the evolution regimes differed in their mean
311 within family CV.
312 Since it is more difficult to detect differences in variances than differences in
313 means, we also performed power calculations on our ability to detect whether within-family
314 CVs differ between the selective regimes. Balanced one-way ANOVA power calculations
315 were performed to estimate the effect sizes possible to detect with power ranging from 0.70
316 to 0.95. Effect sizes, η^2, were obtained for our sample size of N = 48 per selection regime and
317 a significance level of 0.05. η^2 is calculated as the sum of squared explained by the treatment

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318 (here: selection regime) divided by the total sum of squares, and has an equivalent
319 interpretation as an R^2.
320
321 *Genetic variance and correlations*
322 For body size (cross-section area), genetic variance and genetic correlations across
323 temperature were estimated using animal models using the package *MCMCglmm* (Hadfield,
324 2010) in R. Univariate models were used to estimate genetic variance, whereas bivariate
325 models were used to estimate genetic correlations, both using Gaussian family for trait
326 distribution. An inverse Wishart prior with parameters V = 1 and nu = 0.02 were used in both
327 univariate and bivariate models. Pedigree data linking offspring to parents, based on full-sib
328 relationships, was included in the models. Convergence of the models were ensured by
329 evaluating diagnostic plots of posterior distributions, using the convergence diagnostic half-
330 width test by Heidelberger and Welch (1983), and by ensuring that the autocorrelation
331 between MCMC samples was close to zero.
332 For univariate models, body area was used as the response variable. Temperature (as a
333 categorical factor with 2 levels), experimental evolution regime, and an interaction between
334 the two, were included as fixed effects. Genetic variance (V_G), variance due to differences
335 between experimental lines, and residual variances were estimated separately as random
336 effects in the full model for each temperature-by-evolution regime combination. The full
337 model ran for 4.2×10^6 MCMC iterations, 0.2×10^6 samples were discarded as burnin, and the
338 thinning interval was 4000, resulting in a sample size of 1000 MCMC-samples. Reduced
339 models, subset by temperature-by-evolution regime combination, were used to assess the
340 statistical significance of V_G, by comparing the deviance information criterion (DIC) of
341 models with versus without genetic variance included.

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342 Broad sense heritability ($H^2 = V_G/V_P$, where V_P = total phenotypic variance after
343 accounting for variance due to experimental line effects) and broad sense evolvability ($I^2 =$
344 $V_G/mean^2$, Hansen *et al.*, 2003, 2011) were used to estimate the population's evolutionary
345 potential of body size. This was estimated for each temperature-by-evolution regime
346 combination. Evolvability measures the expected percentage change in a trait per generation
347 under unit strength of selection. Compared to heritability, evolvability is independent from
348 the environmental variance and represents a measure of the evolutionary potential that is
349 comparable across traits, populations and species when applied to traits with a natural zero
350 and which are strictly positive (Hansen *et al.*, 2011).

351 Genetic correlations of body size were estimated using bivariate animal models in
352 *MCMCglmm.* The data was split in two subsets based on the experimental evolution regimes,
353 resulting in genetic correlations being estimated separately for the two evolution regimes.
354 Body area was the response variable and was treated as two traits in the models, as area at
355 20°C and area at 25°C. Random effects in the full models included genetic covariance
356 between the two temperatures, whereas V_G, variance due to differences between experimental
357 lines, and residual variances were estimated separately for each temperature. The full models
358 ran for 2.05×10^6 MCMC iterations, 0.05×10^6 samples were discarded as burnin, and the
359 thinning interval was 2000, resulting in a sample size of 1000 MCMC-samples. Reduced
360 models without genetic covariance were used to access the statistical significance of the
361 genetic covariance, by comparing the DIC of models with versus without genetic covariance
362 included. The genetic correlation of body size across temperatures was calculated by dividing
363 the genetic covariance by the product of the genetic standard deviation of the two
364 temperatures. This was done on the posterior distributions, in order to carry the error
365 forwards in the analyses.

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366 To compare posterior distributions of H^2, I^2 and genetic correlations across
367 temperatures and selection regimes, we calculated, within each MCMC sample, the pairwise
368 differences in these measures and checked if the posterior distributions of these differences
369 had a 95% credibility interval that included zero. Pairwise comparisons of distributions were
370 only performed between evolution regimes within temperature, and between temperatures
371 within evolution regimes.

372

373

374 **Results**

375

376 Thermal reaction norms

377 *Size*

378 Size decreased significantly with increasing temperature (Wald $\chi^2 = 309.93$, df = 1, p <
379 0.001, Fig. 1A). There was a significant interaction between temperature and evolution
380 regime, where *Fast temperature cycles* had a steeper slope, meaning that it had evolved
381 increased plasticity in size (Wald $\chi^2 = 5.82$, df = 1, p = 0.016). However, the intercepts
382 (representing size at the mean temperature) were not significantly different between evolution
383 regime (Wald $\chi^2 = 0.09$, df = 1, p = 0.769). The models pseudo $R^2 = 0.51$. Variance
384 components: $V_{dam} = 25.03$, $V_{Line} = 20.92$, and $V_{residual} = 108.19$.

385

386 *Individual fitness (λ_{ind})*

387 The mean total reproduction decreased with temperature (mean ± SE: 20°C, 780 ± 17; 25°C,
388 672 ± 17; p < 0.001 for the difference between the temperatures). However, the individual
389 fitness (λ_{ind}) increased significantly with increasing temperature (Wald $\chi^2 = 3670$, df = 1, p <

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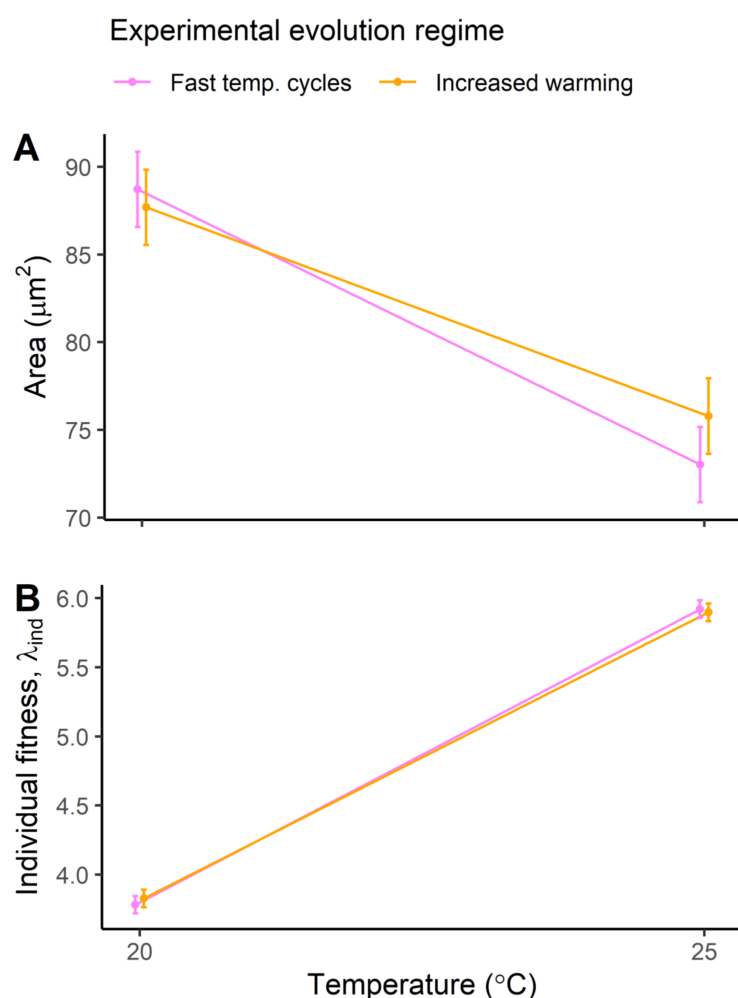
390 0.001, Fig. 1B). The evolution regimes did not differ significantly in intercepts (Wald χ^2 =
391 0.02, df = 1, p = 0.887), nor was there a significant interaction between temperature and
392 evolution regime (Wald χ^2 = 0.97, df = 1, p = 0.324). The best fit models pseudo R^2 = 0.86.
393 Variance components: V_{dam} = 0.05, V_{Line} = 0.01, and $V_{residual}$ = 0.21.

394



395

396 **Figure 1.** Thermal reaction norms showing means ± SE. (A) regression lines (with mean-standardized
397 temperature): Fast; Area = 80.87 ± 2.08 – 3.14 ± 0.22 × Temperature. Increase; Area = 81.74 ± 2.08 – 2.38 ±
398 0.22 × Temperature. (B) regression lines (with mean-standardized temperature): Fast; λ_{ind} = 4.85 ± 0.06 + 0.43 ±
399 0.01 × Temperature. Increase; λ_{ind} = 4.86 ± 0.06 + 0.41 ± 0.01 × Temperature.

400

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401

402

403 Selection

404 There was significant linear and quadratic selection on body size (linear slope: Wald χ^2 =
405 59.4, df = 1, p<0.001. Quadratic term: Wald χ^2 = 40.3, df = 1, p < 0.001). The selection
406 differed significantly between temperatures (Fig. 2), given by a significant overall
407 temperature effect (Wald χ^2 = 2992, df = 1, p < 0.001) and significant interaction effects
408 between temperature and size (linear slope: Wald χ^2 = 13.3, df = 1, p < 0.001. Quadratic
409 term: Wald χ^2 = 12.8, df = 1, p < 0.001). Maximum individual fitness (i.e. the optimal size) is
410 predicted to be 93.73 µm at 20°C [95% bootstrap CI: 87.61, 112.98], and 84.19 µm at 25°C
411 [95% bootstrap CI: 79.81, 92.61]. Selection was however not significantly different between
412 the experimental evolution regimes (p > 0.22 for main effect and interactions between
413 evolution regime and temperature or body size). No 3-way interaction between temperature,
414 body size and evolution regime were significant (p > 0.18). The best fit model's pseudo R^2 =
415 0.85. Variance components: V_{Line} = 0.016, and $V_{residual}$ = 0.204.

416

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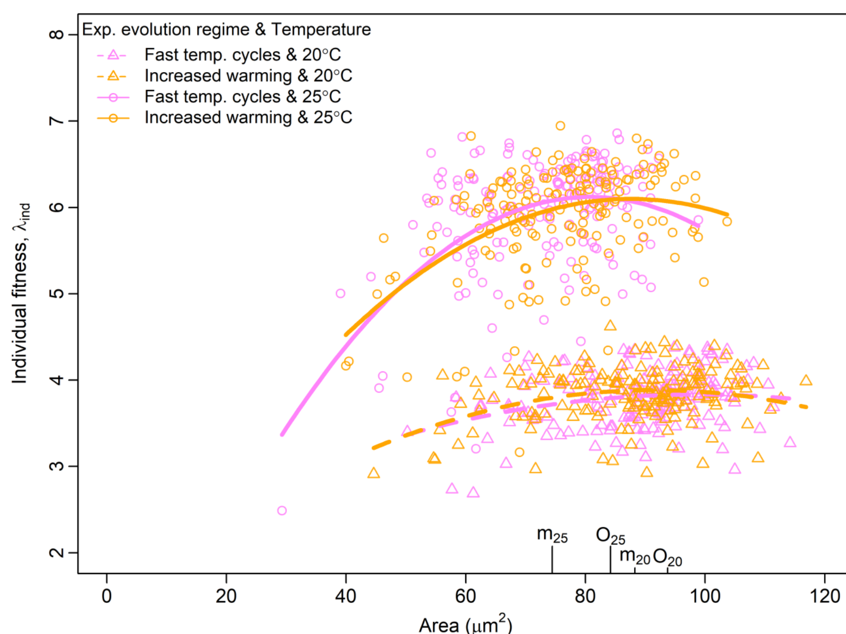

418

419 **Figure 2**. Selection on body size (area). Experimental evolution regimes were not statistically different, but are
420 shown with separate lines. Overall regression line for 20°C: $\lambda_{ind} = 1.596 \pm 0.818 + 0.048 \pm 0.020 \times \text{Area} -$
421 $2.57\times10^{-4} \pm 1.16\times10^{-4} \times \text{Area}^2$. Overall regression line for 25°C: $\lambda_{ind} = 0.138 \pm 0.614 + 0.142 \pm 0.017 \times \text{Area} -$
422 $8.43\times10^{-4} \pm 1.20\times10^{-4} \times \text{Area}^2$. The mean size per temperature (m_{20}, and m_{25}) and optimal size (O_{20}, and O_{25}) are
423 shown for 20°C and 25°C respectively. Individual fitness is higher in 25°C due to decreased development time,
424 even if total reproduction is lower.

425

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428 Within family CV

429 The evolution regimes did not differ significantly in within family CV of body size or of
430 individual fitness at either temperature (Table 1). Moreover, the distributions of within family
431 CV overlapped considerably between evolution regimes (Fig. 3). Power calculations showed
432 that we had a power of 90% to detect effects where the evolutionary regimes explained at
433 least 10% of the variation in within family CV (supplementary figure 2).

434

435

436

437 **Table 1.** Within family coefficient of variance (CV). Size (area) measured in μm^2, fitness as individual lambda
438 (λ_{ind})

439

Trait	Temp. (°C)	Experimental evol. regime	Within family CV (mean±SE)	Difference between evolution regimes	
				F (ndf=1, ddf=96)	p
Area	20	Fast temp. cycles	0.092 ± 0.009	3.799	0.054
		Increased warming	0.115 ± 0.009		
	25	Fast temp. cycles	0.100 ± 0.011	0.014	0.905
		Increased warming	0.102 ± 0.011		
Fitness	20	Fast temp. cycles	0.049 ± 0.005	0.266	0.608
		Increased warming	0.053 ± 0.005		
	25	Fast temp. cycles	0.087 ± 0.011	0.338	0.563
		Increased warming	0.079 ± 0.011		

440

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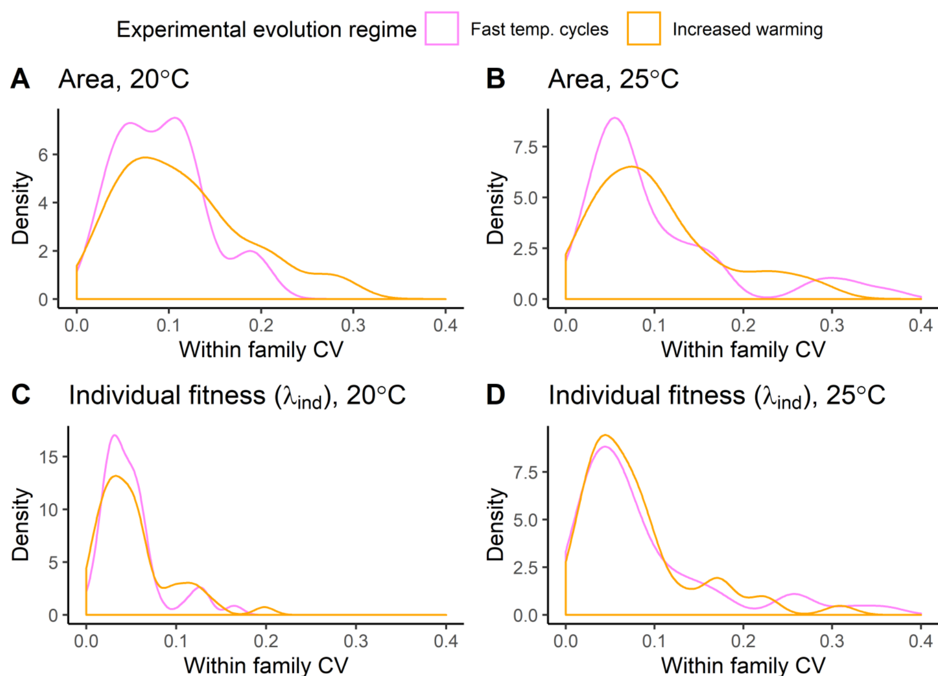

442

443 **Figure 3.** Distribution of within family coefficient of variance (CV) for two traits at two temperatures. The
444 within family CV was estimated within temperature for each family by estimating the standard deviation (σ) and
445 the mean (μ) of the family's offspring trait values, where CV = σ/μ. The density is the number of families.

446

447 Genetic variance and correlations

448 There was overall significant genetic variance for body size (measured as area) for the 4
449 combinations of temperature and evolution regime (models with genetic variance were at
450 least 7 DIC lower compared to models without genetic variance, Table 2). There was also
451 significant genetic covariance between temperatures for both evolution regimes (*fast temp.*
452 *cycle*: model with covariance included was 2.04 DIC lower than model without covariance;
453 *increased warming*: model with covariance was 2.79 DIC lower than model without
454 covariance). However, the pairwise comparisons of the posterior distributions of heritability,
455 evolvability and genetic correlations were not significantly different between the 4 different
456 combinations of temperature and evolution regimes (all 95% credibility intervals of the
457 pairwise differences contained zero).

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458

459 **Table 2.** Heritability (H^2), I^2 (Evolvability, in percentage) and genetic correlations for body size (area).

460

Experimental evol. regime	Temp. (°C)	Posterior mode H^2 (95% CI)	Posterior mode I^2 (95% CI)	Posterior mode genetic correlation across temperatures (95% CI)
Fast temp. cycles	20	0.59 (0.44, 0.70)	1.13 (0.56, 2.01)	0.20 (-0.10, 0.38)
	25	0.56 (0.42, 0.66)	1.30 (0.53, 2.67)	
Increased warming	20	0.50 (0.37, 0.65)	1.08 (0.57, 2.42)	0.20 (-0.12, 0.41)
	25	0.49 (0.42, 0.67)	1.54 (0.69, 2.97)	

461

462

463 ## Discussion

464 We found that evolution in an environment that changed in temperature every 2nd
465 generation (*Fast temperature cycles* regime) resulted in the evolution of increased phenotypic
466 plasticity in body size. In contrast, we did not find any evidence of increased diversifying bet
467 hedging in this evolutionary regime, since there was neither increased phenotypic variance
468 within families nor reduced heritability.

469 Evolution in variable environments with no environmental correlations across
470 generations is predicted to result in increased importance of either phenotypic plasticity or
471 bet-hedging (Tufto, 2015). While phenotypic plasticity should be favored when the
472 environment contains predictable cues for development, bet-hedging should be favored
473 instead in less predictable environments (Botero *et al.*, 2015; Tufto, 2015). Moreover, the
474 timescale of environmental variation relative to the generation time is also important, and
475 when modeled by Tufto, (2015), environmental changes every 2nd generation is identified as

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476 the intersection between the evolution of bet-hedging, reversible plasticity or developmental
477 plasticity. Since the *Fast temperature cycle* regime experienced fluctuations every 2nd
478 generation, they are ideally suited for investigating the evolution of plasticity and bet-hedging
479 in adult peak body size, an irreversible plastic trait closely connected to fitness.
480 We found evolution of increased phenotypic plasticity in the *Fast temperature cycle*
481 *regime*, manifested as a larger size difference between 20°C and 25°C (steeper reaction
482 norm). Evolution of increased phenotypic plasticity in more variable environments is
483 predicted by theory (Moran, 1992; Gavrilets & Scheiner, 1993; Kuijper & Hoyle, 2015), and
484 indeed studies using natural populations (Lind & Johansson, 2007; Lind *et al.*, 2011) or
485 species (Hollander, 2008) have found a positive correlation between the degree of
486 environmental heterogeneity and increased plasticity. Our study, using experimental
487 evolution, supports these results and pinpoint large temporal variation between temperature
488 extremes as the causative selection force underlying evolution of increased plasticity. This
489 has only been showed once before, in a recent experimental evolution study of the microalgae
490 *Thalassiosira pseudonana*, where populations evolving under fast fluctuations evolve
491 increased plasticity in photosynthesis (Schaum *et al.*, 2022). Our results also align with the
492 recent finding that laboratory-adapted populations of Zebra fish (*Danio rerio*), evolving in
493 very stable environments, have reduced physiological plasticity compared to their wild-
494 caught counterparts (Morgan *et al.*, 2022). Together, these studies demonstrate the
495 importance of environmental heterogeneity for the evolution of plasticity. It should however
496 be noted that very fast or unpredictable environmental change can make it impossible to
497 predict the environment, and in these circumstances plasticity may be selected against (Tufto,
498 2015), which was recently demonstrated using experimental evolution in the microalgae
499 *Dunaliella salina* (Leung *et al.*, 2020). Increased environmental variation is however not the
500 only factor that can influence the evolution of plasticity, but plasticity may also evolve when

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501 a population is exposed to a novel (but stable) environment (Lande, 2009; Chevin *et al.*,
502 2010; Corl *et al.*, 2018). Evolution of increased plasticity has been shown for *C. remanei*
503 evolving in very heat-stressed environments (36.8°C), which demonstrates that evolution of
504 plasticity also can be a way to survive novel environment (Sikkink *et al.*, 2014b), a
505 phenomenon called plasticity-led evolution (Levis & Pfennig, 2020).
506
507 In contrast, we did not find any evolution of increased diversifying bet-hedging.
508 While empirical evidence for diversifying bet-hedging is much rarer than for phenotypic
509 plasticity, it is also much harder to detect since it is not trait means but trait variances that
510 needs to be measured. We have the power to detect differences in within-family CV where
511 the selection regimes explain at least 10% of the variation, which corresponds to a large
512 effect (Cohen, 1988). Still, there are a number of examples of bet-hedging, mainly regarding
513 delayed germination in desert plants (Philippi, 1993; Clauss & Venable, 2000; Venable,
514 2007), but also diapause in fish from ephemeral pools (Furness *et al.*, 2015). In addition,
515 diversifying bet-hedging has also evolved as a result of experimental evolution in
516 unpredictable environments in bacteria (Beaumont *et al.*, 2009) and fungi (Graham *et al.*,
517 2014). However, as predicted by Bull, (1987), a common factor in these examples is the very
518 strong fitness differences between environments, where one environmental state (for example
519 dry conditions) results in very low fitness. This contrasts to most examples of phenotypic
520 plasticity, where reproduction is possible to achieve in all environments, even if they are not
521 suitable without plastic adjustment of the phenotype. It has however been found that natural
522 isolates of *C. elegans* differ in their variance of total reproduction, which has been suggested
523 to result from a bet-hedging strategy where lines with more phenotypic variance may
524 originate from more variable environments (Diaz & Viney, 2014). We did not find any
525 difference in reproductive variance between our selection regimes, so further studies are

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526 needed to understand why natural *C. elegans* isolates differ in their phenotypic variance of
527 reproduction.

528 When exposed to increasing temperatures, organisms generally show phenotypic
529 plasticity in size, and develop faster to mature smaller. Although there are exceptions,
530 especially in terrestrial arthropods (Horne *et al.*, 2015), this relationship is so general that it is
531 termed the temperature-size rule (Atkinson, 1994). Therefore, it is not surprising that
532 plasticity in size was present in both evolutionary regimes, and like *C. elegans* (Kammenga *et*
533 *al.*, 2007), we find that *C. remanei* follows the temperature-size rule. Importantly however,
534 the regimes differed in the degree of size plasticity.

535 Whether the temperature-size rule (Atkinson, 1994) reflects an adaptive or non-
536 adaptive response to temperature is not resolved. Arguments for it being non-adaptive center
537 around constraints related to passive by-products of other temperature dependent processes
538 (Atkinson, 1994; van der Have & de Jong, 1996; Forster *et al.*, 2011). For example, small
539 body size can be a result of reduced growth rates which can be selected for as a by-product of
540 increased reproductive investment in warmer temperatures (Heino & Kaitala, 1999;
541 Walczyńska *et al.*, 2015), or, alternatively, as a trade-off between growth rate and resistance
542 to oxidative stress (Kim *et al.*, 2011) which increases due to increased metabolism in higher
543 temperatures (Birnie-Gauvin *et al.*, 2017).

544 However, small size in warm environments can also be adaptive. For example Fryxell
545 *et al.*, (2020) showed that natural selection in warm temperatures favors smaller size in
546 mosquitofish *Gambusia affinis* and a similar result has been reported in snails (Arendt, 2015).
547 A possible advantage of small size in high temperatures can be a regulation of oxygen
548 demand and supply ratio (Walczyńska *et al.*, 2015). In addition, as a body composed of small
549 cells is more efficient in oxygen diffusion (Subczynski *et al.*, 1989; Verberk *et al.*, 2021)
550 there will be a particularly strong selection pressure on organisms such as *Caenorhabditis*

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551 nematodes, which have a fixed number of cells and thus the cell size determines the final
552 body size.

553 To assess whether temperature-induced plasticity in size is adaptive in *C. remanei*, we
554 compared individual fitness of different-sized individuals in both temperatures (Figure 2).
555 We found evidence of directional selection on increased size in both temperatures, but also
556 significant stabilizing selection within each temperature. Stabilizing selection implies that the
557 fitness optimum in both 20°C and 25°C was present in individuals within the data size-range
558 (as opposed to at extreme phenotypes). If small size in warm temperatures were maladaptive,
559 we would expect the largest individuals to have the highest fitness. In contrast, we found that
560 individuals both smaller and larger than the optimum size had decreased fitness. This
561 optimum size in the warm temperature was also substantially smaller than the optimum size
562 at the normal temperature, thus the plastic response to decrease size as a response to warm
563 temperature must be considered adaptive in *C. remanei*.

564 Interestingly, because most individuals raised in 25°C exhibit smaller size than would
565 be optimal (Figure 2.; mean size is smaller than optimal size), we can consider this
566 temperature plasticity to be a hyperplastic response, a special case of plasticity when plastic
567 response overshoots the optimum and brings individuals to the other side of the new fitness
568 peak (King & Hadfield, 2019). Since plasticity nevertheless increases fitness (compared to a
569 hypothetical non-plastic genotype), this hyperplasticity should still be considered adaptive. In
570 addition, we also found linear selection for large size in 20°C with individuals raised in 20°C
571 also having slightly smaller size than would be optimal. A possible explanation is a sexual
572 conflict between male and female worms, as males' optimal size is smaller than females'
573 optimal size in *C. remanei* (Stångberg *et al.*, 2020) so that males drag females from their
574 phenotypic optimum.

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575 In contrast to size, we didn't find any difference in individual fitness between the
576 regimes. While warm temperature caused a drop in total reproduction in both regimes,
577 individuals raised in 25°C had significantly higher rate-sensitive individual fitness, which is a
578 consequence of the temperature-induced alteration of the reproductive schedule, including a
579 faster development time (Sekajova *et al.*, 2022).

580 Previous experimental evolution and artificial selection studies in the SP8 line of *C.*
581 *remanei*, which was our founder population, have documented fast evolutionary responses to
582 selection in life history traits, suggesting a large amount of standing genetic variation (Chen
583 & Maklakov, 2012; Zwoinska *et al.*, 2016; Lind *et al.*, 2020). Accordingly, we found
584 substantial genetic variation for size, for all treatment × temperature combinations, which not
585 only allowed the lines to respond to selection, but also represents a potential for further
586 evolution. Since we used full-sibs, our estimates of genetic variance could potentially be
587 inflated by the presence of dominance variance and epistatic interactions. Epistatic
588 interactions are present for body size in the sister species *C. elegans* (Noble *et al.*, 2017;
589 Maulana *et al.*, 2022), but while most genetic variance for this trait was found to be additive
590 and with similar additive heritability to our estimate using an experimental evolution design
591 (Noble *et al.*, 2017), assessments of narrow-sense heritability using recombinant inbreed lines
592 found that additive effects played a smaller role for body-size (Maulana *et al.*, 2022).
593 Therefore, we assume that our broad sense heritability overestimates the additive genetic
594 effect, but to an unknown degree. Moreover, we didn't observe any significant differences in
595 broad-sense heritability between the lines, which further support our evidence of no evolution
596 of diversified bet-hedging, which comes with the prediction of lowered heritability in traits
597 (Tufto, 2015), nor did we observe any genetic correlations between trait values in the two
598 temperatures, suggesting that traits can largely evolve independently in each environment.
599

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600 **Conclusion**

601 To summarize, we found that 30 generations of experimental evolution in a
602 heterogeneous environment (*Fast temperature cycles)* resulted in the evolution of increased
603 phenotypic plasticity, compared to evolution in a slowly changing environment (*Increased*
604 *warming)*. We showed that plasticity followed the temperature size rule and was adaptive. In
605 addition, substantial amount of standing genetic variation found in the line represents a
606 potential for further evolutionary change.

607

608 **References**

609 Andersson, M. (1994) *Sexual selection*. Monographs in Behavior and Ecology. Princeton
610 University Press, Princeton, New Jersey.

611 Arendt, J. (2015) Why get big in the cold? Size–fecundity relationships explain the
612 temperature-size rule in a pulmonate snail (Physa). *Journal of Evolutionary Biology*, **28**, 169–
613 178.

614 Atkinson, D. (1994) Temperature and organism size: a biological law for ectotherms?
615 *Advances in ecological research*, **25**, 1–58.

616 Bates, D., Mächler, M., Bolker, B.M. & Walker, S.C. (2015) Fitting linear mixed-effects
617 models using lme4. *Journal of Statistical Software*, **67**, 1–48.

618 Beaumont, H.J.E., Gallie, J., Kost, C., Ferguson, G.C. & Rainey, P.B. (2009) Experimental
619 evolution of bet hedging. *Nature*, **462**, 90–93.

620 Berteaux, D., Réale, D., McAdam, A.G. & Boutin, S. (2004) Keeping pace with fast climate
621 change: can arctic life count on evolution? *Integrative and Comparative Biology*, **44**, 140–
622 151.

623 Birnie-Gauvin, K., Costantini, D., Cooke, S.J. & Willmore, W.G. (2017) A comparative and
624 evolutionary approach to oxidative stress in fish: A review. *Fish and Fisheries*, **18**, 928–942.

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625 Botero, C.A., Weissing, F.J., Wright, J. & Rubenstein, D.R. (2015) Evolutionary tipping
626 points in the capacity to adapt to environmental change. *Proceedings of the National*
627 *Academy of Sciences*, **112**, 184–189.

628 Brommer, J.E., Merilä, J. & Kokko, H. (2002) Reproductive timing and individual fitness.
629 *Ecology Letters*, **5**, 802–810.

630 Bull, J.J. (1987) Evolution of phenotypic variance. *Evolution*, **41**, 303–315.

631 Burton, T., Ratikainen, I.I. & Einum, S. (2022) Environmental change and the rate of
632 phenotypic plasticity. *Global Change Biology*, **28**, 5337–5345.

633 Campbell-Staton, S.C., Velotta, J.P. & Winchell, K.M. (2021) Selection on adaptive and
634 maladaptive gene expression plasticity during thermal adaptation to urban heat islands.
635 *Nature Communications*, **12**, 6195.

636 Chen, H. & Maklakov, A.A. (2012) Longer life span evolves under high rates of condition-
637 dependent mortality. *Current Biology*, **22**, 2140–2143.

638 Chevin, L.-M., Lande, R. & Mace, G.M. (2010) Adaptation, plasticity, and extinction in a
639 changing environment: towards a predictive theory. *PLoS Biol*, **8**, e1000357.

640 Clauss, M.J. & Venable, D.L. (2000) Seed germination in desert annuals: an empirical test of
641 adaptive bet hedging. *The American Naturalist*, **155**, 168–186.

642 Cohen, D. (1966) Optimizing reproduction in a randomly varying environment. *Journal of*
643 *Theoretical Biology*, **12**, 119–129.

644 Cohen, J. (1988) *Statistical power analysis for the behavioral sciences*. 2nd edition.
645 Routledge, Hillsdale, N.J.

646 Corl, A., Bi, K., Luke, C., Challa, A.S., Stern, A.J., Sinervo, B., *et al.* (2018) The genetic
647 basis of adaptation following plastic changes in coloration in a novel environment. *Current*
648 *Biology*, **28**, 2970-2977.e7.

649 DeWitt, T.J. & Scheiner, S.M. (2004) *Phenotypic plasticity: functional and conceptual*
650 *approaches*. Oxford University Press, New York, NY, USA.

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.22.523389; this version posted July 17, 2024. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

651 Dey, S., Proulx, S.R. & Teotónio, H. (2016) Adaptation to temporally fluctuating
652 environments by the evolution of maternal effects. *PLoS Biology*, **14**, e1002388.

653 Diaz, S.A. & Viney, M. (2014) Genotypic-specific variance in *Caenorhabditis elegans*
654 lifetime fecundity. *Ecology and Evolution*, **4**, 2058–2069.

655 Dupont, L., Thierry, M., Zinger, L., Legrand, D. & Jacob, S. (2024) Beyond reaction norms:
656 the temporal dynamics of phenotypic plasticity. *Trends in Ecology & Evolution*, **39**, 41–51.

657 Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R. & Mearns, L.O.
658 (2000) Climate extremes: observations, modeling, and impacts. *Science*, **289**, 2068–2074.

659 Forster, J., Hirst, A.G. & Woodward, G. (2011) Growth and development rates have different
660 thermal responses. *The American Naturalist*, **178**, 668–678.

661 Fryxell, D.C., Hoover, A.N., Alvarez, D.A., Arnesen, F.J., Benavente, J.N., Moffett, E.R., *et*
662 *al.* (2020) Recent warming reduces the reproductive advantage of large size and contributes
663 to evolutionary downsizing in nature. *Proceedings of the Royal Society B: Biological*
664 *Sciences*, **287**, 20200608.

665 Furness, A.I., Lee, K. & Reznick, D.N. (2015) Adaptation in a variable environment:
666 Phenotypic plasticity and bet-hedging during egg diapause and hatching in an annual killifish.
667 *Evolution*, **69**, 1461–1475.

668 Gavrilets, S. & Scheiner, S.M. (1993) The genetics of phenotypic plasticity. V. Evolution of
669 reaction norm shape. *Journal of Evolutionary Biology*, **6**, 31–48.

670 Geerts, A.N., Vanoverbeke, J., Vanschoenwinkel, B., Van Doorslaer, W., Feuchtmayr, H.,
671 Atkinson, D., *et al.* (2015) Rapid evolution of thermal tolerance in the water flea *Daphnia*.
672 *Nature Climate Change*, **5**, 665–668.

673 Ghalambor, C.K., Hoke, K.L., Ruell, E.W., Fischer, E.K., Reznick, D.N. & Hughes, K.A.
674 (2015) Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in
675 nature. *Nature*, **525**, 372–375.

676 Graham, J.K., Smith, M.L. & Simons, A.M. (2014) Experimental evolution of bet hedging
677 under manipulated environmental uncertainty in *Neurospora crassa*. *Proceedings of the*
678 *Royal Society of London B: Biological Sciences*, **281**, 20140706.

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679 Hadfield, J. (2010) MCMC methods for multi-response generalized linear mixed models: the
680 MCMCglmm R package. *Journal of Statistical Software*, **33**, 1–22.

681 Hansen, T.F., Pélabon, C., Armbruster, W.S. & Carlson, M.L. (2003) Evolvability and
682 genetic constraint in *Dalechampia* blossoms: components of variance and measures of
683 evolvability. *Journal of Evolutionary Biology*, **16**, 754–766.

684 Hansen, T.F., Pélabon, C. & Houle, D. (2011) Heritability is not evolvability. *Evolutionary*
685 *Biology*, **38**, 258–277.

686 Have, T.M. van der & Jong, G. de. (1996) Adult size in ectotherms: temperature effects on
687 growth and differentiation. *Journal of Theoretical Biology*, **183**, 329–340.

688 Hedrick, A.V. & Temeles, E.J. (1989) The evolution of sexual dimorphism in animals:
689 Hypotheses and tests. *Trends in Ecology & Evolution*, **4**, 136–138.

690 Heino, M. & Kaitala, V. (1999) Evolution of resource allocation between growth and
691 reproduction in animals with indeterminate growth. *Journal of Evolutionary Biology*, **12**,
692 423–429.

693 Hollander, J. (2008) Testing the grain-size model for the evolution of phenotypic plasticity.
694 *Evolution*, **62**, 1381–1389.

695 Horne, C.R., Hirst, Andrew.G. & Atkinson, D. (2015) Temperature-size responses match
696 latitudinal-size clines in arthropods, revealing critical differences between aquatic and
697 terrestrial species. *Ecology Letters*, **18**, 327–335.

698 Kammenga, J.E., Doroszuk, A., Riksen, J.A.G., Hazendonk, E., Spiridon, L., Petrescu, A.-J.,
699 *et al.* (2007) A *Caenorhabditis elegans* wild type defies the temperature–size rule owing to a
700 single nucleotide polymorphism in tra-3. *PLoS Genet*, **3**, e34.

701 Kim, S.-Y., Noguera, J.C., Morales, J. & Velando, A. (2011) Quantitative genetic evidence
702 for trade-off between growth and resistance to oxidative stress in a wild bird. *Evolutionary*
703 *Ecology*, **25**, 461–472.

704 King, J.G. & Hadfield, J.D. (2019) The evolution of phenotypic plasticity when environments
705 fluctuate in time and space. *Evolution Letters*, **3**, 15–27.

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.22.523389; this version posted July 17, 2024. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

706 Kuijper, B. & Hoyle, R.B. (2015) When to rely on maternal effects and when on phenotypic
707 plasticity? *Evolution*, **69**, 950–968.

708 Kussell, E. & Leibler, S. (2005) Phenotypic diversity, population growth, and information in
709 fluctuating environments. *Science*, **309**, 2075–2078.

710 Lachmann, M. & Jablonka, E. (1996) The inheritance of phenotypes: an adaptation to
711 fluctuating environments. *Journal of Theoretical Biology*, **181**, 1–9.

712 Lande, R. (2009) Adaptation to an extraordinary environment by evolution of phenotypic
713 plasticity and genetic assimilation. *Journal of Evolutionary Biology*, **22**, 1435–1446.

714 Leimar, O. & McNamara, J.M. (2015) The evolution of transgenerational integration of
715 information in heterogeneous environments. *The American Naturalist*, **185**, E55–E69.

716 Leung, C., Rescan, M., Grulois, D. & Chevin, L.-M. (2020) Reduced phenotypic plasticity
717 evolves in less predictable environments. *Ecology Letters*, **23**, 1664–1672.

718 Levis, N.A. & Pfennig, D.W. (2020) Plasticity-led evolution: A survey of developmental
719 mechanisms and empirical tests. *Evolution & Development*, **22**, 71–87.

720 Lind, M.I., Ingvarsson, P.K., Johansson, H., Hall, D. & Johansson, F. (2011) Gene flow and
721 selection on phenotypic plasticity in an island system of *Rana temporaria*. *Evolution*, **65**,
722 684–697.

723 Lind, M.I. & Johansson, F. (2007) The degree of phenotypic plasticity is correlated with the
724 spatial environmental heterogeneity experienced by island populations of *Rana temporaria*.
725 *Journal of Evolutionary Biology*, **20**, 1288–1297.

726 Lind, M.I., Zwoinska, M.K., Andersson, J., Carlsson, H., Krieg, T., Larva, T., *et al.* (2020)
727 Environmental variation mediates the evolution of anticipatory parental effects. *Evolution*
728 *Letters*, **4**, 371–381.

729 Lind, M.I., Zwoinska, M.K., Meurling, S., Carlsson, H. & Maklakov, A.A. (2016) Sex-
730 specific trade-offs with growth and fitness following lifespan extension by rapamycin in an
731 outcrossing nematode, *Caenorhabditis remanei*. *Journals of Gerontology Series A:*
732 *Biological Sciences and Medical Sciences*, **71**, 882–890.

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733 Maulana, M.I., Riksen, J.A.G., Snoek, B.L., Kammenga, J.E. & Sterken, M.G. (2022) The
734 genetic architecture underlying body-size traits plasticity over different temperatures and
735 developmental stages in *Caenorhabditis elegans*. *Heredity*, **128**, 313–324.

736 McGraw, J.B. & Caswell, H. (1996) Estimation of individual fitness from life-history data.
737 *The American Naturalist*, **147**, 47–64.

738 Merilä, J. & Hendry, A.P. (2014) Climate change, adaptation, and phenotypic plasticity: the
739 problem and the evidence. *Evolutionary Applications*, **7**, 1–14.

740 Meyers, L.A. & Bull, J.J. (2002) Fighting change with change: adaptive variation in an
741 uncertain world. *Trends in Ecology & Evolution*, **17**, 551–557.

742 Moran, N.A. (1992) The evolutionary maintenance of alternative phenotypes. *American*
743 *Naturalist*, **139**, 971–989.

744 Morgan, R., Andreassen, A.H., Åsheim, E.R., Finnøen, M.H., Dresler, G., Brembu, T., *et al.*
745 (2022) Reduced physiological plasticity in a fish adapted to stable temperatures. *Proceedings*
746 *of the National Academy of Sciences*, **119**, e2201919119.

747 Mylius, S.D. & Diekmann, O. (1995) On evolutionarily stable life histories, optimization and
748 the need to be specific about density dependence. *Oikos*, **74**, 218–224.

749 Noble, L.M., Chelo, I., Guzella, T., Afonso, B., Riccardi, D.D., Ammerman, P., *et al.* (2017)
750 Polygenicity and epistasis underlie fitness-proximal traits in the *Caenorhabditis elegans*
751 multiparental experimental evolution (CeMEE) panel. *Genetics*, genetics.300406.2017.

752 Palmer, T.N. & Räisänen, J. (2002) Quantifying the risk of extreme seasonal precipitation
753 events in a changing climate. *Nature*, **415**, 512–514.

754 Partridge, L. & Coyne, J.A. (1997) Bergmann's rule in ectotherms: is it adaptive? *Evolution*,
755 **51**, 632–635.

756 Philippi, T. (1993) Bet-hedging germination of desert annuals: variation among populations
757 and maternal effects in *Lepidium lasiocarpum*. *The American Naturalist*, **142**, 488–507.

758 Philippi, T. & Seger, J. (1989) Hedging one's evolutionary bets, revisited. *Trends in Ecology*
759 *& Evolution*, **4**, 41–44.

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760 R Core Team. (2015) R: A language and environment for statistical computing.

761 Ray, C. (1960) The application of Bergmann's and Allen's rules to the poikilotherms.
762 *Journal of Morphology*, **106**, 85–108.

763 Schaum, C.-E., Buckling, A., Smirnoff, N. & Yvon-Durocher, G. (2022) Evolution of
764 thermal tolerance and phenotypic plasticity under rapid and slow temperature fluctuations.
765 *Proceedings of the Royal Society B: Biological Sciences*, **289**, 20220834.

766 Sekajova, Z., Rosa, E., Spagopoulou, F., Zervakis, P.-I. & Lind, M.I. (2022) Temperature-
767 induced compensatory growth in the nematode *Caenorhabditis elegans* is regulated by a
768 thermosensitive TRP channel and influences reproductive rate. *Functional Ecology*, **36**,
769 2176–2187.

770 Sikkink, K.L., Ituarte, C.M., Reynolds, R.M., Cresko, W.A. & Phillips, P.C. (2014a) The
771 transgenerational effects of heat stress in the nematode *Caenorhabditis remanei* are negative
772 and rapidly eliminated under direct selection for increased stress resistance in larvae.
773 *Genomics*, Experimental evolution and the use of genomics, **104**, 438–446.

774 Sikkink, K.L., Reynolds, R.M., Ituarte, C.M., Cresko, W.A. & Phillips, P.C. (2014b) Rapid
775 evolution of phenotypic plasticity and shifting thresholds of genetic assimilation in the
776 nematode *Caenorhabditis remanei*. *G3: Genes | Genomes | Genetics*, **4**, 1103–1112.

777 Simons, A.M. (2011) Modes of response to environmental change and the elusive empirical
778 evidence for bet hedging. *Proceedings of the Royal Society of London B: Biological Sciences*,
779 **278**, 1601–1609.

780 Slatkin, M. (1974) Hedging one's evolutionary bets. *Nature*, **250**, 704–705.

781 Stångberg, J., Immonen, E., Moreno, P.P. & Bolund, E. (2020) Experimentally induced
782 intrasexual mating competition and sex-specific evolution in female and male nematodes.
783 *Journal of Evolutionary Biology*, **33**, 1677–1688.

784 Stearns, S.C. (1992) *The evolution of life histories*. Oxford University Press, New York, NY,
785 USA.

786 Stiernagle, T. (2006) Maintenance of *C. elegans*. *WormBook: the online review of C. elegans*
787 *biology*.

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788 Subczynski, W.K., Hyde, J.S. & Kusumi, A. (1989) Oxygen permeability of
789 phosphatidylcholine--cholesterol membranes. *Proceedings of the National Academy of*
790 *Sciences*, **86**, 4474–4478.

791 Teotónio, H., Estes, S., Phillips, P.C. & Baer, C.F. (2017) Experimental evolution with
792 *Caenorhabditis* nematodes. *Genetics*, **206**, 691–716.

793 Tufto, J. (2015) Genetic evolution, plasticity, and bet-hedging as adaptive responses to
794 temporally autocorrelated fluctuating selection: A quantitative genetic model. *Evolution*, **69**,
795 2034–2049.

796 Uller, T., English, S. & Pen, I. (2015) When is incomplete epigenetic resetting in germ cells
797 favoured by natural selection? *Proc. R. Soc. B*, **282**, 20150682.

798 Van Aalst, M.K. (2006) The impacts of climate change on the risk of natural disasters.
799 *Disasters*, **30**, 5–18.

800 Venable, D.L. (2007) Bet hedging in a guild of desert annuals. *Ecology*, **88**, 1086–1090.

801 Verberk, W.C.E.P., Atkinson, D., Hoefnagel, K.N., Hirst, A.G., Horne, C.R. & Siepel, H.
802 (2021) Shrinking body sizes in response to warming: explanations for the temperature–size
803 rule with special emphasis on the role of oxygen. *Biological Reviews*, **96**, 247–268.

804 Walczyńska, A., Labecka, A.M., Sobczyk, M., Czarnoleski, M. & Kozłowski, J. (2015) The
805 temperature–size rule in *Lecane inermis* (Rotifera) is adaptive and driven by nuclei size
806 adjustment to temperature and oxygen combinations. *Journal of Thermal Biology*, **54**, 78–85.

807 West-Eberhard, M.J. (2003) *Developmental plasticity and evolution*. Oxford University Press
808 Inc, USA.

809 Wolf, D.M., Vazirani, V.V. & Arkin, A.P. (2005) Diversity in times of adversity:
810 probabilistic strategies in microbial survival games. *Journal of Theoretical Biology*, **234**,
811 227–253.

812 Zwoinska, M.K., Lind, M.I., Cortazar-Chinarro, M., Ramsden, M. & Maklakov, A.A. (2016)
813 Selection on learning performance results in the correlated evolution of sexual dimorphism in
814 life-history. *Evolution*, **70**, 342–357.

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