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Evolution of phenotypic plasticity during environmental fluctuations

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Abstract

Evolution in variable environments is predicted to disfavour genetic canalization and instead select for alternative strategies, such as phenotypic plasticity or possibly bet-hedging, depending on the accuracy of environmental cues and type of variation. While these two alternatives are often contrasted in theoretical studies, their evolution are seldom studied together in empirical work. We used experimental evolution for 30 generations in the nematode worm *Caenorhabditis remanei* to simultaneously study the evolution of plasticity and bet-hedging in environments differing only in their temperature variability, where one regime is exposed to faster temperature cycles between 20 and 25 °C, with little autocorrelation between parent and offspring environment, while the other regime had slowly increasing temperature with high autocorrelation in temperature between parent and offspring. These two environments had the same average temperature over evolutionary time, but one varied with larger magnitude on a shorter time scale. After experimental evolution, we scored adult size and fitness in full siblings reared in two different temperatures, optimal 20 °C and mildly stressful 25 °C. Experimental evolution in fast temperature cycles resulted in the evolution of increased body size plasticity but not increased bet-hedging, compared to evolution in the slowly changing environment. Plasticity followed the temperature–size rule as size decreased with increasing temperature and this plastic response was adaptive. In addition, we documented substantial standing genetic variation in body size, which represents a potential for further evolutionary change.

Keywords: adaptation, bet-hedging, *Caenorhabditis remanei*, experimental evolution, phenotypic plasticity, temperature

Introduction

Natural environments are generally not stable, but can vary both spatially and temporally, and a developing organism needs to take this environmental variation into account when developing their phenotype. If the environment varies with a large magnitude on a relatively short spatial or temporal scale, and the developmental environment provides reliable cues for the selective environment, theory predicts the evolution of adaptive phenotypic plasticity (Gavrilets & Scheiner, 1993; Moran, 1992; Simons, 2011) which is the ability of a genotype to produce different phenotypes depending on environmental conditions. If plasticity is present, individuals will canalize fitness between environments by adjusting their phenotype according to the environment.

As an alternative to plasticity in variable environments, individuals may express bet-hedging, which is an adaptive response that acts to reduce variation in fitness (especially to avoid very low fitness values in certain environmental states) at the cost of lowered arithmetic mean fitness, often by producing offspring with a range of phenotypes (diversified bet-hedging), where some of the offspring matches the environment and is successful (Philippi & Seger, 1989). Diversified bet-hedging generally requires large fitness differences between environmental states (Bull, 1987) and in contrast to plasticity, bet-hedging is favoured when environmental cues are not predictive of the selective environment (Cohen, 1966; Kussell & Leibler, 2005; Slatkin, 1974; Tufto, 2015).

While both phenotypic plasticity and bet-hedging can evolve as adaptive responses to increased environmental variation (Furness et al., 2015; Simons, 2011), most empirical studies of evolution in variable environments focus on the evolution of plasticity. While plasticity is common and well documented (DeWitt & Scheiner, 2004), few studies have investigated its evolution. These studies generally follow two lines, either they investigate whether increased plasticity evolves in more variable environments (Moran, 1992; Tufto, 2015), or whether increased plasticity evolves as a step in adaptation to a novel but stable environment (Chevin et al., 2010; Lande, 2009). Studies focusing on the role of environmental heterogeneity have found stronger phenotypic plasticity in natural populations (Lind & Johansson, 2007) or species (Hollander, 2008) from more variable environments. Moreover, recent experimental evolution studies in microalgae have shown that variable environments with short cycles (3–4 generations) select for increased plasticity compared to slow cycles (40 generation cycles) (Schaum et al.,

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2022), but very unpredictable environments can select against plasticity (Leung et al., 2020). Studies focusing on evolution of plasticity during adaptation to new stable environments have found that plasticity can rapidly evolve (Corl et al., 2018; Sikkink et al., 2014b), but also that maladaptive plasticity can play a role during adaptation (Ghalambor et al., 2015).

In contrast, empirical studies documenting bet-hedging are rare (Simons, 2011), and it seems more likely to evolve if the environments differ dramatically in fitness (Bull, 1987), such as delayed germination in desert plants (Philippi, 1993; Venable, 2007), diapause in killifish (Furness et al., 2015), or experimental evolution in fluctuating environments with large fitness differences (Beaumont et al., 2009; Graham et al., 2014).

One environmental factor that is known to result in evolutionary adaptations (Berteaux et al., 2004), but also alternative strategies, is temperature. Not only is temperature gradually increasing due to the ongoing climate change, but climate change also results in increased temperature variability (Easterling et al., 2000) potentially favouring evolution of increased plasticity or possibly bet-hedging. Indeed, most documented responses to climate change in natural populations are caused by pre-existing plasticity, while genetic adaptation seems rare (Merilä & Hendry, 2014).

Among traits showing plastic responses to temperature, body size is of immense importance to reproductive fitness. For females, a large body generally translates into increased egg production, and also males often benefit from large size (Andersson, 1994). Therefore, perhaps surprisingly, in warm environments organisms generally increase growth rate, accelerate maturation but mature at a smaller size, which is called the temperature–size rule (Atkinson, 1994; Ray, 1960; Verberk et al., 2021). This has been argued to be a passive by-product of other temperature-dependent processes (Atkinson, 1994). However, small size may actually be actively regulated and beneficial in warm environments being advantageous for thermoregulation (Partridge & Coyne, 1997), or allowing better regulation of oxygen demand and supply (Verberk et al., 2021; Walczyńska et al., 2015).

We set out to investigate whether exposure to fast and intense temperature cycles results in evolution of increased phenotypic plasticity compared to evolution in an environment with slowly increased temperature and whether any plasticity in body size is adaptive, using experimental evolution in the nematode *Caenorhabditis remanei*. *C. remanei* has a fast generation time and, being dioecious, harbors substantial standing genetic variation. In addition, *C. remanei* has been shown to respond to manipulations in temperature (Lind et al., 2020; Sikkink et al., 2014a), can respond plastically to new environmental conditions (Lind et al., 2020), and its plasticity to withstand heat-shock can itself evolve (Sikkink et al., 2014b). Body size of *C. remanei* is under directional upward selection under standard temperature (Stångberg et al., 2020), and pharmacologically lowered body size results in lower female reproduction (Lind et al., 2016). Body size in *Caenorhabditis* nematodes is a plastic trait that can be continuously adjusted during the whole growth period, both in juveniles (Sekajova et al., 2022) and adults (Lind et al., 2016), and follow the temperature–size rule in *C. elegans* (Kammenga et al., 2007). Body size plasticity is actively regulated by mTOR (Lind et al., 2016), insulin/IIS (Zečić & Braeckman, 2020), and TRPA-1 (Sekajova et al., 2022) signalling in response to environmental factors, including temperature. As an alternative to phenotypic plasticity, we investigated if populations evolving in variable environments had evolved increased diversifying bet-hedging.

We used previously established experimental evolution populations of *C. remanei* (described in Lind et al., 2020). During experimental evolution, replicate populations were exposed to 30 generations in one of two regimes; *fast temperature cycles* or *increased warming*. The *fast temperature cycles* regime was switched between two temperatures (20 and 25 °C) every second generation, and although these large fluctuations are deterministic, it represents an uncorrelated (and therefore unpredictable) fluctuating environment each generation, as the next generation will either be in the same or in a different temperature (Supplementary Figure 1). This was compared to the *increased warming* regime, where worms were exposed to experimental evolution in a gradually increasing temperature which slowly raised from 20 to 25 °C over 30 generations, and which served as a control, as the temperature variation was of very low magnitude (0.17°C increase per generation). Importantly, these two regimes had the same average temperature (22.5 °C) over evolutionary time, and only differed in the intensity and predictability of environmental change. As a result, the populations evolving in a predictable environment with low variation (the *increased warming regime*) have an anticipatory parental effect on reproduction, while this parental effect was lost in populations evolving in fast fluctuations (Lind et al., 2020). While no theoretical model to our knowledge have investigated evolution of plasticity in fast cycling versus slowly changing environments, it corresponds to environments with different degrees of temporal variation, which is well explored theoretically. In general, plasticity will evolve in the more variable environment both when modelling spatial (Moran, 1992) and temporal (Gavrilets & Scheiner, 1993; Tufto, 2015) variation. After the 30 generations of experimental evolution, we reared full-siblings in either standard 20 °C, or warm 25 °C, and scored them for reproduction and body size.

We predict that worms evolving in fast temperature cycles (every second generation) would evolve relatively increased phenotypic plasticity (compared to the *increased warming* regime), since the environment varies across a wide temperature range at short timescales and the timescale of this environmental variation is well within the parameter space where plasticity (and bet-hedging) is favoured (Tufto, 2015). We acknowledge, however, that we cannot separate the effect of environmental variability and predictability (the fast cycle regime is both more variable and less predictable). We predict that evolution of phenotypic plasticity is more likely than the evolution of bet-hedging, since the difference in fitness between the two temperatures is likely to be relatively small (Lind et al., 2020). If increased plasticity has evolved, we predict that the *fast temperature cycle* populations would show increased size difference between temperatures, but not increased phenotypic variance within one temperature. If instead increased diversifying bet-hedging had evolved, we predict that the *fast temperature cycle* populations will show (1) increased within-family variance within each temperature and (2) decreased heritability of size. The latter prediction is because bet-hedging will increase the phenotypic variance expressed by a given genotype in any environment, and thus the

proportion of the variance expressed by genotype within an environment will be lower, resulting in decreased heritability (Tufto, 2015). We also predict that any plasticity in body size will follow the temperature–size rule, and be adaptive.

Methods

Experimental evolution

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

Each evolutionary regime consisted of six replicate populations. The populations were maintained on 92 mm Petri dishes poured with Nematode Growth Medium (NGM) agar in climate chambers set to 60% relative humidity. In order to prevent bacterial and fungal contamination, the agar and bacterial Lysogeny Broth (LB) contained the antibiotics streptomycin and kanamycin and the fungicide nystatin. The plates were seeded with 2 ml of an antibiotic-resistant OP50-1 (pUC4K) strain of *E. coli* (Stiernagle, 2006) that served as a source of food. Every 1–2 days, a piece of agar containing approximately 150 worms of mixed ages was cut and transferred to a new plate containing fresh bacteria. This resulted in populations with overlapping generations that were maintained in a constant exponential growth phase. After the experimental evolution, populations were expanded for two generations and frozen in −80 °C for later revival and subsequent phenotypic assays.

Experimental set-up

Each replicate population of each of the two selection regimes was run in a separate block resulting in 12 experimental blocks in total. For logistic reasons we focus on females, since they are responsible for population growth rate and their fitness is straightforward to measure.

Briefly, populations were revived from freezing and exposed to 25 °C for three generations, to avoid any maternal effects associated with freezing. The third generation were split into eight families, each family consisting of one male and one female worm. From each family, we randomly picked eight offspring females (full siblings) and placed four females in 20 °C and four in 25 °C. Since our focus was evolution in females, their fitness was assessed by mating them with standardized males from the ancestral line. For the detailed description of the experimental set up, see Supplementary Figure 2.

Phenotypic assays

Daily reproduction

Female and male worms were transferred to a new plate every 24 hr, and viable offspring were counted 2 days later. The female worm was discarded after dying, or when reproduction ended.

Body size

Worms in 20 °C reach their peak size at day 4 of adulthood (Lind et al., 2016). The peak size in 25 °C is on day 2 of adulthood, which was determined during pilot assays (Supplementary Figure 3). Photographs of worms were taken during their peak size using a Lumenera Infinity2-5C digital microscope camera mounted on a Leica M165C stereomicroscope. Body size was measured from the photographs using *ImageJ 1.46r* (https://imagej.nih.gov/ij/) as total cross-section area.

Statistical analyses

All statistical analyses were conducted in R 3.6.1 (R Core Team, 2019).

Individual fitness

We used the age-specific reproduction data to calculate rate-sensitive individual fitness λ_{ind} for each individual (McGraw & Caswell, 1996), which is analogous to the intrinsic rate of population growth. Individual fitness was calculated by constructing a population projection matrix for each individual, and then calculating the dominant eigenvalue of this matrix, following McGraw & Caswell (1996). Since we kept the population size and age structure constant during experimental evolution, individual fitness is the most appropriate fitness measure for this study (Mylius & Diekmann, 1995).

Thermal reaction norms

To test whether the degree of phenotypic plasticity has evolved, we used linear mixed-effect models to separately estimate the thermal reaction norms of body size and individual fitness, using the package *lme4* (Bates et al., 2015) in R. The models included either body size (area) or individual fitness (λ_{ind}) as response variables. The full model included three fixed effects: mean-standardized temperature as a covariate, the experimental evolution regime as a categorical factor, and their interaction. We expect this interaction to be significant if the degree of plasticity has evolved. Experimental line and dam identity were included as random effects. Significance of the fixed effects was evaluated using Wald χ^2 tests. Pseudo-R^2 values were calculated as the squared correlation coefficient between fitted values from the model and observed values.

Selection

To test if temperature responses in size are adaptive, we estimated the selection on body size and compared it to the observed temperature response. Selection on body size (area) was estimated using mixed-effect models in R with the package *lme4* and individual fitness (λ_{ind}) as the response variable. The full model included the following fixed effects: area, area^2, temperature, experimental evolution regime, and all interactions except for interactions involving both area and

area^2 together. Experimental line was included as random effect. Significance of fixed effects was evaluated using Wald χ^2 tests. From the full model, we obtained temperature-specific estimates of the slope and the squared term between λ_{ind} and body size. For each temperature, the optimal size (i.e., the area that maximizes fitness) was calculated as: $-b/(2 \times c)$, where b = the temperature-specific slope from the full model (i.e., the linear selection gradient, estimating the relationship between body area and λ_{ind}) and c = the temperature-specific squared term from the full model (i.e., the quadratic selection gradient, estimating the relationship between area^2 and λ_{ind}). Confidence intervals of the temperature-specific optimal sizes were generated by bootstrapping, implemented in the *boot* package using 10,000 bootstrap replicates.

Within family coefficient of variance

To test whether the degree of diversifying bet-hedging has evolved, we tested whether the experimental evolution regimes differed in the mean within family variance within temperatures. For each family, we used the trait values of the offspring (within a temperature) to calculate the within family variance. To account for differences in trait means, we used within family means to mean-standardize the variance by calculating the within family coefficient of variance (CV): CV = σ/μ, where σ = within family standard deviation and μ = within family mean. An Analysis of Variance (ANOVA) was used within each temperature to test if the evolution regimes differed in their mean within family CV.

Since it is more difficult to detect differences in variances than differences in means, we also performed power calculations on our ability to detect whether within-family CVs differ between the selective regimes. Balanced one-way ANOVA power calculations were performed to estimate the effect sizes possible to detect with power ranging from 0.70 to 0.95. Effect sizes, η^2, were obtained for our sample size of $N = 48$ per selection regime and a significance level of 0.05. η^2 is calculated as the sum of squared explained by the treatment (here, selection regime) divided by the total sum of squares and has an equivalent interpretation as an R^2.

Genetic variance and correlations

For body size, genetic variance and genetic correlations across temperature were estimated using animal models in the package *MCMCglmm*. Univariate models were used to estimate genetic variance, whereas bivariate models were used to estimate genetic correlations, both models using Gaussian family for trait distribution. An inverse Wishart prior with parameters V = 1 and nu = 0.02 were used in both univariate and bivariate models. Pedigree data linking offspring to parents, based on full-sib relationships, was included in the models. Convergence of the models were ensured by evaluating diagnostic plots of posterior distributions, using the convergence diagnostic half-width test by Heidelberger & Welch (1983), and by ensuring that the autocorrelation between Markov chain Monte Carlo (MCMC) samples was close to zero.

For univariate models, body area was used as response variable. Temperature (categorical), experimental evolution regime, and their interaction, were included as fixed effects. Genetic variance (V_G), variance due to differences between experimental lines, and residual variances were estimated separately as random effects in the full model for each temperature-by-evolution regime combination. The full model ran for 4.2×10^6 MCMC iterations, 0.2×10^6 samples were discarded as burnin, and the thinning interval was 4000, resulting in a sample size of 1000 MCMC-samples. Reduced models, subset by temperature-by-evolution regime combination, were used to assess statistical significance of V_G, by comparing deviance information criterion (DIC) of models with versus without genetic variance included.

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

Genetic correlations of body size were estimated using bivariate animal models in *MCMCglmm*. Genetic correlations was estimated separately for the two regimes. Body size was the response variable and was treated as two traits (size at 20 and 25 °C). Random effects included genetic covariance between the temperatures, whereas V_G, variance due to differences between experimental lines, and residual variances were estimated separately for each temperature. The full models ran for 2.05×10^6 MCMC iterations (burnin: 0.05×10^6 samples, thinning interval: 2000 samples), resulting in a sample size of 1000 MCMC-samples. Reduced models without genetic covariance were used to access the statistical significance of the genetic covariance, by comparing the DIC of models with versus without genetic covariance included. The genetic correlation of body size across temperatures were calculated by dividing the genetic covariance by the product of the genetic standard deviation of the two temperatures. This was done on the posterior distributions, in order to carry the error forwards in the analyses.

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

Results

Thermal reaction norms

Size

Size decreased significantly with increasing temperature (Wald $\chi^2 = 309.93$, df = 1, $p < .001$; Figure 1A). There was a significant interaction between temperature and evolution regime, where *fast temperature cycles* had a steeper slope, meaning that it had evolved increased plasticity in size (Wald $\chi^2 = 5.82$, df = 1, $p = .016$). However, the intercepts (representing size at the mean temperature) were not significantly different between evolution regimes (Wald $\chi^2 = 0.09$, df = 1, $p = .769$). The model's pseudo $R^2 = 0.51$. Variance components: $V_{dam} = 25.03\ \mu m^4$, $V_{Line} = 20.92\ \mu m^4$, and $V_{residual} = 108.19\ \mu m^4$.

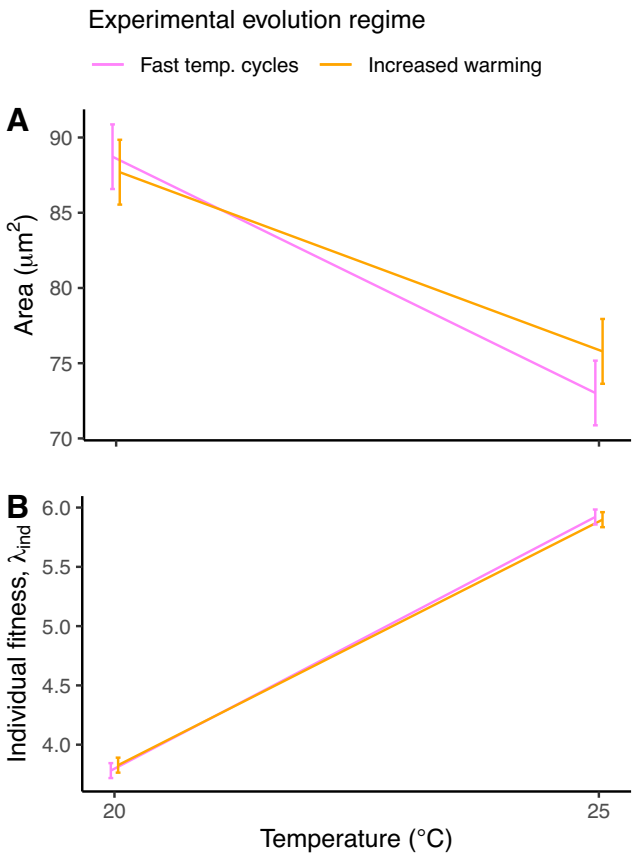

Figure 1. Thermal reaction norms showing means ± Standard Error (SE). (A) Regression lines (with mean-standardized temperature): Fast; Area = 80.87 ± 2.08–3.14 ± 0.22 × Temperature. Increase; Area = 81.74 ± 2.08–2.38 ± 0.22 × Temperature. (B) Regression lines (with mean-standardized temperature): Fast;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

Individual fitness (λ_{ind})

The mean total reproduction decreased with temperature (mean ± SE: 20 °C, 780 ± 17; 25 °C, 672 ± 17; $p < .001$ for the difference between the temperatures). However, the individual fitness (λ_{ind}) increased significantly with increasing temperature (Wald $\chi^2 = 3670$, df = 1, $p < .001$; Figure 1B). The evolution regimes did not differ significantly in intercepts (Wald $\chi^2 = 0.02$, df = 1, $p = .887$), nor was there a significant interaction between temperature and evolution regime (Wald $\chi^2 = 0.97$, df = 1, $p = .324$). The best-fitting models pseudo $R^2 = 0.86$. Variance components: $V_{dam} = 0.05$, $V_{Line} = 0.01$, and $V_{residual} = 0.21$.

Selection

There was significant linear and quadratic selection on body size (linear slope: Wald $\chi^2 = 59.4$, df = 1, $p < .001$. Quadratic term: Wald $\chi^2 = 40.3$, df = 1, $p < .001$). The selection differed significantly between temperatures (Figure 2), given by a significant overall temperature effect (Wald $\chi^2 = 2992$, df = 1, $p < .001$) and significant interaction effects between temperature and size (linear slope: Wald $\chi^2 = 13.3$, df = 1, $p < .001$. Quadratic term: Wald $\chi^2 = 12.8$, df = 1, $p < .001$). Maximum individual fitness (i.e., the optimal size, measured as cross-section area) is predicted to be 93.73 μm² at 20 °C [95% bootstrap CI: 87.61, 112.98], and 84.19 μm² at 25 °C [95% bootstrap confidence interval (CI): 79.81, 92.61]. Selection was, however, not significantly different between the experimental evolution regimes ($p > .22$ for main effect and interactions between evolution regime and temperature or body size). No three-way interactions were significant ($p > .18$). The best-fitting model's pseudo $R^2 = 0.85$. Variance components: $V_{Line} = 0.016$ and $V_{residual} = 0.204$.

Within family CV

The regimes did not differ significantly in within family CV of body size or individual fitness at either temperature (Table 1). Moreover, the distributions of within family CV overlapped considerably between regimes (Figure 3). Power calculations showed that we had a power of 90% to detect effects where the evolutionary regimes explained at least 10% of the variation in within family CV (Supplementary Figure 4).

Genetic variance and correlations

There was overall significant genetic variance for body size for the four combinations of temperature and evolution regime (models with genetic variance were at least seven DIC lower compared to models without genetic variance; Table 2). There was also significant genetic covariance between temperatures for both regimes (*fast temperature cycle*: model with covariance included was 2.04 DIC lower than model without covariance; *increased warming*: model with covariance was 2.79 DIC lower than model without covariance). However, pairwise comparisons of the posterior distributions of heritability, evolvability, and genetic correlations were not significantly different between the four different combinations of temperature and regimes (all 95% credibility intervals contained zero).

Discussion

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

Evolution in variable environments is predicted to result in increased importance of either phenotypic plasticity or bet-hedging (Tufto, 2015). While phenotypic plasticity should be favoured when the environment contains predictable cues for development, bet-hedging should be favoured instead in less predictable environments (Botero et al., 2015; Tufto, 2015). Moreover, the timescale of environmental variation relative to the generation time is also important, and when modeled by Tufto (2015), environmental changes every second generation is identified as the intersection between the evolution of bet-hedging, reversible plasticity, or developmental plasticity. Since the *fast temperature cycle* regime experienced fluctuations every second generation, they are ideally suited for investigating the evolution of plasticity and bet-hedging in adult peak body size, an irreversible plastic trait closely connected to fitness.

We found evolution of increased phenotypic plasticity in the *fast temperature cycle regime*, manifested as a larger size difference between 20 and 25 °C (steeper reaction norm). Evolution of increased plasticity in more variable environments is predicted by theory (Moran, 1992), and studies using natural populations (Lind & Johansson, 2007) or species (Hollander,

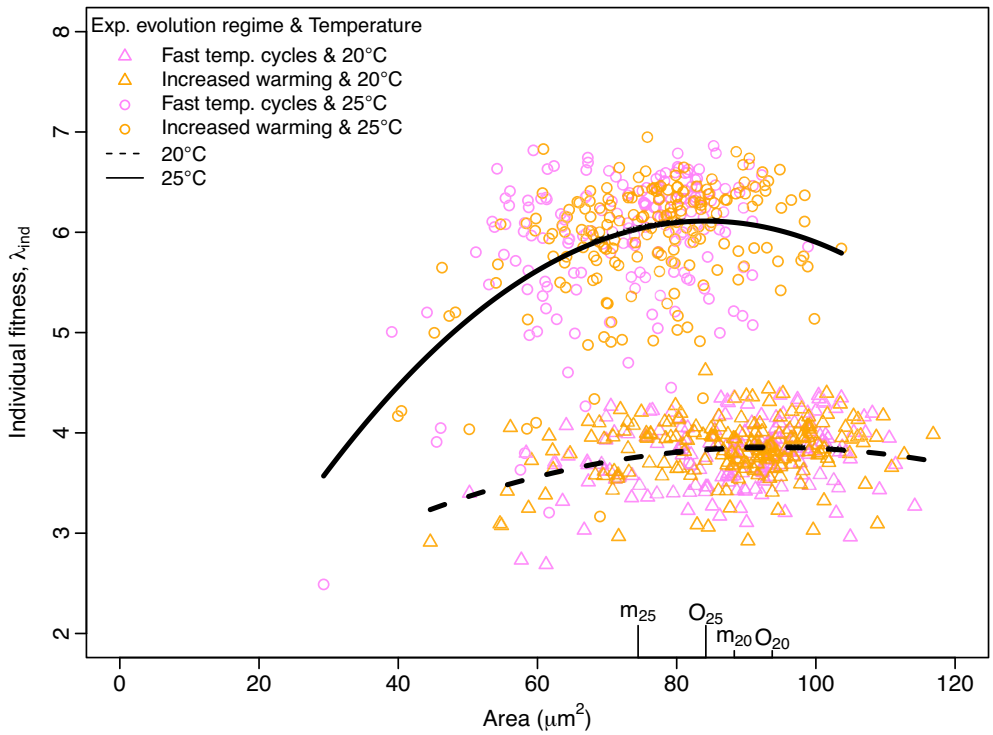

Figure 2. Selection on body size (cross-section area). Experimental evolution regimes are shown with different colours and line types. Overall regression line for 20 °C: $\lambda_{ind} = 1.596 (\pm 0.818) + 0.048 (\pm 0.020) \times Area - 2.57 \times 10^{-4} (\pm 1.16 \times 10^{-4}) \times Area^2$. Overall regression line for 25 °C: $\lambda_{ind} = 0.138 (\pm 0.614) + 0.142 (\pm 0.017) \times Area - 8.43 \times 10^{-4} (\pm 1.20 \times 10^{-4}) \times Area^2$. The mean size per temperature (m_{20} and m_{25}) and optimal size (O_{20} and O_{25}) are shown for 20 and 25 °C, respectively. Individual fitness is higher in 25 °C due to decreased development time, even if total reproduction is lower.

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

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

2008) have found increased plasticity in more variable environments compared to less variable environments. Our study, using experimental evolution, supports these results and pinpoint repeated temporal fluctuations of large magnitude between environmental states as the causative selection force underlying evolution of increased plasticity. This has only been showed once before, in a recent experimental evolution study of the microalgae *Thalassiosira pseudonana*, where populations evolving under fast temperature fluctuations (3–4 generation cycles) evolve increased plasticity in photosynthesis compared to populations under long fluctuation (40 generation cycles) (Schaum et al., 2022). This design is very similar to ours, as the selection regimes have the same average temperature and temperature range and only differed in their degree of short-scale variation. Our results also align with the recent finding that laboratory-adapted populations of Zebra fish (*Danio rerio*), evolving in very stable environments, have reduced plasticity compared to their wild-caught counterparts (Morgan et al., 2022). Together, these studies demonstrate the importance of the relative degree of environmental heterogeneity for the evolution of plasticity. However, very fast or unpredictable environmental change can make it impossible to predict the environment, and then plasticity may be selected against (Tufto, 2015), as demonstrated in the microalgae *Dunaliella salina* (Leung et al., 2020). Increased environmental variation is, however, not the only factor that influence the evolution of plasticity, but plasticity may also evolve when a population is exposed to a novel (but stable) environment (Chevin et al., 2010; Corl et al., 2018; Lande, 2009). Evolution of increased plasticity has been shown for *C. remanei* evolving in very heat-stressed environments (36.8°C), which demonstrates that evolution of plasticity also can be a way to survive novel environment (Sikkink et al., 2014b). To summarize, we found that the *fast temperature cycle* regime has no

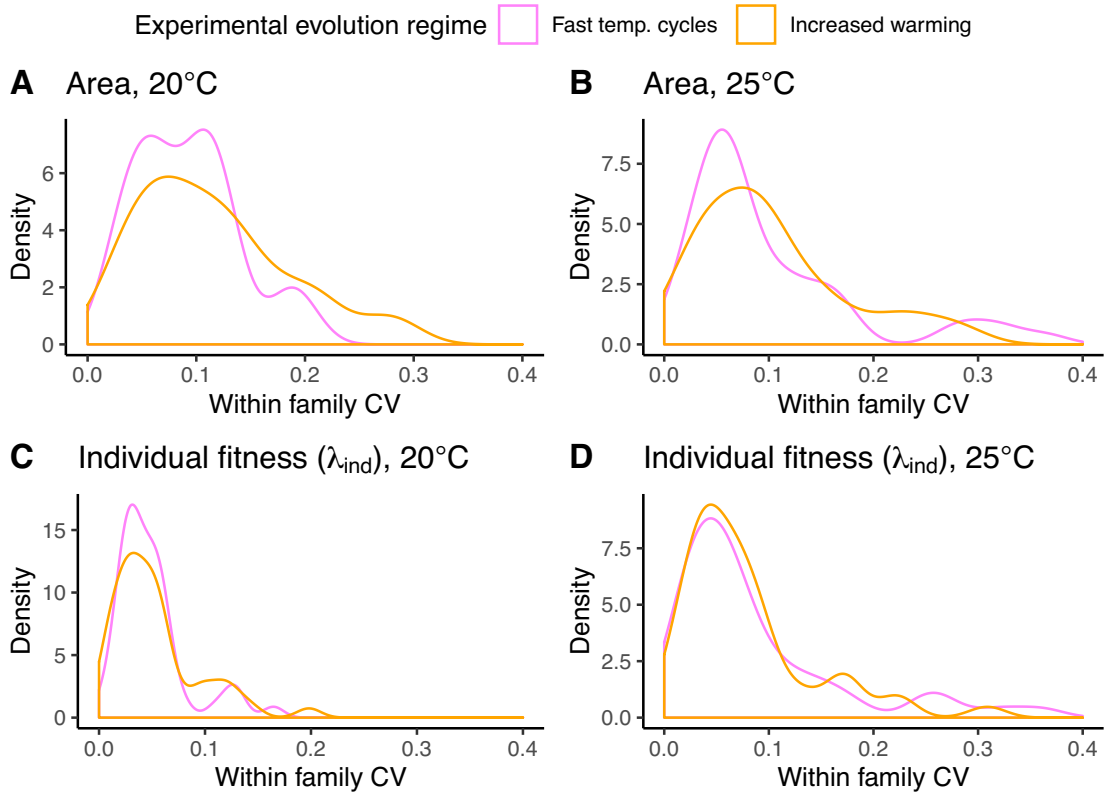

Figure 3. Distribution of within family coefficient of variance (CV) for two traits at two temperatures. The within family CV was estimated within temperature for each family by estimating the standard deviation (σ) and the mean (μ) of the family's offspring trait values, where CV = σ/μ. The density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

Table 2. Posterior mode and 95% credibility interval (CI) of genetic variance (V_G), total phenotypic variance (V_P), heritability (H^2), evolvability (I^2, in percentage), and genetic correlations for body size (area [μm^2]).

Experimental evolution regime	Temperature (°C)	V_G (95% CI)	V_P (95% CI)	H^2 (95% CI)	I^2 (95% CI)	Genetic correlation across temperatures (95% CI)
Fast temperature cycles	20	89.3 (64.0, 124.7)	157.9 (133.4, 202.8)	0.59 (0.44, 0.70)	1.13 (0.56, 2.01)	0.20 (−0.10, 0.38)
	25	80.6 (53.4, 107.0)	142.5 (116.3, 174.5)	0.56 (0.42, 0.66)	1.30 (0.53, 2.67)	
Increased warming	20	97.3 (65.9, 145.6)	201.6 (162.4, 251.6)	0.50 (0.37, 0.65)	1.08 (0.57, 2.42)	0.20 (−0.12, 0.41)
	25	85.8 (63.7, 129.8)	171.4 (140.1, 213.6)	0.49 (0.42, 0.67)	1.54 (0.69, 2.97)	

temperature induced maternal effects on reproduction (Lind et al., 2020) but high phenotypic plasticity of body size (this study) which would be adaptive when evolving in an environment that varies substantially over short timescales but when parent and offspring environment is not correlated. Consequently, the high environmental correlation between generations in the *increased warming* resulted in the evolution of a strong maternal effects on reproduction (Lind et al., 2020), but less phenotypic plasticity (this study). Since there is a negative autocorrelation between grandparent and offspring environment in the *fast temperature cycle* regime (Supplementary Figure 1), one could imagine the evolution of a negative grandparental effect that is not expressed in the parental generation. We did not investigate grandparental effects, but in *C. elegans* maternal effects are much stronger than effects spanning more generations (Burton et al., 2021).

In contrast to plasticity, we did not find any evolution of increased diversifying bet-hedging. While empirical evidence for diversifying bet-hedging is much rarer than for plasticity, it is also harder to detect since not trait means but trait variances needs to be measured. We have the power to detect differences in within-family CV where the selection regimes explain at least 10% of the variation, which corresponds to a large effect (Cohen, 1988). Still, there are a number of examples of bet-hedging, mainly regarding delayed germination in desert plants (Philippi, 1993; Venable, 2007), but also diapause in killifish (Furness et al., 2015). In addition, diversifying bet-hedging has also evolved in unpredictable environments in bacteria (Beaumont et al., 2009) and fungi (Graham et al., 2014). However, as predicted by Bull (1987), these examples have strong fitness differences between environments, where one environmental state results in very low fitness. This

contrasts to most examples of phenotypic plasticity, where reproduction is possible to achieve in all environments, even if some environments are unfavourable.

When exposed to increasing temperatures, organisms generally develop faster to mature smaller. Although exceptions exist, this relationship is general enough to be termed the temperature–size rule (Atkinson, 1994; Verberk et al., 2021). Unsurprisingly therefore, plasticity in size was present in both evolutionary regimes, and like *C. elegans* (Kammenga et al., 2007), *C. remanei* follows the temperature–size rule.

Whether this rule reflects an adaptive or non-adaptive response to temperature is not resolved. Arguments for it being non-adaptive centre around constraints related to passive by-products of other temperature dependent processes (Atkinson, 1994). However, small size in warm environments can be adaptive (Arendt, 2015; Fryxell et al., 2020). One advantage is better regulation of oxygen demand and supply ratio (Walczyńska et al., 2015). Additionally, since a body composed of small cells is more efficient in oxygen diffusion (Verberk et al., 2021), there will be a particularly strong selection pressure on organisms such as *Caenorhabditis* nematodes, which have a fixed number of cells and thus the cell size determines the final body size.

To assess whether plasticity in size is adaptive, we compared individual fitness of different-sized individuals in both temperatures (Figure 2). We found directional selection on increased size in both temperatures, but also significant stabilizing selection within each temperature. Stabilizing selection implies that the fitness optimum in both temperatures was present in individuals within the data size-range (as opposed to at extreme phenotypes). If small size in warm temperatures were maladaptive, we would expect the largest individuals to have the highest fitness. Instead, individuals both smaller and larger than the optimum size had decreased fitness. This optimum size in the warm temperature was also substantially smaller than the optimum size at the normal temperature, thus the plastic response to decreased size in warm temperature must be considered adaptive in *C. remanei*.

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

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

Previous selection studies in the SP8 line of *C. remanei*, which was our founder population, have documented fast evolutionary responses to selection in life history, suggesting substantial standing genetic variation (Lind et al., 2020). We found substantial genetic variation for size, for all treatment × temperature combinations, which allowed response to selection, and represents a potential for further evolution. Since we used full-sibs, our estimates of genetic variance could potentially be inflated by dominance variance and epistatis. Epistatic interactions are present for body size in the sister species *C. elegans* (Maulana et al., 2022; Noble et al., 2017), but while most genetic variance for size was additive with similar additive heritability to our estimate (Noble et al., 2017), assessments of narrow-sense heritability using recombinant inbreed lines found that additive effects played a smaller role for body-size (Maulana et al., 2022). Therefore, we assume that our broad sense heritability overestimates the additive genetic effect, but to an unknown degree. Moreover, we did not observe any differences in heritability between regimes, which further support our evidence of no evolution of diversified bet-hedging, which comes with the prediction of lowered heritability in traits (Tufto, 2015), nor did we observe any genetic correlations between trait values in the two temperatures; therefore, traits can largely evolve independently in each environment.

Conclusion

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

Supplementary material

Supplementary material is available at *Journal of Evolutionary Biology* online.

Data availability

The data underlying this article are available in the Dryad Digital Repository, at https://dx.doi.org/10.5061/dryad.2jm63xt1g

Author contributions

Zuzana Sekajova (Conceptualization [equal], Investigation [lead], Methodology [lead], Project administration [equal], Writing – original draft [equal], Writing – review & editing [supporting]), Erlend I. F. Fossen (Formal analysis [lead], Methodology [supporting], Visualization [lead], Writing – original draft [supporting], Writing – review & editing [equal]), Elena Rosa (Investigation [equal], Methodology [supporting], Writing – original draft [supporting]), Irja I. Ratikainen (Conceptualization [equal], Writing – original draft [supporting]), Manon Tourniaire-Blum (Investigation [supporting]), Elisabeth Bolund (Conceptualization [supporting], Formal Analysis [supporting], Methodology [supporting]), and Martin I Lind (Conceptualization [lead], Data curation [supporting], Formal Analysis [supporting], Funding

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Conflicts of interest

None declared.

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