An empirical test for a zone of canalization in thermal reaction norms

Authors: Erlend I. F. Fossen*, Christophe Pélabon*, Sigurd Einum*

* Centre for Biodiversity Dynamics, Department of Biology, NTNU, Norwegian University of Science and Technology, Trondheim, Norway

Correspondence: E. I. F. Fossen, Centre for Biodiversity Dynamics, Department of Biology, NTNU, Norwegian University of Science and Technology, 7491 Trondheim, Norway. Tel.: +47 73596066; e-mail: erlend.f.fossen@ntnu.no

Running title: Zone of canalization in reaction norms

Acknowledgments

Financial support was provided by the Research Council of Norway, FRIPRO programme, project 'Eco-evolutionary dynamics of thermal reaction norms' (Project 230482), and partly by the Research Council of Norway through its Centres of Excellence funding scheme, project number 223257/F50 and the Norwegian University of Science and Technology (NTNU). We thank V. Yashchenko for technical assistance, J. Tufto and Ø. N. Kielland for valuable discussion and comments on the manuscript, and two anonymous reviewers for valuable comments on earlier versions of the manuscript.

Data accessibility

Data deposited at Dryad: https://doi.org/10.5061/dryad.gp77fh7

Abstract

Theoretical models on the evolution of phenotypic plasticity predict a zone of canalization where reaction norms cross and genetic variation is minimized in the environment a population most frequently encounter. Empirical tests of this prediction are largely missing, in particular for life-history traits. We addressed this prediction by quantifying thermal reaction norms of three life-history traits (somatic growth rate, age and size at maturation) of a Norwegian population of *Daphnia magna* and testing for the occurrence of an intermediate temperature (T_m) at which genetic variance in the traits is minimized. Size at maturation changed relatively little with temperature compared to the other traits, and there was no genetic variance in the shape of the reaction norm. Consequently, age at maturation and somatic growth rate were strongly negatively correlated. Both traits showed a strong genotype-environment interaction and the estimated T_m was 14°C for both age at maturation and growth rate. This value of T_m corresponds well with mean summer temperatures experienced by the population and suggests that the population has evolved under stabilizing selection in temperatures that fluctuate around this mean temperature. These results suggest local adaptation to temperature in the studied population and allow predicting evolutionary trajectories of thermal reaction norms under changing thermal regimes.

Keywords: Daphnia, genotype-environment interaction, life-history evolution, phenotypic plasticity, thermal adaptation, thermal performance curves, thermal reaction norm, zone of canalization

Introduction

For ectotherms, temperature is an important environmental characteristic that varies over long (among year), medium (throughout season) and short (day to day and hour to hour) temporal scales, and directly affects morphological, physiological and life-history traits (e.g. wing length, de Moed *et al.*, 1997; metabolic rate, Gillooly *et al.*, 2001; somatic growth rate, Kingsolver *et al.*, 2004). Thermal reaction norms represent the phenotypic changes of a genotype with changes in temperature. Genetic variation in reaction norms is necessary for these to evolve, and quantifying such variation enables predictions about evolutionary responses to changes in the mean and variance of thermal regimes.

Thermal reaction norms are usually dome shaped, with performance increasing nearly linearly towards an optimum temperature and decreasing rapidly with further increase in temperature (Martin & Huey, 2008; Angilletta, 2009). Models describing variation in these performance curves (e.g. template modes of variation, Izem & Kingsolver, 2005) quantify variation in the overall elevation of the reaction norms (vertical shifts, "faster-slower"), in the optimal temperatures (horizontal shifts, "hotter-colder"), and in the width of the performance curves (generalist-specialist). These contrast with quantitative genetics models on the evolution of phenotypic plasticity that assume linear reaction norms (e.g. de Jong, 1990; Gavrilets & Scheiner, 1993; de Jong & Gavrilets, 2000; Lande, 2009; Ergon & Ergon, 2016). Although quantitiative genetic models exist for reaction norms with shapes that can be approximated as polynomials (e.g. Gavrilets & Scheiner, 1993; de Jong, 1999), linear models may be particularly relevant for understanding the evolution of thermal reaction norms because the range of temperatures normally experienced by ectotherms is commonly below the optimum temperature, especially for temperate ectotherms (e.g. Campbell *et al.*, 1974; Deutsch *et al.*, 2008; Kingsolver, 2009; Dell *et al.*, 2011; Nilsson-Örtman *et al.*, 2012; Thomas *et al.*, 2012;

Richter-Boix *et al.*, 2015; Mitchell & Bergmann, 2016; Amarasekare & Johnson, 2017). Therefore, evolution of thermal reaction norms for these organisms should essentially concern the temperature range where performance increases monotonically and close to linearly. Yet, these models are seldom considered in empirical literature describing thermal performance curves, and their predictions have rarely been tested. For instance, these models predict a zone of canalization within the range of temperatures encountered by the populations. Specifically, if a population has evolved under stabilizing selection in a range of environments fluctuating around an average environment, genetic variance is expected to be minimized at the intermediate environment (T_m) most frequently encountered (de Jong, 1990; Lande, 2009; Ergon & Ergon, 2016). This is a form of genetic canalization, where the effect of an allele substitution on a phenotype depends on the environment and is minimized at the zone of canalization (T_m). This contrasts to environmental canalization, in which a phenotype changes relatively little with the environment (Wagner *et al*., 1997; Flatt, 2005).

Empirical studies assessing the model by de Jong (1990) remain rare. Recent quantitative genetic models examining the evolution of phenotypic plasticity and genetic assimilation have assumed the existence of such an intermediate environment where reaction norms cross (Lande, 2009; Ergon & Ergon, 2016), but this has, to our knowledge, only been shown for morphological traits in *Drosophila melanogaster* (Noach *et al*., 1996; Karan *et al*., 1999; Imasheva *et al*., 2000). To fully understand evolution of thermal reaction norms, more empirical studies are needed to assess the validity of this model.

Here, we provide an empirical test of the model by de Jong (1990) using the crustacean zooplankton *Daphnia magna*, a keystone organism of many freshwater ecosystems (Lampert, 2011). Ten genotypes from a single population were exposed to eight different temperatures

(range 12 – 28 °C, i.e. within the monotonically changing part of the reaction norms), and genotype-specific thermal reaction norms were estimated for three life-history traits: somatic growth rate, age and size at maturation. For each trait, we tested for genotype-by-environment interactions in the reaction norms, and whether an intermediate temperature of minimum genetic variance could be detected.

Materials and methods

Study animals and husbandry

Ephippia of *Daphnia magna* Straus, 1820, containing up to two sexually produced resting eggs, were collected in November 2014 from the surface sediment of a shallow pond at Værøy Island (Sandtjønna, 1.0 ha, 67.687°N 12.672°E), northern Norway. Ten genotypes, hereby referred to as clones, each from a separate ephippia, were hatched in December 2014 and cultured separately for three asexual generations at 17°C with a 16L:8D photoperiod in 250 mL jars containing a modified ADaM medium (Klüttgen *et al.*, 1994, SeO_2 concentration reduced by 50%). Each clone line started from animals born in different jars to ensure independent replicates of clones. The clone lines, containing five adults per jar, with 13 to 14 replicated jars per clone, were fed three times a week with Shellfish Diet 1800 (Reed Mariculture Inc, USA) at a final algae concentration of 4×10^5 cells mL^{-1}, and the medium was changed weekly.

Experimental design

Fourth generation female neonates (<24 hours old) from the second or later clutches born at 17°C were transferred to individual 50 mL centrifuge tubes with 17°C ADaM medium. These juveniles were haphazardly chosen within each clonal line and from different mothers within the same clone to minimize maternal effects in the estimation of the genetic (clonal) variance.

A photograph was taken of each individual under a stereomicroscope and thereafter the animals were placed in a Memmert Peltier cooled incubator IPP 260plus (Memmert, Germany) climate cabinet with the air temperature set to one of eight different experimental temperatures (12.0°C, 15.0°C, 17.0°C, 19.0°C, 22.0°C, 24.0°C, 26.0°C or 28.0°C). All temperature treatments were run simultaneously. The chosen temperatures are all within the monotonically changing part of the reaction norm for somatic growth rate with relatively low mortality (unpublished data), and were chosen to cover the whole range of temperatures the animals experience in the wild (see discussion). The position of the tubes in the cabinets was randomized, and animals were fed a specific amount of food every second day (concentrations $\times 10^5$ cells/mL: 12°C, 2.00; 15°C, 2.38; 17°C, 2.62; 19°C, 2.88; 22°C, 3.24; 24°C, 3.50; 26°C, 3.76; 28°C, 4.00). Feeding regimes represent *ad libitum* concentrations during the juvenile growth stage (unpublished data). Individuals that died were not replaced. We checked individuals daily at approximately the same time of day to estimate the age at maturation (defined as the time when eggs were first visible in the brood chamber). Mature individuals were photographed for size measurements. The gut lengths (*GL*, mm, measured from the top of midgut to the bottom of hindgut when the animal is relaxed) of each individual as neonate and mature were measured using ImageJ v1.48 (National Institutes of Health, Bethesda, MD). These length measurements were then transformed to dry mass (*DM*, mg) using the following relationship between dry mass (*DM*) and gut length (*GL*): $DM = 0.00679GL^{2.75}$ (modified from Yashchenko *et al.*, 2016, see Appendix S1). Using dry mass of neonates (DM_{start}), dry mass at maturation (DM_{end}) and the number of days between the two measurements (*duration*), the somatic growth rate (SGR) was calculated as:

$$\mathrm{SGR} = \frac{\ln(DM_{end}) - \ln(DM_{start})}{duration} \quad (1)$$

By transforming the data using natural log, SGR×100 can be interpreted as the percentage increase in dry mass per day. This estimate of somatic growth rate correlates well with the instantaneous rate of increase (*r*) in *D. magna* (Lampert & Trubetskova, 1996). We quantified the thermal reaction norms of three life-history traits: somatic growth rate, age and size at maturation. Maturing at a larger size tends to increase fecundity and the survival of the offspring produced, but often at the cost of delaying maturation and being exposed to juvenile mortality for a longer time (Stearns, 2000). This delay can be compensated by having high somatic growth, but this can also be costly because it involves reallocating resources into growth from other traits and functions (Dmitriew, 2011). Thus, because of trade-offs with other traits, these traits are expected to have an optimum phenotype and be under stabilizing selection within different environments. This makes them ideal candidates for testing for a zone of canalization.

We used eight replicates per clone per temperature for a total sample size of 640 (8 temperatures × 10 clones × 8 replicates). The experiment lasted for about one month during May-June 2015, but due to logistic reasons most temperature-treatments started at different days. Each temperature-treatment was separated into two blocks with 4 replicates of each clone per temperature in each block, giving a total of 15 start dates over a span of 22 days. The starting date order of the treatments was decided by stratified randomization to avoid any systematic order within each block.

Statistical analyses

The average juvenile mortality among temperature treatments was 14% (range: 9-20%) with no apparent bias among treatments. Dead animals were treated as not available in statistical analyses. All statistical analyses were conducted in R v.3.1.1 (R Core Team, 2014).

Quantifying thermal reaction norms

Both linear and nonlinear mixed effects models were used to estimate the thermal reaction norms using the package lme4 (v. 1.1-7, Bates *et al.*, 2015) in R. Mixed effect models have been shown to give more accurate estimates of variances than alternative two-step approaches (Morrissey & Liefting, 2016). Temperature was used as a covariate whereas start date (a categorical factor with 15 levels representing the starting dates of experimental treatments) and clone were used as random effects. Start date was assumed to only affect the elevation of the reaction norms, while clone identity could affect both the elevation, the slope (in linear models) and the curvature (in non-linear models). The estimated clonal variance of the regression parameters (elevation, slope and curvature) are estimates of the total (broad sense) genetic variance in the parameters of the reaction norm. We used Akaike information criterion corrected for small sample sizes (AICc) in model selection. We first selected models with different random effect structures using the full model fitted with restricted maximum likelihood (REML). We then compared linear, log-linear and quadratic models using maximum likelihood (ML) depending on the observed relationship between the trait and temperature. Finally we estimated the reaction norm parameters from the best fit model using REML. Clone-specific intercepts and slopes were obtained from the random effects as Best Linear Unbiased Predictions (BLUPs). Pseudo R^2 values were calculated as the squared correlation coefficient between fitted values from the model and observed values.

Somatic growth rate showed signs of heteroscedasticity, so we used a weighted least squares regression with weights = 1/(somatic growth rate)^2. Age at maturation was log-transformed and centered at 11°C so that $e^{\text{intercept}}$ corresponds to the age at maturation at 12°C when using a log-linear model. We note that although log-transforming the data changes the shape of the

reaction norms, it does not affect the point where they cross and consequently not our estimate of the temperature where genetic variance is minimized.

Estimating the temperature with the minimum genetic variance (Tm)

We estimated the temperature with the minimum amount of genetic variance (T_m) as:

$$T_m = - G_{Cov(a,b)}/G_b \quad (2)$$

where $G_{Cov(a,b)}$ is the genetic covariance between the intercepts and slopes and G_b is the genetic variance in the slopes (Lande, 2009). This analysis was restricted to traits with linear or log-linear reaction norms harboring genetic variation in the slope.

To quantify how sensitive our estimate of T_m is to the specific temperature treatments we used, T_m was estimated for different subsets of the data where data from different combinations of temperatures were excluded. One to four temperatures were excluded at a time, where the higher temperatures (24-28°C, less often experienced by the population in the wild) and 17°C (which did not need to acclimate to a new temperature) were excluded more often.

Estimating evolutionary potential

The effect of T_m on the population's evolutionary potential was further illustrated by calculating the broad sense evolvability (clonal variance/mean^2) at each temperature (Hansen *et al.*, 2003; Hansen *et al.*, 2011). Evolvability is the expected percentage change in a trait per generation per unit strength of selection. Compared to heritability, evolvability has the advantage of being independent from the environmental variance and therefore represents a measure of the evolutionary potential that is comparable across traits, populations and species

(Hansen *et al.*, 2011). We estimated temperature-specific evolvability for each trait by using the predicted trait values of each clone to calculate the clonal variance. Because age at maturation is estimated on a natural log scale its clonal variance can be directly interpreted as broad sense evolvability.

Results

Somatic growth rate and age at maturation were remarkably more variable across temperatures than size at maturation (growth rate: CV = 0.34; age at maturation: CV = 0.45; size at maturation: CV = 0.17).

Thermal reaction norms and T_m

The effect of temperature on age at maturation was best described by a log-linear regression model with statistically significant differences among clones in intercept and slope (Fig. 1A, Table 1, Table S1), indicating that clones react differently to a change in temperature. Overall, the effect of temperature was large with a decrease in the age at maturation of 68.4 % from 12°C to 28°C. Age at maturation was estimated to have the minimum amount of genetic variance at T_m = 14.11°C (range of estimates: 13.5°C, 15.8°C), where the slowest clone matured 5.4 % later than the fastest clone.

For somatic growth rate, the reaction norm was linear with statistically significant differences in slope among clones (Fig. 1B, Table 1, Table S1). Genetic variance in growth rate was minimum at T_m = 13.96°C (range of estimates: 12.3°C, 16.1°C), where the fastest clone grew 8.8 % faster than the slowest clone. Overall, the effect of temperature was dramatic with an increase in somatic growth rate of 169 % from 12°C to 28°C. The clone with the strongest

response to temperature (b = 0.0216 day^{-1} $°C^{-1}$) had a slope 40.6 % steeper than that of the clone with the shallowest slope (b = 0.0153 day^{-1} $°C^{-1}$).

The effect of temperature on size at maturation was best described by a quadratic model with statistically significant differences among clones in the elevation, but not in the curvature (Fig. 1C, Table 1, Table S1). Compared to the two other traits, the effect of temperature was small with an increase in size at maturation by only 24.1 % when going from the smallest size (at 28°C) to the largest size (at 18.28°C). At the temperature with the largest size, the largest clone was 14.7 % larger than the smallest clone.

Evolvability

The broad sense evolvability of the traits changed across temperatures as a result of changes in clonal variance and/or in trait means (Fig. S1). For size at maturation, where broad sense genetic variance was constant across temperatures, evolvability ranged from 0.22 % (at the temperature with the largest mean size) to 0.35 % at the highest temperature where individuals matured at the smallest size. For somatic growth rate and age at maturation, which both displayed a genotype × temperature interaction, evolvability was minimum at T_m (approximately 0.05%), increasing towards lower and higher temperatures where evolvability reached 0.37 % at 28°C.

Discussion

Quantitative genetic models on the evolution of phenotypic plasticity predict that for a given population there exists a zone of canalization at intermediate values of environmental variables, where reaction norms tend to cross each other and genetic variance is minimized (de Jong, 1990). By exposing clones of *Daphnia magna* to temperatures that largely cover the

range of temperatures the population experiences in the wild, we provide empirical support for the occurrence of such a zone of canalization (at T_m ~14°C) in two of the three traits studied (age at maturation and somatic growth rate). The fact that these two traits have virtually identical estimates of T_m may appear striking. However, there was considerably less variance in both size at maturation and neonate size (CV = 0.18, Fig. S2) compared to age at maturation and growth rate. Additionally, the shape of the reaction norm for size at maturation did not vary across clones. As a result, age at maturation and somatic growth rate increases almost proportionally or inversely with *duration*, respectively (see Eq. 1). Consequently, these two traits became strongly negatively correlated ($r = -0.90$, $t_{527} = -46.7$, $P < 0.001$).

To our knowledge, only three other empirical studies have shown the existence of T_m (Noach *et al.*, 1996; Karan *et al.*, 1999; Imasheva *et al.*, 2000), all of them for morphological traits (thorax length, wing length and other wing related traits) in *Drosophila melanogaster*. However, these results have been variable. Noach *et al.* (1996) found a good match between T_m and environmental temperatures for one of two populations of *D. melanogaster*, but only for 2 of the 6 traits they studied. In contrast, Karan *et al.* (1999) found an ecologically relevant T_m for three of three traits studied in a different population of *D. melanogaster*. Lastly, Imasheva *et al.* (2000) studied two closely related species of *Drosophila*, *D. melanogaster* and *D. simulans*, but only observed a zone of canalization in *D. melanogaster*. With a very different organism (*Daphnia magna*), living in highly contrasting environments, we show that T_m also occurs for life-history traits. Life-history traits and other thermal performance traits are often assumed to be direct surrogates of fitness, but it can be argued that most will be under stabilizing selection due to trade-offs with other traits (Stearns, 1989). Our results therefore provide further support to the model by de Jong (1990) to explain the

evolutionary dynamics of thermal reaction norms when the temperature encountered by the population is limited to the monotonic part of the reaction norm, as in the case of most temperate ectotherms.

We found a temperature of minimum genetic variance (T_m) that corresponds well with the mean summer temperature the population has experienced over the last 10 years (Fig. 2 and Appendix S2). Although this match may be coincidental since we only studied one population, it suggests that the population has undergone local adaptation to these temperatures in response to stabilizing selection. Importantly, this would not be possible to detect using thermal performance curve approaches (e.g. template modes of variation, Izem & Kingsolver, 2005) because of the large mismatch between average environmental temperature and the optimal temperature of our population (>26°C for somatic growth rate and age at maturation). Such a mismatch between the average environmental temperature experienced by a population and the optimal temperature is commonly observed (e.g. Campbell *et al.*, 1974; Lamb & Gerber, 1985; Dell *et al.*, 2011; Mitchell & Bergmann, 2016). This may result from the left-skewed shape of the reaction norm, causing fitness costs of experiencing temperatures above the optimum (causing mortality events) to greatly exceed those of experiencing temperatures below the optimum (Martin & Huey, 2008). Given that individuals of many populations rarely or never experience their optimal temperature, selection should mostly affect the monotonically changing part of reaction norms, making quantitative genetic models of plasticity (e.g. de Jong, 1990; Gavrilets & Scheiner, 1993; de Jong & Gavrilets, 2000; Lande, 2009; Ergon & Ergon, 2016) relevant for most thermal reaction norms.

Finding a temperature of minimum genetic variance (T_m) enables us to use quantitative genetic models (Lande, 2009; Ergon & Ergon, 2016) to make predictions about evolutionary

trajectories of populations under climate change. For instance, the expected increase in the mean temperature in Norway of 3.3-6.4°C (2-2.5°C for Værøy, where our population is from) within 2100 (Hanssen-Bauer *et al.*, 2015), should bring the mean summer temperature above T_m. Such an increase in temperature, assuming that higher growth rate at the new temperature is beneficial, should select for clones with steeper slopes ("warm-specialists"). Therefore, the mean slope of the reaction norm should increase until the new optimum phenotype is almost reached. Then, stabilizing selection around the new optimum should favor intermediate plasticity, leading to a progressive decrease of the mean slope and an increase in the elevation of the average reaction norm. This process, referred to as genetic assimilation, should result in an optimum phenotype being reached in the new environment (Lande, 2009; Ergon & Ergon, 2016). Furthermore, if there is genetic variation in how the organism perceives the environment, T_m itself should respond to selection and over time become equal to the new mean environmental temperature (see Fig. 1 in Ergon & Ergon, 2016). Alternatively, T_m may change to the new mean environment through genetic drift, increased fitness costs of maintaining plasticity in the new environment or by changes in the genetic architecture of reaction norms (Lande, 2009; Ergon & Ergon, 2016).

Size at maturation appears to be environmentally canalized, changing relatively little with temperature. Yet, the trait has a significant degree of genetic variance, with an evolvability ranging from 0.22-0.35 % across temperatures, which is similar to what is found in other traits, although somewhat lower than what is typically found for size measures and life-history traits (Hansen *et al.*, 2011). This environmental canalization suggests that size at maturation is a particularly important trait for fitness (Stearns & Kawecki, 1994; Stearns *et al.*, 1995), but still harbors genetic variation allowing it to respond to selection. It also supports the idea of a threshold size that *Daphnia* need to reach for maturing (Ebert, 1994; 1997).

The animals used in this experiment were all born at 17°C before being moved into new temperature treatments as newborns, meaning that the reaction norms we quantified included acclimation to new temperatures. Rearing temperature has been shown to affect the shape (both elevation and curvature) of reaction norms in various animal taxa (e.g. Angilletta, 2009; Cavieres *et al.*, 2016), but it is unknown if, or to what degree, acclimation affects the pattern of expressed genetic variation across temperature and in turn the temperature of minimum genetic variance (T_m). It is also unknown whether non-additive genetic variation (due to epistasis and dominance) included in our estimate of genetic variance has affected our estimate of T_m. Although total genetic variance is typically larger than additive genetic variance (Lynch & Walsh, 1998), it should not affect our estimate of T_m unless the relative proportion of additive to dominance and epistatic variance changes with temperature.

In this study, we tested a fundamental prediction for the evolution of phenotypic plasticity, namely that genetic variation in reaction norms for performance traits should be lowest at the most common environment experienced by the population (de Jong, 1990; Lande, 2009; Ergon & Ergon, 2016). We found support for a temperature of minimum genetic variance (T_m) in life-history traits, and the observed value of T_m corresponds well with the population's environmental summer temperatures, showing that T_m is ecologically relevant. Comparative estimates of T_m from populations of different thermal origins should provide a fruitful approach for further empirical evaluations of these models.

References

Amarasekare, P. & Johnson, C. 2017. Evolution of Thermal Reaction Norms in Seasonally Varying Environments. *Am. Nat.* **189:** 31-45.

Angilletta, M.J. 2009. *Thermal adaptation : a theoretical and empirical synthesis*. Oxford University Press, Oxford.

Bates, D., Mächler, M., Bolker, B. & Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **67:** 48.

Campbell, A., Frazer, B.D., Gilbert, N., Gutierrez, A.P. & Mackauer, M. 1974. Temperature Requirements of Some Aphids and Their Parasites. *J. Appl. Ecol.* **11:** 431-438.

Cavieres, G., Bogdanovich, J.M. & Bozinovic, F. 2016. Ontogenetic thermal tolerance and performance of ectotherms at variable temperatures. *J. Evol. Biol.* **29:** 1462-1468.

de Jong, G. 1990. Quantitative Genetics of reaction norms. *J. Evol. Biol.* **3:** 447-468.

de Jong, G. 1999. Unpredictable selection in a structured population leads to local genetic differentiation in evolved reaction norms. *J. Evol. Biol.* **12:** 839-851.

de Jong, G. & Gavrilets, S. 2000. Maintenance of genetic variation in phenotypic plasticity: the role of environmental variation. *Genet. Res.* **76:** 295-304.

de Moed, G.H., de Jong, G. & Scharloo, W. 1997. The phenotypic plasticity of wing size in *Drosophila melanogaster*: the cellular basis of its genetic variation. *Heredity* **79:** 260-267.

Dell, A.I., Pawar, S. & Savage, V.M. 2011. Systematic variation in the temperature dependence of physiological and ecological traits. *Proc. Natl. Acad. Sci. USA* **108:** 10591-10596.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C. & Martin, P.R. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. USA* **105:** 6668-6672.

Dmitriew, C.M. 2011. The evolution of growth trajectories: what limits growth rate? *Biol. Rev.* **86:** 97-116.

Ebert, D. 1994. A Maturation Size Threshold and Phenotypic Plasticity of Age and Size at Maturity in *Daphnia magna*. *Oikos* **69:** 309-317.

Ebert, D. 1997. The evolution and genetics of maturation in *Daphnia*. In: *Evolutionary Ecology of Freshwater Animals* (T. Streit, T. Städler & C. M. Lively, eds), pp. 151-178. Birkhäuser Verlag, Basel, Switzerland.

Ergon, T. & Ergon, R. 2016. When three traits make a line: evolution of phenotypic plasticity and genetic assimilation through linear reaction norms in stochastic environments. *J. Evol. Biol.* **30:** 486–500.

Flatt, T. 2005. The evolutionary genetics of canalization. *Q. Rev. Biol.* **80:** 287-316.

Gavrilets, S. & Scheiner, S.M. 1993. The genetics of phenotypic plasticity. V. Evolution of reaction norm shape. *J. Evol. Biol.* **6:** 31-48.

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M. & Charnov, E.L. 2001. Effects of size and temperature on metabolic rate. *Science* **293:** 2248-2251.

Hansen, T.F., Pelabon, C., Armbruster, W.S. & Carlson, M.L. 2003. Evolvability and genetic constraint in *Dalechampia* blossoms: components of variance and measures of evolvability. *J. Evol. Biol.* **16:** 754-766.

Hansen, T.F., Pelabon, C. & Houle, D. 2011. Heritability is not Evolvability. *J. Evol. Biol.* **38:** 258-277.

Hanssen-Bauer, I., Førland, E., Haddeland, I., Hisdal, H., Mayer, S., Nesje, A. *et al*. 2015. Klima i Norge 2100: Kunnskapsgrunnlag for klimatilpasning oppdatert i 2015. *NCCS report*. **no. 2/2015:** 1-203.

Imasheva, A.G., Moreteau, B. & David, J.R. 2000. Growth temperature and genetic variability of wing dimensions in *Drosophila*: opposite trends in two sibling species. *Genet. Res.* **76:** 237-247.

Izem, R. & Kingsolver, J.G. 2005. Variation in continuous reaction norms: Quantifying directions of biological interest. *Am. Nat.* **166:** 277-289.

Karan, D., Morin, J.-P., Gravot, E., Moreteau, B. & David, J.R. 1999. Body size reaction norms in *Drosophila melanogaster*: temporal stability and genetic architecture in a natural population. *Genet. Sel. Evol.* **31:** 491.

Kingsolver, J.G., Ragland, G.J. & Shlichta, J.G. 2004. Quantitative genetics of continuous reaction norms: Thermal sensitivity of caterpillar growth rates. *Evolution* **58:** 1521-1529.

Kingsolver, J.G. 2009. The Well-Temperatured Biologist. *Am. Nat.* **174:** 755-768.

Klüttgen, B., Dülmer, U., Engels, M. & Ratte, H.T. 1994. ADaM, an artificial freshwater for the culture of zooplankton. *Water Res.* **28:** 743-746.

Lamb, R.J. & Gerber, G.H. 1985. Effects of temperature on the development, growth, and survival of larvae and pupae of a north-temperate chrysomelid beetle. *Oecologia* **67:** 8-18.

Lampert, W. & Trubetskova, I. 1996. Juvenile growth rate as a measure of fitness in *Daphnia*. *Funct. Ecol.* **10:** 631-635.

Lampert, W. 2011. *Daphnia : development of a model organism in ecology and evolution*. International Ecology Institute, Oldendorf/Luhe.

Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *J. Evol. Biol.* **22:** 1435-1446.

Lynch, M. & Walsh, B. 1998. *Genetics and analysis of quantitative traits*. Sinauer, Sunderland, Ma.

Martin, T.L. & Huey, R.B. 2008. Why "Suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. *Am. Nat.* **171:** E102-E118.

443 Mitchell, A. & Bergmann, P.J. 2016. Thermal and moisture habitat preferences do not
444 maximize jumping performance in frogs. *Funct. Ecol.* **30:** 733-742.
445 Morrissey, M.B. & Liefting, M. 2016. Variation in reaction norms: Statistical considerations
446 and biological interpretation. *Evolution* **70:** 1944-1959.
447 Nilsson-Örtman, V., Stoks, R., De Block, M. & Johansson, F. 2012. Generalists and
448 specialists along a latitudinal transect: patterns of thermal adaptation in six species of
449 damselflies. *Ecology* **93:** 1340-1352.
450 Noach, E.J.K., de Jong, G. & Scharloo, W. 1996. Phenotypic plasticity in morphological traits
451 in two populations of *Drosophila melanogaster*. *J. Evol. Biol.* **9:** 831-844.
452 R Core Team 2014. R: A language and environment for statistical computing. *R Foundation*
453 *for Statistical Computing*. Vienna, Austria.
454 Richter-Boix, A., Katzenberger, M., Duarte, H., Quintela, M., Tejedo, M. & Laurila, A. 2015.
455 Local divergence of thermal reaction norms among amphibian populations is affected
456 by pond temperature variation. *Evolution* **69:** 2210-2226.
457 Stearns, S.C. 1989. Trade-offs in life-history evolution. *Funct. Ecol.* **3:** 259-268.
458 Stearns, S.C. & Kawecki, T.J. 1994. Fitness Sensitivity and the Canalization of Life-History
459 Traits. *Evolution* **48:** 1438-1450.
460 Stearns, S.C., Kaiser, M. & Kawecki, T.J. 1995. The differential genetic and environmental
461 canalization of fitness components in *Drosophila melanogaster*. *J. Evol. Biol.* **8:** 539-
462 557.
463 Stearns, S.C. 2000. Life history evolution: successes, limitations, and prospects.
464 *Naturwissenschaften* **87:** 476-486.
465 Thomas, M.K., Kremer, C.T., Klausmeier, C.A. & Litchman, E. 2012. A Global Pattern of
466 Thermal Adaptation in Marine Phytoplankton. *Science* **338:** 1085-1088.

Wagner, G.P., Booth, G. & Bagheri-Chaichian, H. 1997. A population genetic theory of canalization. *Evolution* **51:** 329-347.

Yashchenko, V., Fossen, E.I., Kielland, Ø.N. & Einum, S. 2016. Negative relationships between population density and metabolic rates are not general. *J. Anim. Ecol.* **85:** 1070-1077.

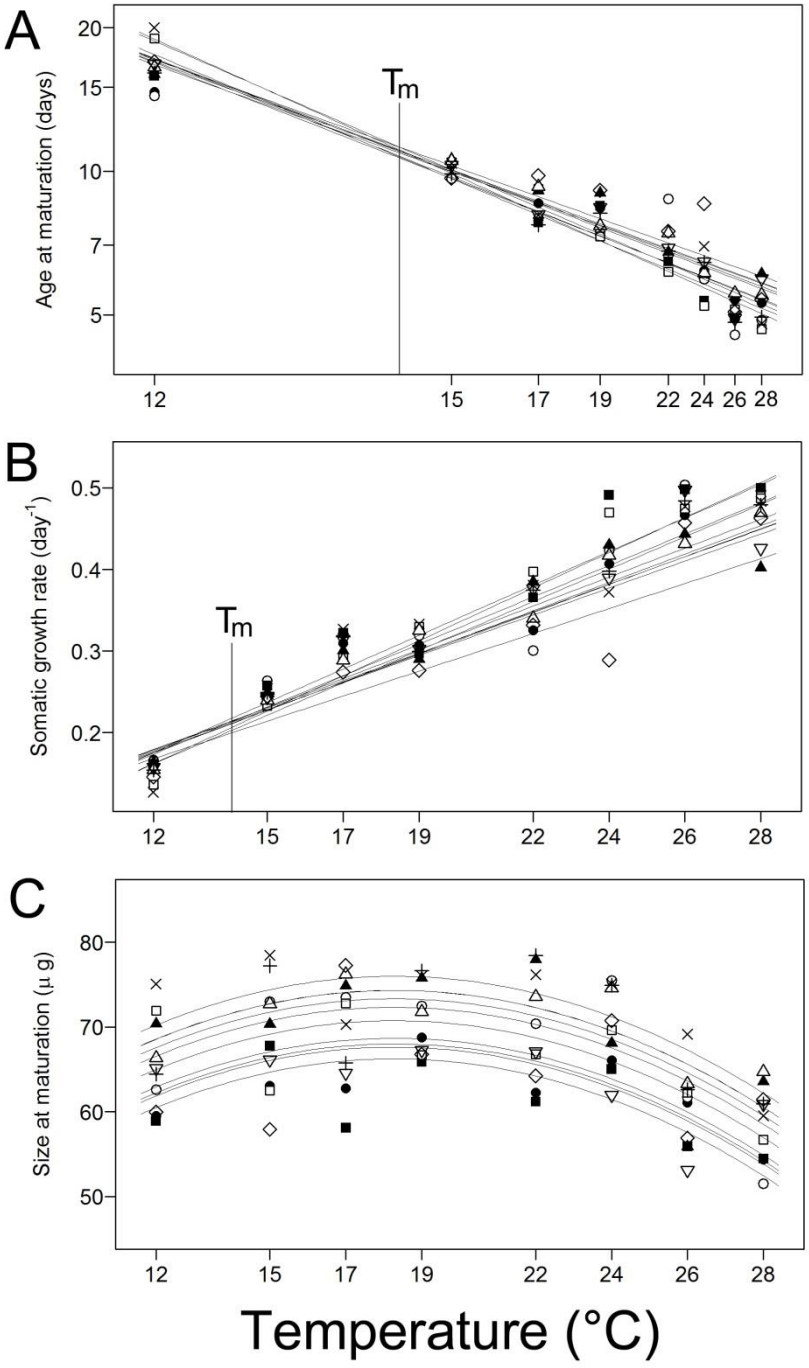
Table 1. Variance components of thermal reaction norm parameters for three life-history traits in a population of *Daphnia magna*. Variance components were obtained from mixed effect models. Only variance in intercept (predicted trait value at 18.28°C) is reported for size at maturation because there was no genetic variance in curvature parameters. V_{clone} = clonal variance; $V_{start\ date}$ = variance due to starting date; $V_{residual}$ = residual variance.

Trait	Intercept			Slope
	V_{clone}	$V_{start\ date}$	$V_{residual}$	V_{clone}
Size at maturation (μg^2)	13.02	8.62	2.86	-
Somatic growth rate (day^{-2})	0.56×10^{-4}	11.31×10^{-4}	1.06×10^{-4}	4.97×10^{-6}
Age at maturation $(\ln days)^2$	0.00333	0.00750	0.02648	0.00155

Figure legends

Figure 1. Thermal reaction norms of three life-history traits in a population of *Daphnia magna*. Each point is the mean of a clone for a given temperature with symbols representing different clones. Each line represents a clone, fitted from BLUPs of the random effects from a mixed effect model. T_m is the temperature at which genetic variance is minimized. See Table S2 for clonal regression lines. **A**) Reaction norms of age at maturation. Regression line for the whole population (± SE); ln (age at maturation) = 2.86 (± 0.04) – 0.407 (± 0.019) × ln (T), where T = temperature centered at 11°C. Pseudo $R^2 = 0.84$. **B**) Reaction norms of somatic growth rate. Regression line for the whole population (± SE); somatic growth rate = 0.209 (± 0.010) + 0.0182 (± 0.001) × T, where T = temperature centered at $T_m = 13.96$°C. Pseudo $R^2 = 0.80$. **C**) Reaction norm of size at maturation. Regression line for the whole population (± SE); size at maturation = 71.15 (± 1.69) - 0.0001 (± 0.1876) × T - 0.146 (± 0.039) × T^2, where T = temperature centered at 18.28°C. Pseudo $R^2 = 0.29$.

Figure 2. Predicted daily mean water temperatures at pond Sandtjønna, Værøy, from mid-April to mid-October for the period 2006-2015. Bold horizontal line shows estimated T_m = 14°C, dashed lines show the maximum and minimum estimate of T_m when using subsets of the data (see Material and Methods for details).



497

498 Figure 1

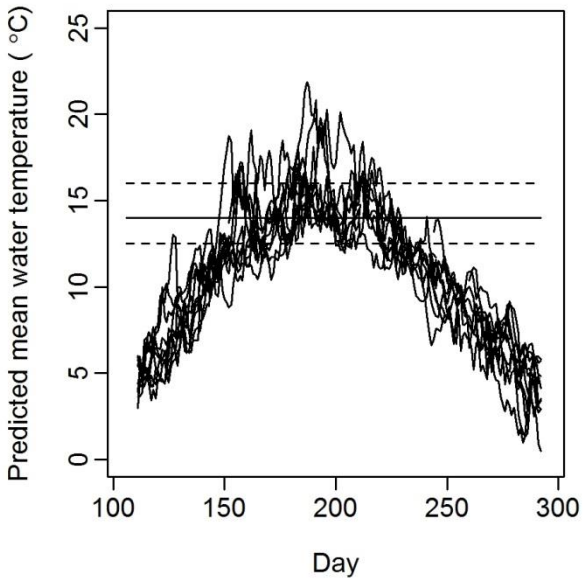

Predicted mean water temperature (°C)
0
5
10
15
20
25
100
150
200
250
300
Day

Figure 2